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PRESENCE OF LONG-LASTING PERIPHERAL ADAPTATION IN OBLIQUE-BANDED LEAFROLLER, *Choristoneura rosaceana* AND ABSENCE OF SUCH ADAPTATION IN REDBANDED LEAFROLLER, *Argyrotaenia velutinana*

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Abstract—Pre-exposure of male oblique-banded leafrollers, *Choristoneura rosaceana* (Harris), to the main component of their pheromone blend and traces of its geometric isomer (*Z*11–14:Ac and *E*11–14:Ac, respectively) at 36 ± 12 ng/ml air for durations of 15 and 60 min in sealed Teflon chambers with continuous air exchange significantly reduced peripheral sensory responses to these compounds as measured by electroantennograms (EAGs). The EAG responses of *C. rosaceana* to all tested dosages of pheromonal stimuli and blank controls were lowered by 55–58% and made a linear recovery to 70–100% of the pre-exposure amplitude within 12.5 min at a rate of 3–4 %/min. Exposures of 5 min were insufficient to maximally adapt *C. rosaceana*; however, exposures of 15 and 60 min reduced sensory responsiveness to the same minimum. In contrast, EAG responses of redbanded leafroller, *Argyrotaenia velutinana* (Walker), after identical pheromone exposure for 5 and 60 min yielded no long-lasting peripheral sensory adaptation as measured by EAGs, even though this species shares the same main pheromone components with *C. rosaceana*. We postulate that the long-lasting peripheral adaptation observed for *C. rosaceana* is a mechanism that impedes central nervous system habituation in this species. In contrast, *A. velutinana* may be more susceptible to central nervous system habituation because it lacks the capacity for minutes-long adaptation. We propose that long-lasting adaptation may be a mechanism explaining some of the variation in efficacy of pheromone-based mating disruption across taxa.

Key Words—Long-lasting adaptation, adaptation chamber, electroantennogram, NO/cGMP signal transduction pathway.

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INTRODUCTION

Pheromone-based mating disruption for economically important lepidopteran pests has been successfully demonstrated and promises to make important contributions to biorational pest control (Cardé et al., 1975; Deland et al., 1994; Cardé and Minks, 1995). However, in certain cases, utility of pheromone mating disruption may be limited by such factors as high population densities of moths (Schmitz et al., 1995a; Weissling and Knight, 1996; Suckling and Angerelli, 1996; Knight and Turner, 1999) that increases competition between calling females and pheromone dispensers; migration of mated females into treated areas; variable canopy structure; wind direction, which affects pheromone plume structure (Cardé and Minks, 1995) and retention; and the specific tuning of the pheromone blends employed (Pfeiffer et al., 1993; Knight et al., 1998; Knight and Turner, 1999; Evenden et al., 1999a). A challenge for pest managers is to determine which pests are most conducive to management by pheromones as opposed to other strategies.

The most popular hypotheses concerning mechanisms for disruption of pheromone-based communication are: (1) sensory adaptation at the peripheral level affecting olfactory receptors, (2) habituation affecting processing of and normal responsiveness to olfactory information reaching the central nervous system, (3) camouflage of female-produced plumes, and (4) false-trail-following of synthetic pheromone plumes by male moths (Rothchild, 1981; Bartell, 1982; Cardé, 1990).

The effects of short and prolonged exposures of moths to their species-specific synthetic pheromones and geometric isomers have been the targets of various investigations (Bartell and Roelofs, 1973; Bartell and Lawrence, 1976a–c; Linn and Roelofs, 1981; Sanders, 1985). These and other studies established that prolonged exposure to pheromone decreased such male behavioral responses as wing fanning and rapid walking (Bartell and Roelofs, 1973), recapture rates in mark–release studies, and orientation in wind tunnels (Rumbo and Vickers, 1997; Daly and Figueredo, 2000). For *Trichoplusia ni* (Hübner), these effects occurred with no corresponding decrease in responses of olfactory receptor neurons as measured by EAGs (Kuenen and Baker, 1981). In a later study, male *Cydia* (=*Grapholita*) *molesta* (Busck) exhibited days-long habituation after exposure to its pheromone (Figueredo and Baker, 1992). Such studies provide evidence that habituation of the central nervous system can be an important mechanism for observed decreases in males' responsiveness to female pheromones under conditions of mating disruption. In contrast, other studies suggest that adaptation and habituation, although inducible, had no influence on the effectiveness of mating disruption in the field (Novak and Roelofs, 1985; Schmitz et al., 1995a,b, 1997). The main explanations proposed for mating disruption of these species are competition between females and pheromone dispensers as well as camouflage of female pheromone signals.

The recent review by Zufall and Leinders-Zufall (2000) has formally defined three distinct types of olfactory adaptation, using vocabulary likely to be adopted for animals generally. The categories are characterized by differing temporal dynamics. The two short-lived variants have onset times on the order of 100 msec and 4 sec and corresponding recovery times of 10 sec and 1.5 min, respectively. The third type of adaptation is termed "long-lasting"; onset occurs after repetitive exposure for 25 sec and subsequent recovery intervals are about 6 min. As shown by Zufall and Leinders-Zufall (2000), these three types of adaptation are further distinguished by separate molecular mechanisms. For example, in salamander (*Ambystoma tigrinum*) olfactory receptor neurons, the long-lasting adaptive effect depends on the carbon monoxide (CO)/cGMP second messenger system, and can be uncoupled from excitation and completely eliminated by inhibitors of CO synthesis (Zufall and Leinders-Zufall, 1997). Likewise, elevated concentrations of cGMP were observed in moth antennae [*Heliothis virescens* (F.)], after application of high doses of pheromone (Boekhoff et al., 1993). Furthermore, Boekhoff et al. (1993) established that the IP_3 pathway mediates the primary transduction of pheromone signaling in antennal neurons, whereas activation of the cGMP-cascade is a secondary effect thought to be involved in adaptation and tuning of receptor neuron sensitivity.

