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Concentration of air-borne pheromone required for long-lasting peripheral adaptation in the obliquebanded leafroller, *Choristoneura rosaceana*

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Abstract. Electroantennogram (EAG) responses of male obliquebanded leafrollers, *Choristoneura rosaceana* (Harris), to the main component of its pheromone blend and traces of geometric isomer ((*Z*)11-14:Ac and (*E*)11-14:Ac, respectively) were recorded before and after 1 h of continuous exposure to pheromone in laboratory experiments, and 24 h of exposure under field conditions. Concentrations of pheromone ranging from 56 to below 1 ng mL^{-1} air in Teflon chambers with regulated air exchange reduced peripheral sensory responses by 40–60% as measured by amplitudes of the EAG. Adaptation did not increase in a dosage-dependent fashion over most of this range; an identical reduction of responsiveness was observed at each exposure to an effective concentration. Exposure of *C. rosaceana* at a loading dosage of 1 ng of pheromone in 100 μL of mineral oil (air concentration below the GLC detection limit) did not induce measurable adaptation. Caging *C. rosaceana* in apple trees adjacent to one, two or four Isomate OBLR/PLR Plus polyethylene pheromone dispensers for 24 h resulted in long-lasting adaptation similar to that seen in laboratory experiments. Adaptation was not observed for *C. rosaceana* caged at a distance of 2 m from Isomate dispensers in 1-ha plots treated with 500 dispensers per ha. Whenever observed, this type of adaptation was expressed for more than 5 min after exposure to pheromone ceased. Collectively, this adaptation phenomenon in *C. rosaceana* is consistent with the third of Zufall & Leinders-Zufall's types of olfactory adaptation that is 'long-lasting'. Although the dosage of pheromone required to induce long-lasting adaptation in this moth is judged high relative to that for normal sexual communication, we suggest this type of adaptation may come into play for some but not all moths under pest-control regimes using the tactic of pheromone-disruption, particularly those using high-dosage release technologies like pheromone rope dispensers or Microsprayers.

Key words. Adaptation chamber, electroantennogram (EAG), cGMP signal-transduction pathway, pheromone-based mating disruption.

Introduction

Under natural conditions, adaptation of pheromonal and other olfactory neurones is thought to enable an animal's sensory system to adjust levels of sensitivity to allow appropriate behavioural responses at varying stimulus intensities (Zufall & Leinders-Zufall, 2000). Moreover, adaptation may preclude: saturation of cellular transduction processes, and/or overwhelming of sensory processing centres during continuous high levels of stimulation. In some cases, adaptation is reported to improve the detection of signal in a background of noise, e.g. in lobsters (Atema *et al.*, 1989).

Sensory adaptation and central nervous system habituation are also thought to be important under unnatural conditions, e.g. when semiochemicals like sex attractant

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pheromones are broadcast throughout a crop to control insects by disrupting mate-finding (Cardé, 1990; Cardé & Minks, 1995). For example, Baker *et al.* (1988, 1989) reported that adaptation of antennal neurones was responsible for stopping the flight of male *Agrotis segetum* (Schiffermüller) up a pheromone plume. It has become clear that insights into processes of adaptation will be important from both the applied as well as the basic perspective.

The molecular basis and temporal dynamics of three distinct types of adaptation have recently been elucidated in vertebrate olfactory neurones (Zufall & Leinders-Zufall, 2000). They include: Ca^{2+} influx through cyclic nucleotide-gated channels and Ca^{2+}-dependent ion channel modulation; Ca^{2+}/calmodulin kinase II-dependent phosphorylation; and activation of the carbon monoxide (CO)/cGMP second messenger system. These different types of adaptation can be distinguished on the basis of their onset and recovery times. Two short-lived variants exhibit onset times on the order of 100 ms and 4 s and corresponding recovery times of 10 s and 1.5 min, respectively. The third type of adaptation is characterized as 'long-lasting'; onset requires repetitive exposures of at least 25 s and recovery is seen after 6 min.

Morphologically and physiologically, the olfactory neurones of vertebrates and insects are similar both peripherally and in the initial steps of central processing (Lancet, 1986; Anholt, 1987; Boeckh & Ernst, 1987; Homberg *et al.*, 1989; Zufall & Leinders-Zufall, 2000). Moreover, insects and vertebrates are thought to share many olfactory transduction mechanisms (Stengl *et al.*, 1992). Thus, it is reasonable to search in insects for types of sensory adaptation already established for vertebrates.

We recently discovered (Stelinski *et al.*, 2003) in a moth of the family Tortricidae a form of pheromonal sensory adaptation corresponding to the 'long-lasting' adaptation reported by Zufall & Leinders-Zufall (2000) for the tiger salamander, *Ambystoma tigrinum*. Pre-exposure of male obliquebanded leafrollers, *Choristoneura rosaceana* (Harris), to components of its pheromone blend ((*Z*)11-14:Ac and (*E*)11-14:Ac) at a concentration of 36 ± 12 ng mL^{-1} air for durations of 15 or 60 min significantly reduced peripheral sensory responses to these compounds as measured by electroantennograms (EAG). The EAG amplitudes of *C. rosaceana* were reduced by 55–58% and recovered linearly to 70–100% of the pre-exposure response within 12.5 min at a rate of 3–4% min^{-1}. Exposures of 5 min were insufficient to elicit maximal adaptation in *C. rosaceana*; however, exposures of 15 or 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 or 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*.

The concentration of pheromone (*c.* 40 ng per mL of air) used to induce long-lasting adaptation in *C. rosaceana* in our initial study was well above levels used in normal sexual communication by these tortricid moths (Miller & Roelofs, 1980). The objectives of the current study were to characterize the dose–response relationship for long-lasting adaptation in *C. rosaceana*, define its threshold, and determine whether this type of adaptation could be coming into play under certain regimes of mating disruption in the field.

Materials and methods

Insect source. *C. rosaceana* were drawn from a 4-year-old laboratory colony collected originally as 1st and 2nd generation pupae from apple orchards in south-western Michigan. Moths were reared at 24 °C on pinto bean diet (Shorey & Hale, 1965) in a LD, 16 : 8-h photocycle. Male pupae of each species were segregated in 1-L plastic cages containing a 5% sucrose solution in plastic cups with dental cotton wick protruding from their lids.

Laboratory electroantennograms. The EAG system and test protocols were identical to those detailed by Stelinski et al. (2003). Briefly, 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 micropipettes (10-μL microhematocrit capillary tubes) containing 0.5 M KCl. Moths were 2–4 days post-eclosion when used for electroantennography. EAGs were measured as the maximum amplitude of depolarization elicited by 1-mL puffs of air through EAG cartridges of various pheromone loadings directed over live-insect preparations. The time interval to expel 1 mL of stimulus odour or clean air from the syringe was 120 ± 0.02 (SD) ms ($n = 20$) (Stelinski *et al.*, 2003).

Pheromone source and purity. (Z)11-14:Ac (lot # 10010) was obtained from Shin Etsu (Tokyo, Japan); its purity was determined with gas chromatography to be 96.1% (Z)11-14:Ac and 3.9% (E)11-14:Ac.

Laboratory adaptation experiments. Moths were placed in adaptation chambers consisting of cylindrical, 1-L Teflon transfer containers (Jensen, Coral Springs, FL, U.S.A.) equipped with two 64-mm ports in their lids. Glass inlets and outlets were affixed to the lids, allowing pressurized air that had been filtered through carbon to pass through the chambers at 30 mL min^{-1}. Chambers were divided with wire mesh such that insects were confined in upper halves, while one 2-cm-diameter × 0.5-cm-deep stainless steel planchette loaded with a given dosage of pheromone was placed in the lower half. This arrangement reduced variation in pheromone exposure relative to that in a static container, where distance of the moth from the pheromone source might influence pheromone uptake. The highest dosage of pheromone tested was 100 μL of neat pheromone in the planchette. Successively lower dosages were achieved by serially diluting 100 μL of mineral oil (Aldrich Chemical Company,

Milwaukee, WI, U.S.A.); loadings ranged in decade steps from 10 mg to 1 ng of pheromone. Chambers always equilibrated for 60 min prior to insertion of insects.