Few studies have characterized sensory adaptation with the intention of distinguishing long-lasting versus short-lived variants of peripheral adaptation. Kuenen and Baker (1981) documented a short-lived form of pheromonal adaptation in *T. ni* using EAG; full receptor cell recovery occurred within 1 min of exposure.Schmitz et al. (1997) characterized longer-lasting sensory adaptation in *Lobesia botrana* (Denis and Schiffermüller), from which EAG responses returned to 70% of their pretreatment amplitude after 5 min.

The obliquebanded leafroller, *Choristoneura rosaceana* (Harris), and the redbanded leafroller, *Argyrotaenia velutinana* (Walker), share the major components of their pheromone blends: *Z*11–14:Ac and *E*11–14:Ac in a 98:2 ratio for *C. rosaceana* and 93:7 ratio for *A. velutinana* (Roelofs and Arn, 1968; Roelofs and Tette, 1970; Cardé and Roelofs, 1977; Hill and Roelofs, 1979). *A. velutinana* is reported to be easily disrupted, in some cases using only the main pheromone component, *Z*11–14:Ac (Novak et al., 1978; Reissig et al., 1978; Novak and Roelofs, 1985; Miller et al., unpublished data). In contrast, *C. rosaceana* is often described as difficult to disrupt in the field as measured by lowered captures of males by synthetically baited traps, fruit and foliar damage, and mating reductions of tethered females (Novak et al., 1978; Reissig et al., 1978; Roelofs and Novak, 1981; Deland et al., 1994; Agnello et al., 1996; Lawson et al., 1996; Miller et al., unpublished data) and possibly requiring the full natural blend of pheromone components. However, populations of *C. rosaceana* from Western Canada and Washington state, USA, which are characterized by a slightly different blend of pheromone components compared with those from central and Eastern North America (Vakenti et al.,

1988; Thomson et al., 1991), have shown some potential for successful mating disruption in small-plot trials (Knight et al., 1998; Evenden et al., 1999b,c).

We seek to understand the mechanisms underlying the differences in susceptibility to mating disruption in these two sympatric tortricids. In this first paper of a series, we characterize the differences in the capacity for long-lasting peripheral adaptation and disadaptation in *C. rosaceana* and *A. velutinana* using EAG measurements performed on moths before and after exposure to pheromone for various time intervals.

METHODS AND MATERIALS

Insect Colonies. C. rosaceana were drawn from a 4-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, New York, USA, by W. Roelofs. Both species were reared at 24°C on a pinto bean diet (Shorey and Hale, 1965) under a 16L : 8D photoperiod. Male pupae of each species were segregated in 1-liter plastic cages containing a 5% sucrose solution in plastic cups with dental cotton wick protruding from their lids.

Electroantennograms. Our EAG system consisted of a data acquisition interface board (type IDAC-02) and universal single-ended probe (Type PRS-1) from Syntech (Hilversum, The Netherlands). The recording and indifferent electrodes consisted of silver-coated wire in glass micropipets (10 μl microhematocrit capillary tubes) containing 0.5 M KCl. Micropipets were prepared by a Flaming/Brown micropipet puller (model P-97, Sutter Instrument Co.). The pipets were pulled at 308°C under time and velocity settings of 150 and 80, respectively. EAG data were recorded, stored, printed, and quantified using a Gateway 2000 (P-75) computer equipped with an interface card and software (PC-EAG version 2.4) from Syntech. The interface card contained a software-controlled amplifier, and an A/D conversion circuit; it operated with 12-bit resolution.

Male insects of both species were 2–4 days old when used for electroantennograms. EAGs were measured as the maximum amplitude of depolarization elicited by the applied stimulus. EAGs were conducted on live-insect preparations (Figure 1). Insects were restrained on a wax-filled, 3.5-cm-diam. Petri dish by placing clay (10 × 3 mm) over their thorax and abdomen. The terminal two segments of the antenna destined for recording were removed with fine scissors and the recording electrode was placed over the severed end. The reference electrode was inserted into the neck (Figure 1).

Stimulus Delivery. This apparatus consisted of a glass Y-tube (each arm 2 cm in length, the base 1 cm long, 0.5 cm diam.) positioned approximately 5 mm from the antenna (Figure 1). Carbon-filtered and humidified air (50 ml/min) was delivered

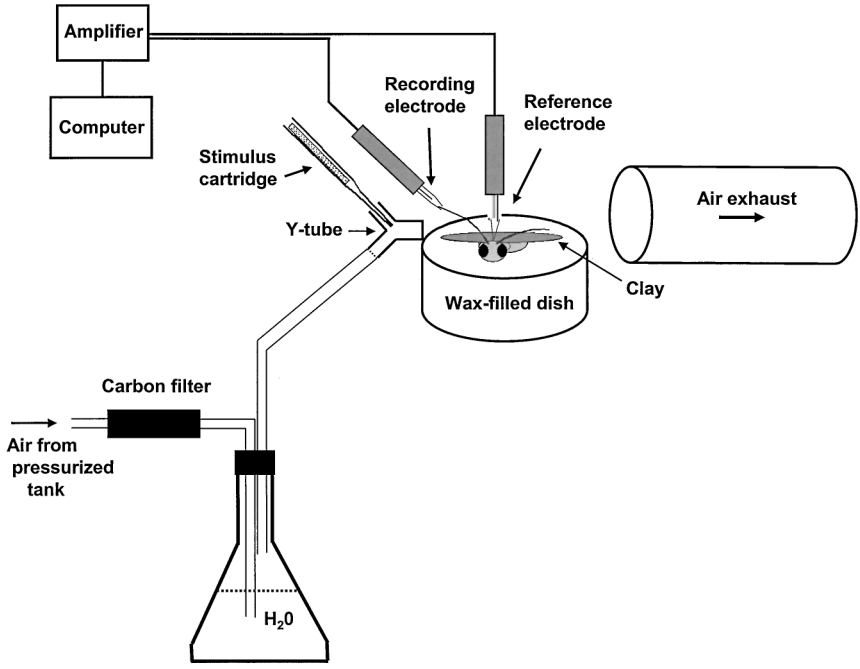

FIG. 1. Design of electroantennogram recording and stimulus delivery apparatus. Insect and electrodes are enlarged.

continuously into one arm of the Y-tube via Tygon tubing, while pheromone stimuli were delivered through the second arm of the Y-tube. *Z*11–14:Ac (lot # 10010) was obtained from Shin Etsu (Tokyo, Japan) and purity was determined with GLC (96.1% *Z*11–14:Ac and 3.9% *E*11–14:Ac). Various concentrations of this mixture of pheromone in hexane (20 μl total solution) were pipetted onto 1.4- $\times$ 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 pipets, sealed with Parafilm, and allowed to equilibrate for 24 hr prior to use. A given stimulus pipet (cartridge) was inserted into the Y-tube such that its tip was positioned at the junction of the Y-tube and 1.5 cm from antennal preparations (Figure 1). Stimulus puffs (1 ml) were generated through the cartridges with a clean hand-held 20-ml glass syringe connected to the pipets with a 1-cm piece of Tygon tubing. The time interval to expel 1 ml of stimulus odor or clean air from the syringe was quantified with video-cinematography and slow-motion playback. The 1-ml puff of air was expelled from the syringe in 120 ± 0.02 (SD) msec ($N = 20$).

Adaptation Experiments. Prior to adaptation experiments, dose–response curves were obtained for both moth species. Pheromone dosages were delivered

to individual moths (N10) either in ascending or descending order. Four puffs of each dosage spaced 10 sec apart were administered to yield duplicate depolarization amplitudes at each dosage. Appropriate dosages were chosen for further experimentation and the ascending order of stimulus–dose application was employed in all later studies.

To test for inducement of adaptation, males of both species were placed in adaptation chambers consisting of 1-liter Teflon transfer containers (Jensen, Coral Springs, Florida, USA) equipped with two 0.64-cm ports in their lids (Figure 2). Glass inlets and outlets were affixed to the lids, allowing for carbon-filtered air (30 ml/min) to pass through the chambers. Chambers were divided with wire mesh (Figure 2), such that insects were confined in the upper halves while a rubber septum impregnated with 5 mg (solvent-free) of the same pheromone blend used in EAG cartridges was placed in the lower half (Figure 2). This arrangement was designed to reduce variation in pheromone exposure relative to that possible in a static container, where moths might touch the dispenser.

All chambers were allowed to equilibrate for 15 min prior to insertion of insects. To assay the onset of peripheral adaptation, EAGs were performed on both species ($N \geq 18$) after confinement for 5, 15, or 60 min. Sham treatments ($N \geq 18$) were administered in separate, pheromone-free chambers. EAGs were performed on all insects prior to confinement, exactly 1 min after confinement, and then 12.5 or 60 min postconfinement.

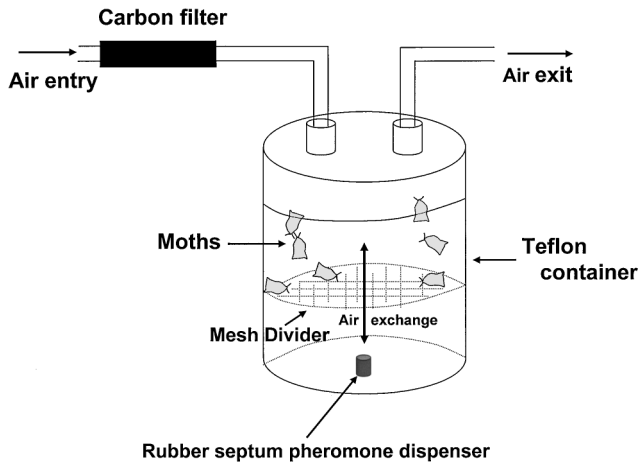

FIG. 2. Adaptation chamber: 1-liter Teflon container with glass inlet and outlet. Wire mesh divider prevents insects from contacting pheromone dispenser but allows exchange of air at throughput of 30 ml/min.

Disadaptation Study. Because minutes-long adaptation did not occur in *A. velutinana* (See Results below), only *C. rosaceana* was used for characterization of disadaptation. EAGs were performed on groups of six male *C. rosaceana* prior to confinement in adaptation chambers for 15 min of continuous pheromone exposure. After exposure, entire groups were removed from adaptation chambers and placed into clean-air containers. Individuals were assayed 1, 2.5, 5, 7.5, 10, 12.5, and 30 min thereafter. A total of 7 groups of six individuals was assayed in this manner.