Twelve male *C. rosaceana* were assayed at each pheromone concentration. EAGs were performed on all insects (left antenna) prior to confinement, exactly 1 min after the 60-min confinement (right antenna), and after 5- or 15-min post-confinement intervals in clean air (left antenna 2nd time). Two control treatments were performed on additional groups of moths ($n = 12$). In the first control, we employed adaptation chambers containing planchettes with 100 μL mineral oil only, and followed all other procedures as described above. In the second control, we sought to establish a baseline for consistency of EAG readings between antennae for a given individual. We chose to perform EAGs on the left antenna, and then right antenna 1 h later without exposure in adaptation chambers, followed by a second reading from the left antenna 15 min later.

In previous work (Stelinski et al., 2003), *C. rosaceana* exhibited adaptation lasting up to 7.5 min after 15–60 min constant exposures to its pheromone at a concentration of $36 \pm 12\,\text{ng}\,\text{mL}^{-1}$ air achieved by loading adaptation chambers with single rubber septa impregnated with 5 mg of pheromone. In the present study, we sought to determine whether decreasing the loading dosage in adaptation chambers would alter the longevity of adaptation to pheromone in *C. rosaceana*, which was previously established at an arbitrary and high pheromone dosage.

Measurement of pheromone concentration in adaptation chamber. Planchettes containing each dosage of pheromone tested above were placed one at a time in adaptation chambers. After equilibration for 60 min, the exhaust port was replaced with a port sealed with a clean rubber septum. Immediately thereafter, 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 stainless steel needle. In rapid succession, 5 mL of hexane wash containing 14:Ac at $6.4\,\text{ng}\,\mu\text{L}^{-1}$ (used as an internal standard) was drawn into the syringe. The solution within the syringe was carefully shaken for 30 s then expelled into a 2-mL glass vial designed for gas liquid chromatography (Amber Crimp Vial, Hewlett-Packard Co.). This entire procedure was replicated five times at 23 °C and c. 35% RH with separate planchettes for each pheromone dosage tested. Prior to analysis, samples were concentrated under nitrogen by a factor of 50 (10 μL final volume). Samples were analysed by a gas liquid chromatograph (GLC) (HP-6890, Hewlett-Packard Co.) with flame ionization detection to reveal the concentration of pheromone present in adaptation chambers. The GLC was fitted with a DBWAXETR polar column (model #122-7332, J & W Scientific, Folsom, CA, U.S.A.) of length 30 m and internal diameter 250 μm. The initial oven temperature was held at 100 °C for 3 min and then programmed to increase $10\,^{\circ}\text{C}\,\text{min}^{-1}$ up to 250 °C where it was held for 3 min; the carrier gas was He. We calculated the concentration ($\text{ng}\,\mu\text{L}^{-1}$) 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: the concentration of internal standard present in the concentrated hexane wash; and 100 to account for GLC analysis of only 1% of the total concentrated sample.

Field adaptation experiments. All field experiments were conducted in June 2002 at Michigan State University's Trevor Nichols Research Complex, Fennville, MI, U.S.A. Experiments were conducted within a 15-year-old planting of Red Delicious apple trees spaced 3 m within and 6 m between rows. Male *C. rosaceana* (2–4 days post-eclosion), taken from the same colony as used in laboratory experiments, were placed in $6 \times 4 \times 2$-cm wire mesh cages (three per cage) containing a 2-cm piece of dental wick moistened with sugar-water. Cages were hung in branches of apple trees at 1.5–2 m above ground under various pheromone exposure regimes for 24 h (Table 1). Isomate OBLR/PLR Plus pheromone rope dispensers (Pacific Biocontrol Co., Vancouver, Washington) containing 227 mg of (Z)11-14:Ac were used to deliver pheromone in all field exposure trials. Treatments included cages hung in untreated 1-ha plots on: trees containing no dispensers; 2 m from one pheromone dispenser within the same tree; adjacent to one dispenser; adjacent to two dispensers; and adjacent to four dispensers surrounding the cage. The final treatment consisted of cages hung 2 m from pheromone dispensers within 1-ha plots treated with pheromone at the recommended label rate of 500 dispensers per ha.