Pheromone Concentration in Adaptation Chamber. Rubber septa impregnated with 5 mg of pheromone were placed into adaptation chambers and allowed to equilibrate for 15 min with air flowing through at a rate of 30 ml/min, as previously described. After equilibration, the exhaust port was replaced with a port sealed with a clean rubber septum. Immediately following port replacement, 15 ml of air was withdrawn from adaptation chambers through the septum-sealed port into a 20-ml glass syringe fitted with a 22-gauge needle. In rapid succession, 5 ml of hexane wash containing an internal standard of saturated 14:Ac at 6.4 ng/μl was drawn into the syringe. The solution within the syringe was carefully shaken for 30 sec, then expelled into a gas chromatography vial. This entire procedure was repeated 5 times with separate pheromone-impregnated septa. Prior to analysis, samples were concentrated under nitrogen by a factor of 50. Samples of 1 μl were analyzed by gas chromatography (HP-6890, Hewlett-Packard Co.) with flame ionization detection (FID) to measure the concentration of pheromone present in adaptation chambers. The GC was fitted with a DBWAXETR polar column (model 122-7332, J&W Scientific, Folsom, California, USA) 30 m long and 250 μm internal diam. The initial GC temperature was held at 100°C for 3 min and the program ran at a rate of 10°C/min from 100 to 250°C and was held for 3 min; the carrier gas was He. We calculated nanograms of pheromone present in the 15 ml of adaptation chamber air by multiplying the ratio of peak areas of the target compound (*Z*11–14:Ac) over the standard (saturated 14:Ac) by: (1) nanograms of internal standard present in 1 μl of concentrated hexane wash, and (2) 100 to account for GLC analysis of only 1% of the total concentrated sample.

The dosage of pheromone in adaptation chambers relative to pheromone cartridges was also compared to that of EAG cartridges. Adaptation chambers containing rubber septa impregnated with 5 mg of pheromone and equilibrated for 15 min were used as stimulus cartridges in EAG-assays of both *C. rosaceana* and *A. velutinana;* $N = 10$ for both species. EAGs were also carried out on individuals of both species ($N = 10$) using control chambers lacking pheromone. Chambers were modified by replacing the glass exhaust tube with a 5-cm Teflon tube. The in-current port was disconnected from the continuous airflow 1 min prior to assay and connected to a 20-ml glass syringe via a 25 cm piece of Tygon tubing. The Teflon exhaust tube was positioned ca. 1 cm from antennal preparations and 1-ml puffs were delivered through the chambers.

Statistical Analysis. 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 comparisons test (SAS Institute, 1989). The relationship between percent recovery from adaptation and time interval after exposure to pheromone was analyzed using linear regression. All values are ± SEM unless otherwise designated.

RESULTS

EAG Dose–Response Curves. Profiles for both species are presented in Figure 3 as unnormalized millivolt responses in both ascending and descending orders of stimulus-dosage applied. Under both regimes, responses of the two species overlapped for pheromone dosages 0–2.0 μg (Figure 3). Beginning at 20 μg, the dosage–response curves diverged; *A. velutinana* consistently produced higher EAG amplitudes at the high dosages than did *C. rosaceana.* Under the ascending regime, the mean responses reached a plateau of ca. 6 and 5 mV for *A. velutinana* and *C. rosaceana*, respectively, at the 200-μg dose. The mean EAG amplitudes were significantly ($P < 0.05$) different at the 200-μg and 2-mg dosages. Quite linear dosage–response curves were observed when dosages were applied in descending order, and the divergence in EAG amplitude between species was significantly ($P < 0.05$) different at the 200-μg, 2-mg, and 20-mg dosages. The maximum mean millivolt response for a given species was consistent irrespective of the order for dosage presentation (Figure 3).

Pheromone Exposure Studies. EAG amplitudes of male *C. rosaceana* prior to and 1 min after 5 min of confinement in adaptation chambers were not different

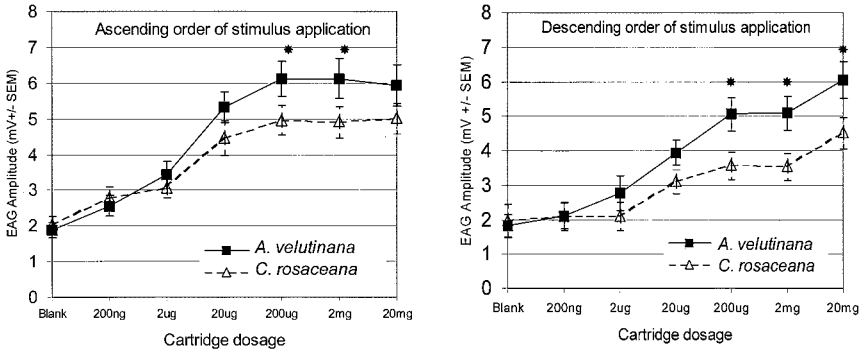

FIG. 3. Dose–response relationships for *Choristoneura rosaceana* and *Argyrotaenia velutinana* live-insect antennal prepartions in both ascending and descending orders of stimulus application. *Significant differences between pairs of means ($P < 0.05$).