After 24 h of field exposure under the various pheromone regimes, male *C. rosaceana* were assayed by EAG in the field to measure the possible onset of adaptation. The field-EAG system and stimulus-delivery methods were as similar as possible to those used in the laboratory set-up. Briefly, the micromanipulators used to manoeuvre the reference and recording electrodes were mounted on a 1-m × 1-m × 2-cm steel plate placed in the rear compartment of a Minivan automobile. A $0.75 \times 1 \times 1$-m Faraday cage covered the electrodes, micromanipulators and a dissecting scope, all situated on the steel plate. A Syntech data acquisition interface board (Type IDAC-02, Hilversum, the Netherlands) and computer used to record and store data were placed adjacent to the Faraday cage. Power outlets located throughout the research orchard supplied electricity. The van was always positioned c. 12–15 m from test plots. The field EAG system differed from our laboratory arrangement in that a constant stream of charcoal-filtered and humidified air was not continuously delivered over antennal preparations.

One live *C. rosaceana* out of a possible three was randomly removed from each wire mesh cage and immediately mounted for EAG analysis. Cases in which more than 1 min elapsed between cage retrieval and mounting the individual for EAG recording were discarded because of possible onset of disadaptation. As in laboratory experiments, the tip of an EAG cartridge was positioned c. 5 mm from antennal preparations. Stimulus puffs (1 mL) were generated through

Table 1. Prevalence and degree of 'long-lasting' adaptation in laboratory-reared *Choristoneura rosaceana* upon differing levels of exposure to Isomate OBLR/PLR Plus pheromone dispensers in the field.

			EAG amplitude (mean mV ± SE) upon stimulation with 1 mL of air through pheromone cartridge (200 μg loading dosage)		
				Adapters	
Pheromone exposure (24 h)	*n*	Number adapted	Non-adapters	Pre-recovery[1]	Post-recovery[2]
Directly from lab, no pheromone	17	0	3.26 ± 0.23a[3]	–	–
Field exposure, no pheromone	16	0	3.41 ± 0.33a	–	–
1 rope @2 m, single treated tree	16	0	3.28 ± 0.27a	–	–
1 dispenser @2 m, treated plot	16	0	3.14 ± 0.25a	–	–
1 dispenser adjacent	22	5	3.51 ± 0.25a	2.00 ± 0.25b	3.62 ± 0.47a
2 dispensers adjacent	16	7	3.46 ± 0.36a	1.69 ± 0.28b	3.70 ± 0.30a
4 dispensers adjacent	16	12	3.48 ± 0.30a	1.78 ± 0.15b	3.33 ± 0.25a

[1]Mounted and assayed within 1 min of field exposure.
[2]10 min after pheromone exposure and first EAG assay.
[3]Means across all columns not followed by the same letter are significantly different ($P < 0.05$) by ANOVA followed by Tukey's multiple-comparisons test.

pheromone cartridges (200 μg loading dosage) with a clean hand-held 20-mL glass syringe (Stelinski *et al*., 2003). *C. rosaceana* responding at least 1 mV below the mean amplitude obtained from individuals having no pheromone exposure were left mounted in pheromone-free air for10 min and EAG-assayed a second time to quantify disadaptation.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) and differences in selected pairs of means over time and between treatments for laboratory and field experiments were separated using Tukey's multiple comparisons test (SAS Institute, 1989). The relationship between loading dosage of pheromone diluted in mineral oil and pheromone concentration per mL of air in adaptation chambers was analysed using the trendline feature of Microsoft Excel.