(Figures 4A and 5A). However, EAG responses to the 200-μg and 2-mg dosages were ($P < 0.05$) lower in *C. rosaceana* assayed 1 min after 15 min of confinement compared with their pre-exposure responses (Figures 4B and 5B). The mean EAG responses of these individuals returned to their pre-exposure amplitudes within 30 min after exposure (Figures 4B and 5B). *C. rosaceana* exposed for 60 min in adaptation chambers produced lower mean EAG amplitudes 1 min after confinement compared with their pre-exposure responses to the 2-μg, 200-μg, and 2-mg doses (Figures 4C and 5C) ($P < 0.05$). After a 60-min post-exposure interval in clean air, the mean EAG responses of *C. rosaceana* exposed for 60 min were not different from their pre-exposure amplitudes for all dosages except 2 mg (Figures 4C and 5C).

EAG responses of *A. velutinana* prior to exposure in adaptation chambers and after exposure for 5- and 60-min durations were not different at any of the stimulus dosages applied (Figures 4E, F, and 5D).

Disadaptation Study. C. rosaceana EAG amplitudes after 15 min of exposure were lower compared with pre-exposure amplitudes for the 200-μg and 2-mg doses at 1, 2.5, and 5 min after exposure (Figures 4D) ($P < 0.05$). At 7.5 min after exposure, the mean EAG amplitudes were no longer different from pre-exposure amplitudes and responses returned to amplitudes nearly equal to pre-exposure responses within 12.5 min after exposure (Figure 4D). The mean EAG amplitudes elicited by the blank and 2-μg doses also decreased after 15 min of exposure and followed a similar trend of increase after exposure; however, the mean differences were not significant in simple pair-wise comparisons. A significant ($P < 0.05$) linear relationship was found in the percent recoveries of the mean EAG responses elicited by each dosage including the blank when regressed over time after 15 min of exposure in adaptation chambers (Figure 6). Percent recovery was calculated as the ratio of the mean EAG amplitude at the various time intervals at which individuals were assayed after exposure over the mean pre-exposure amplitude. The process of recovery was decidedly linear; recovery rate was a constant 3–4%/min and was nearly complete within 12.5 min (Figure 5).

Pheromone Concentration in Adaptation Chamber. The retention times for the 14:Ac (internal standard) and *Z*11–14:Ac were 13.7 ± 0.012 and 14.3 ± 0.008 min, respectively. The trace amount of *E*11–14:Ac present in the pheromone used to impregnate the rubber septa was undetected by FID in the air samples taken from adaptation chambers. The mean peak areas in thousands for the 14:Ac standard and *Z*11–14:Ac were 454 ± 28 and 8 ± 3, respectively. The calculated concentration of *Z*11–14:Ac present in our adaptation chambers was 36 ± 12 ng/ml of air.

For *C. rosaceana*, adaptation chambers containing pheromone elicited higher EAG responses (2.5 ± 0.24 mV) compared with depolarizations elicited by control chambers lacking pheromone (1.1 ± 0.13 mV) ($P < 0.05$). The mean amplitude elicited by the adaptation chamber closely corresponded to the mean response