Results

Adaptation experiments in the laboratory. The EAG responses of male *C. rosaceana* to the 2 μg, 200 μg, 2 mg and blank cartridges were significantly ($F = 21.0$; d.f. = 19, 351; $P < 0.05$) reduced (between 40 and 60%) after 60 min of exposure at pheromone dosages ranging from the maximum loading of 100 μL neat pheromone to 100 μL of mineral oil containing 100 ng pheromone (0.0001% by volume) (Fig. 1a). A slight reduction in EAG amplitude was recorded for *C. rosaceana* exposed in chambers containing the 10 ng pheromone dosage when tested with 1-mL puffs of air through EAG stimulus-cartridges loaded with 200 μg of pheromone (Fig. 1a). No adaptation was recorded for moths exposed in chambers containing the 1 ng dosage. Adapted *C. rosaceana* recovered their pre-exposure EAG amplitudes within 15 min post-exposure (Fig. 1b), but not by 5 min post-exposure (Fig. 2). The change in amplitude was the only notable shift in EAG profile visible for moths exposed to dosages that caused adaptation (Fig. 3a–g) vs. dosages of pheromone that did not (Fig. 3h,i). On average, responses to blank cartridges ranged from 0.9 to 1.7 mV; Stelinski *et al*. (2003) have discussed such responses to blank cartridges.

Pheromone concentration in the adaptation chamber. The retention times for the unsaturated 14:Ac (internal standard) and (Z)11-14:Ac were 13.7 ± 0.001 and 14.5 ± 0.003 min, respectively. Sample detection and quantification by the flame-ionization detector required 0.1 ng of pheromone per 1 μL of sample injected onto the GLC. Concentrations of pheromone in adaptation chamber air were detectable down to but not below the 1 μg loading (Fig. 4). The air-borne concentration of pheromone in adaptation chambers reached a plateau at the 1 mg loading dosage; there were no significant ($P > 0.05$) differences between the measured concentrations of pheromone achieved by the three highest loading dosages (1, 10 and 100 mg) (Fig. 4). Although not statistically different with the current small sample size, we found a surprising trend toward a drop in atmospheric concentration when the planchette was loaded with neat compound. Nevertheless, the pheromone concentration in air was well approximated ($R^2 = 0.8$) by the expression: $y = 3.42\text{Ln}(x) + 11.5$. On this basis, we estimate that the air concentration required for full adaptation (100 ng loading in 100 μL mineral oil) was *c*. 0.5 ng mL^{-1} air.

Adaptation experiments in the field. No long-lasting adaptation, as measured by decreased EAGs, was observed in the field for male *C. rosaceana* caged for 24 h at a distance of 2 m from Isomate pheromone dispensers in otherwise untreated plots (Table 1). Also, no adaptation was found for *C. rosaceana* caged 2 m from Isomate dispensers in 1-ha plots treated with pheromone at a rate of 500 Isomate dispensers per ha (Table 1). However, adaptation was observed in *C. rosaceana* caged adjacent to one, two or