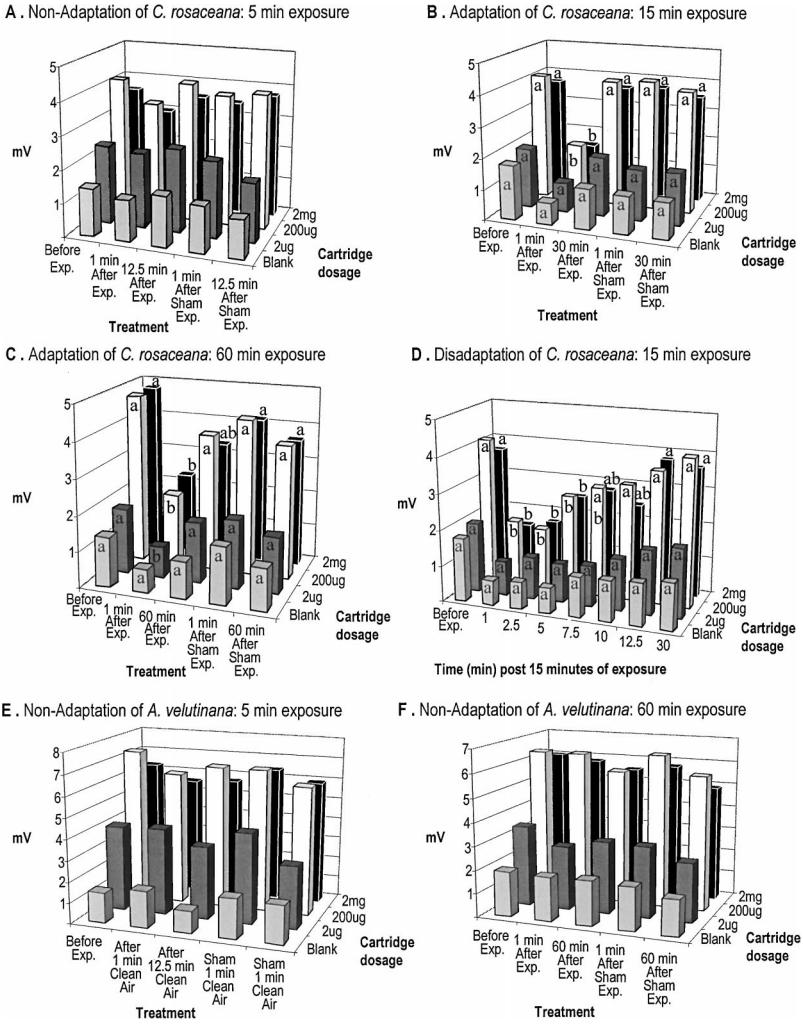

FIG. 4. (A) Effect of 5 min of confinement of *Choristoneura rosaceana* in adaptation chambers. There were no significant differences between treatment means for responses within or across dosages. (B) Effect of 15 min of confinement of *C. rosaceana* in adaptation chambers. Bars with different letters indicate significant differences between treatment means within a given dosage. (C) Effect of 60 min of confinement of *C. rosaceana* in adaptation chambers. Bars with different letters indicate significant differences between treatment means within a given dosage. (D) Disadaptation of *Choristoneura rosaceana* after 15 min of confinement in adaptation chambers. Bars with different letters indicate significant differences between treatment means within a given dosage. (E and F) Effect of 5 and 60 min, respectively of confinement of *Argyrotaenia velutinana* in adaptation chambers. There were no significant differences between treatment means for responses within a given dosage.

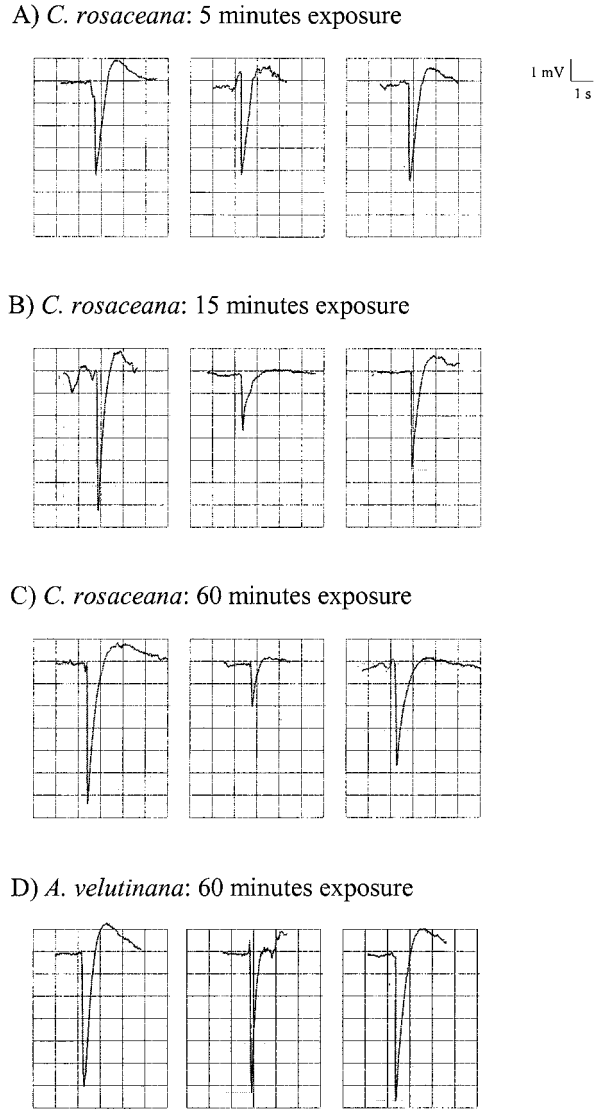

FIG. 5. Representative EAG tracings of *Choristoneura rosaceana* and *Argyrotaenia velutinana* responding to 1-ml puffs from pheromone cartridges loaded with 200 μg of pheromone. Tracings on the left within each row are responses of *C. rosaceana* or *A. velutinana* prior to exposure in adaptation chambers. Center tracings within each row are responses of the same individuals after various exposure intervals within adaptation chambers. Tracings on the right within each row were measured after 30- to 60-min intervals within pheromone-free air after exposure in adaptation chambers. Each horizontal tick mark represents 1 sec and each vertical tick mark represents 1 mV of depolarization.

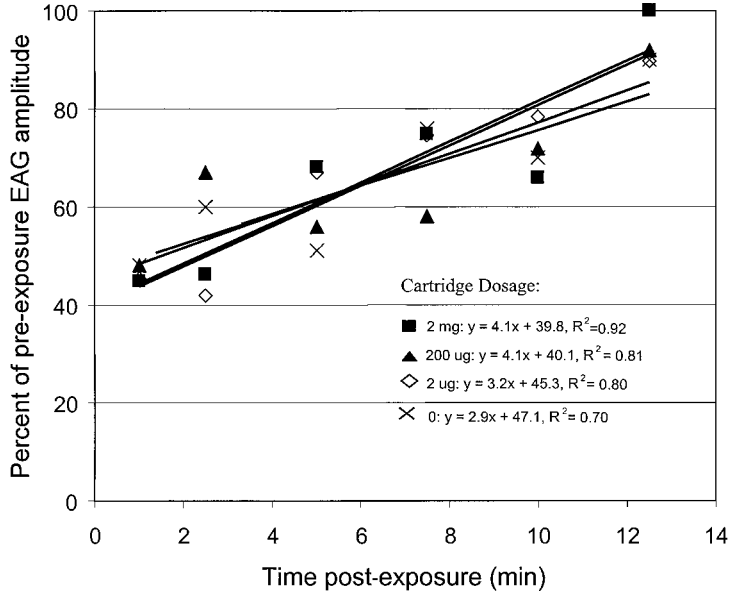

FIG. 6. Linear regressions of percent recovery of EAG responses of *Choristoneura rosaceana* after 15 min of confinement in adaptation chambers over time. Percent recovery was calculated as the ratio of the mean EAG amplitude at the various time intervals at which individuals were assayed post-exposure over the mean pre-exposure amplitude. All regressions were significant at $P < 0.01$.

generated by our stimulus cartridges at the 200-ng dosage (Figure 3). For *A. velutinana*, a response of 4.1 ± 0.4 mV was generated by the adaptation chamber with pheromone, which was also higher than the responses elicited by control chambers lacking pheromone (1.0 ± 0.13 mV) ($P < 0.05$). For this species, the adaptation chambers induced a response that closely corresponded to responses generated by stimulus cartridges charged with the 2-μg dosage (Figure 3).

DISCUSSION

Dynamics of Long-Lasting Adaptation in C. rosaceana. Pre-exposure of male *C. rosaceana* to the main components of its pheromone blend (*Z*11- and *E*11–14:Ac) decreased EAG responses to the pheromone for up to 10 min after exposure (Figure 4D). Assuming that an EAG consists of depolarization of receptor potentials summed across the antennal olfactory neurons (Roelofs, 1984), the observed decrease in response by *C. rosaceana* can be attributed to decreased sensitivity of pheromone receptor neurons. The EAG response of pre-exposed *C. rosaceana* to our stimuli was reduced by 55–58% for all cartridge dosages tested including

the blank (control). Responses returned in a linear fashion to 70–100% of the pre-exposure response within 12.5 min (Figure 6). These results establish that the process of recovery from adaptation took place at a constant rate. At our exposure dosage of 36 ± 12 ng/ml, exposures of only 5 min were not sufficient to induce adaptation in *C. rosaceana*; however, exposures of 15 and 60 min reduced sensory responsiveness to the same degree (60%). Given that exposure duration of 60 min did not increase adaptation, it seems that a plateau was reached at or even before 15 min. Currently, we have no physiological explanation for why long-lasting adaptation in *C. rosaceana* peaks at 60%. Perhaps the titer of some inhibitory signaling agent (see below) is set so as not to turn off signaling transduction completely. Alternatively, we have not ruled out the possibility that certain populations of receptors adapt completely while others do not. Given the adaptive significance of male sensitivity to female pheromone, it would seem disadvantageous for male *C. rosaceana* to become completely anosmic to its pheromone. Characterization of the dose–response relationship and threshold for long-lasting adaptation in *C. rosaceana* will be treated further in a subsequent publication.

Similarities of C. rosaceana Adaptation Response to Those of Other Moth Species. By performing recordings from single antennal neurons, Baker et al. (1989) showed that male *Agrotis segetum* (Schiffermüller) olfactory receptor neurons adapted when they were exposed to high pheromone concentrations known to cause in-flight arrestment of progress toward the source. Using the same technique, they also showed that antennal neurons from *H. virescens* failed to adapt regardless of concentration. Baker et al. (1989) proposed that, given the low emission rate of *Z*11–16:Ald from the rubber septa employed in their study, it was unlikely that *H. virescens* neurons were challenged to the same degree as *A. segetum* had been by the more volatile pheromone of that species. Alternatively, we suggest that *A. segetum* and *H. virescens* may differ in their susceptibility to peripheral sensory adaptation, as we have observed with *C. rosaceana* and *A. velutinana*. Specifically, certain species, such as *C. rosaceana* and *A. segetum*, may exhibit greater capacity for sensory adaptation at the peripheral level than others such as *A. velutinana* and possibly *H. virescens.* Other electrophysiological studies have also demonstrated differential degrees of peripheral adaptation among moth species (Kuenen and Baker, 1981; Schmitz et al., 1997).

Molecular Basis of Long-Lasting Adaptation. Adaptation of moth olfactory receptor neurons has long been recognized to occur following intense pheromonal stimulation, be it constant or pulsed (e.g., Baker et al., 1989; Figuerdo and Baker, 1992); however, delineation of the particular types of adaptation as recently defined by Zufall and Leinders-Zufall (2000) has just begun. Both invertebrate and vertebrate animal models reveal that both rapid and slower, longer-lasting forms of odor adaptation exist (Getchell, 1986; Marion-Poll and Tobin, 1992). The molecular basis and temporal dynamics of some of these differences has recently been described (Zufall and Leinders-Zufall, 2000). The rapid forms of adaptation result

from Ca^{2+}-dependent cyclic nucleotide-gated (CNG) ion channel modulation and Ca^{2+}/calmodulin kinase II-dependent phosphorylation, respectively. In contrast, long-lasting adaptation is mediated by the carbon monoxide (CO)/cGMP second messenger system. Thus, odor adaptation is a complex phenomenon mediated by diverse molecular processes; these may differ within and among taxa depending on the nature of the signal-transduction cascades mediating olfaction in those species. Boekhoff et al. (1993) showed that in moth antennae cGMP formation is a consecutive reaction to pheromone-induced elevation in IP_3 concentration. Consistent with this interpretation, exogenously applied cGMP abolished the phasic but not the tonic component of the pheromone-stimulated IP_3 signal in *H. virescens* [Figure 5B of Boekhoff et al., (1993)]. Sustained pheromonal stimulation is also known to induce cGMP signals in *Antheraea polyphemus* and *Bombyx mori* (Ziegelberger et al., 1990), and pheromone-activated cation channels sensitive to cGMP have been found in insect olfactory cilia (Zufall and Hatt, 1991). Furthermore, an IP_3–mediated increase in intracellular Ca^{2+} could be the direct stimulus for shifts in Ca^{2+}/calmodulin interactions or, alternatively, could activate NO synthase, leading to the generation of NO and subsequently activating cytoplasmic guanylyl cyclase (Steinlen et al., 1990; Boekhoff et al., 1993). Thus, as proposed by Zufall and Leinders-Zufall (2000), there is good reason to believe that insects and vertebrates share parallel mechanisms yielding long-lasting adaptation. The NO/cGMP pathway has been implicated in odor processing in vertebrates and invertebrates (Breer et al., 1992; Boekhoff et al., 1993) and should be considered along with the (CO)/cGMP second-messenger system as possible mechanisms leading to the long-lasting form of adaptation such as that observed in *C. rosaceana* in the present study. Pharmacological inhibitors of CO and NO formation (Zufall and Leinders-Zufall, 1997) will be useful in testing this hypothesis.