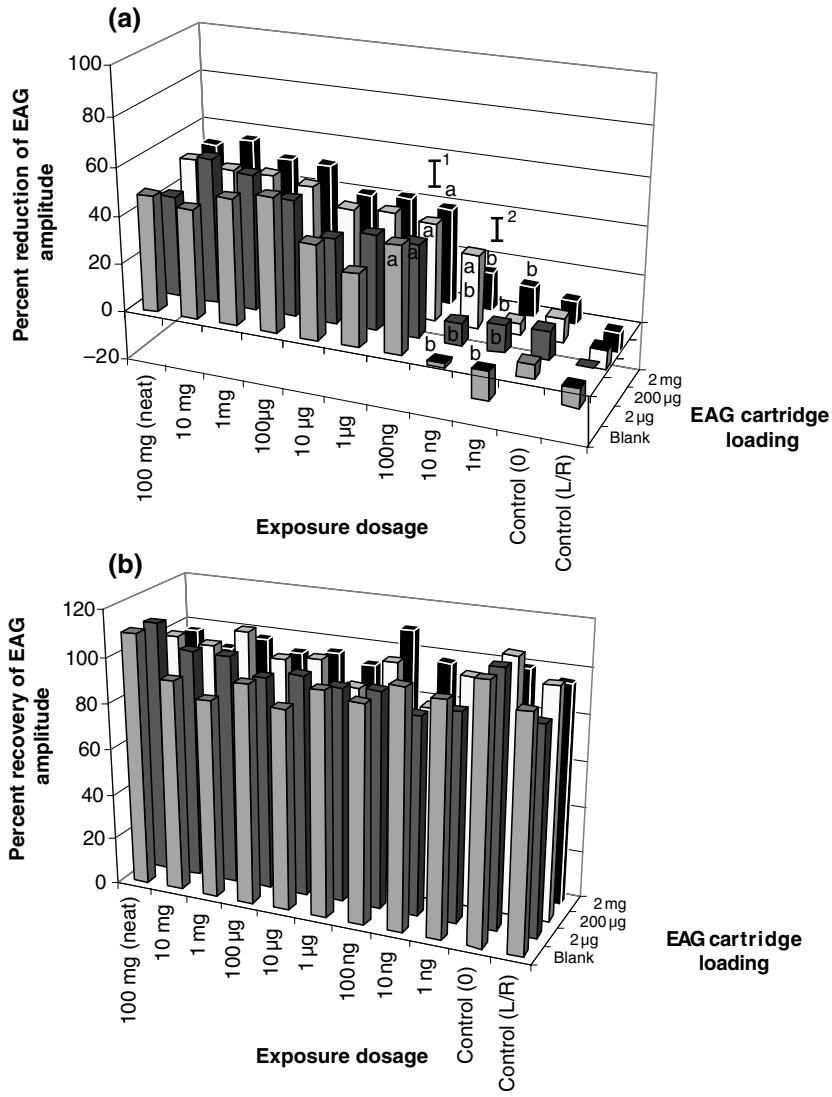

Fig. 1. a. Effect of 60 min of confinement of *Choristoneura rosaceana* ($n = 12$ per treatment) in adaptation chambers with various pheromone-loading dosages. Bars with different letters indicate significant ($P < 0.05$) differences between treatment means within a given cartridge dosage. b. Effect of 15 min of recovery of *C. rosaceana* in pheromone-free air after 60 min confinement in adaptation chambers. There were no significant differences between treatment means for responses within or across dosages. Exposure dosages of 10 ng per planchette and lower were non-adapting; these became the reference point for calculating percentage recovery. [1]Average standard error of the mean percentages of reduction of EAG amplitude for all exposure dosages ranging from 100 mg to 100 ng and assayed by the 2 mg EAG cartridge loading. [2]Average standard error of the mean percentages of reduction of EAG amplitude for 10 and 1 ng exposure dosages and the two control treatments assayed by the 2 mg cartridge loading. The average standard errors for treatments assayed by the other cartridge loadings were lower than those shown here for the 2 mg cartridge loading.

four Isomate dispensers in otherwise untreated plots (Table 1). The frequency with which adaptation was observed increased as the number of Isomate pheromone dispensers placed adjacent to cages increased. Most individuals adapted at the four-dispenser treatment (Table 1). As observed in the lab, the EAG response of adapted individuals returned to pre-exposure levels within 10 min after pheromone exposure (Table 1, Fig. 5).

Discussion

Cumulative characteristics of long-lasting adaptation in C. rosaceana

Adaptation of moth olfactory receptor neurones has long been recognized to occur following intense pheromonal stimulation, be it constant or pulsed (e.g. Baker *et al.*,