Adaptation to Blank Air Puffs. Throughout this study, blank (negative control) puffs of clean air elicited EAG responses of 1–2 mV from both tortricids when applied from either clean disposable Pasteur pipets or clean adaptation chambers (Figure 3), and adaptation resulted in reduced responses to both pheromonal and clean air (control) stimulus puffs (Figure 4). We took utmost care to assure that there was no pheromone contamination in controls. Pioneer studies on *B. mori* showed that EAG depolarizations occur in response to blank (control) puffs of clean pheromone-free air (Schneider 1962, Figures 2 and 3) and more contemporary studies using *A. velutinana* also showed EAG responses to 1-ml puffs of clean air (Baker and Roelofs 1976, Figure 3); however, these responses were smaller than in the present study. Mayer et al. (1984) also obtained pronounced EAG responses to their blank control stimulus puffs and proposed that stimuli such as water vapor, extraneous room contaminants, or delivery-line plasticizers may have been responsible for eliciting these responses. If the blank responses in our study were due to such extraneous contaminants, then adaptation to pheromone may

have resulted in cross-adaptation to other unknown chemical or physical stimuli. In addition, the 1-ml puffs of air momentarily trembled the filiform antennae of both species, and such movement of the antennae may have added to the apparent EAG depolarization.

Proposed Impacts of Long-Lasting Adaptation on Susceptibility to Pheromone Disruption. Exposure of *A. velutinana* to the components of its pheromone blend results in distortion and inhibition of the normal sexual response (Bartell and Roelofs, 1973). However, our results indicated that long-term exposure of *A. velutinana* to the main component of its pheromone blend and the geometric isomer had no effect on peripheral sensitivity even 1 min after exposure. Our results with *A. velutinana* are similar to those of Kuenen and Baker (1981), who showed that adaptation of receptor response in *T. ni* was also short-lived and returned to control levels 1 min after cessation of pheromone stimulation. In both cases, sensory processes at the peripheral level appear not to have declined after pheromone exposure, whereas central nervous system habituation appears to be the longer lasting and fundamental cause of sexual response-inhibition. Long-term habituation has also been shown to occur with *C. molesta* in wind-tunnel trials (Figueredo and Baker, 1992) and in field experiments (Rumbo and Vickers, 1997). Similarly, wind-tunnel and field experiments using *H. virescens* implicated central nervous system habituation, lasting as long as 96 h, as the major mechanism for modulating male moth response to female pheromone and as the underlying means for pheromone-based mating disruption (Daly and Figueredo, 2000).

Bartell and Lawrence (1977) suggested that male moth exposures to pulsed pheromonal stimuli would result in greater reductions of sexual response compared with constant stimulation, because peripheral adaptation would be circumvented so as to allow for greater central habituation. Kuenen and Baker (1981) obtained data supporting this hypothesis for *T. ni* by showing that pulsed rather than constant pre-exposure resulted in greater disorientation. They also demonstrated decreased EAG amplitudes with concurrent exposure, indicating that receptor adaptation was taking place. They concluded that receptor adaptation might have been an impediment for central nervous system habituation. Therefore, we postulate that the long-lasting peripheral adaptation documented in this study for *C. rosaceana* could be a mechanism precluding central nervous system habituation in this species and reducing susceptibility to pheromone-based mating disruption, as observed in field studies (Novak et al., 1978; Reissig et al., 1978; Roelofs and Novak, 1981; Deland et al., 1994; Agnello et al., 1996; Lawson et al., 1996; Gut and Miller, unpublished data). In contrast, *A. velutinana*, which is easily disoriented in lab and field studies (Bartell and Roelofs, 1973; Cardé et al., 1975; Novak et al., 1978; Reissig et al., 1978; Novak and Roelofs, 1985; Gut and Miller, unpublished data), may be more susceptible to central nervous system habituation, as it appears to lack the capacity for long-lasting peripheral adaptation. In addition, it is possible that the onset of long-lasting adaptation in *C. rosaceana* may result in a decrease in perception of

the active space of synthetic pheromone point sources and, therefore, a decreased inclination for false-trail-following. The opposite effect would be expected for *A. velutinana*, resulting in sustained false-trail-following or pheromone-induced excitation and arrestment precluding movement into zones of pheromone free air.

The concentration of pheromone per milliliter of air in our adaptation chambers was judged high, as it was within the range of pheromone present per abdominal tip extract of female *A. velutinana* (Roelofs et al., 1975; Miller and Roelofs, 1980). Future studies must be extended into the field to document whether similar adaptation takes place at pheromone concentrations realized within a pheromone-treated crop. Finally, studies employing intracellular recordings from central nervous system processing centers in the olfactory lobe (Anton and Gadenne, 1999; Gadenne et al., 2001), obtained from pheromone-exposed *C. rosaceana* and *A. velutinana*, may illuminate whether adaptation actually precludes habituation.

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