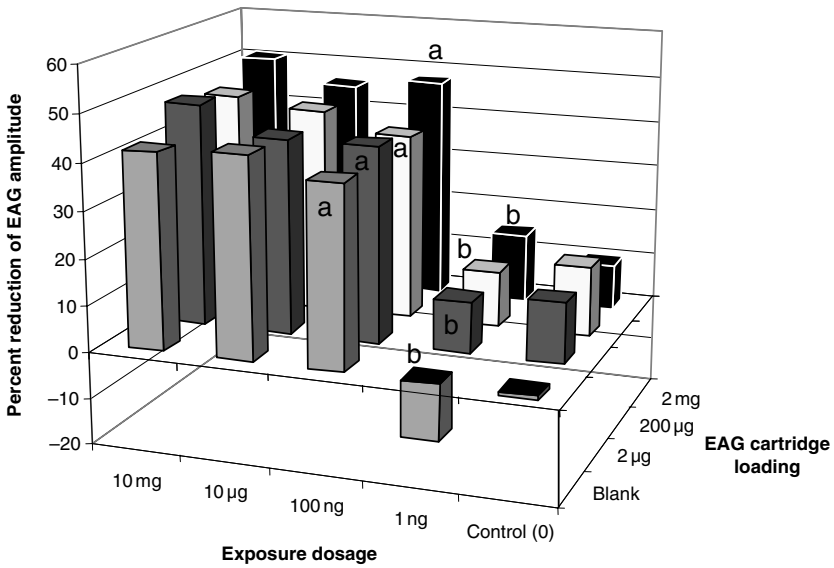

Fig. 2. Lack of effect of 5 min of recovery of *Choristoneura rosaceana* in pheromone-free air after 60 min confinement in adaptation chambers. Bars with different letters indicate significant ($P < 0.05$) differences between treatment means within a given dosage.

1988; Figueredo & Baker, 1992; Marion-Poll & Tobin, 1992); however, delineation of the particular types of adaptation as recently defined by Zufall & Leinders-Zufall (2000) has just begun. The 'long-lasting' variant of adaptation to sex pheromone we report for *C. rosaceana* has this emerging set of characteristics. As reported previously (Stelinski *et al.*, 2003) it can be measured by EAGs as a reduction in summated depolarization across unexcised antennal mounts in response to pheromone puffs of various dosages following sustained pheromone exposure of whole moths; its onset occurs after > 5 min exposure to pheromone and, like the adaptation reported for some other moths [*Trichoplusia. ni* (Kuenen & Baker, 1981); *Lobesia botrana* (Schmitz *et al.*, 1997)], it plateaus at *c.* 40–60% response reduction regardless of longer exposures; it is a 'long-lasting' (*sensu* Zufall & Leinders-Zufall, 2000) phenomenon measurable in full for up to 5 min after pheromone exposure ceases; moreover, the effect decays linearly over a period of 12.5 min.

The current study extends this list to include the following features: the maximal 40–60% EAG amplitude reduction was evident for all concentrations of pheromone at which adaptation occurred [from *c.* 0.5 to 50 ng mL^{-1} of air (Fig. 1a)]; and concentrations only slightly lower than 0.5 ng mL^{-1} produced no long-lasting adaptation. Rather than a typical dose–response phenomenon with a graded effect extending across orders of magnitude, this dosage pattern suggests a distinct threshold concentration above which long-lasting adaptation occurs and is immediately maximal, albeit never total. In that sense this long-lasting adaptation as measured in *C. rosaceana* is more quantal than gradual. We estimate the threshold concentration for onset of long-lasting adaptation (*c.* 500 pg mL^{-1} air) to be at least five orders of magnitude higher than the threshold for positive anemotactic flight by tortricidae in a wind tunnel (Miller & Roelofs, 1978), as estimated by response to known release rates of pheromone from microcapillary tubes (J. Miller, unpublished data). Wide separation between the dosage of pheromone-triggering long-lasting adaptation and that for normal sexual communication makes sense, as it is difficult to envisage any circumstance in which it would be advantageous for a male moth to dampen sensitivity and responsiveness for minutes at a time when competing for a mate.

Mechanisms of long-lasting adaptation in vertebrates

In vertebrates, long-lasting adaptation is mediated by the carbon monoxide (CO)/cGMP second messenger system (Zufall & Leinders-Zufall, 1997). The onset of long-lasting adaptation results in reduced amplitude and prolonged kinetics of the cAMP-mediated excitatory odour response and the generation of a persistent current-component that lasts several minutes; these effects are attributed to cyclic nucleotide-gated channel activation by cGMP. Therefore, cGMP mediates this type of olfactory adaptation by modulating the signalling properties of olfactory receptor neurones and thus controlling the sensitivity of the excitatory cAMP cascade (Zufall & Leinders-Zufall, 1997).

Proposed mechanisims of long-lasting adaptation in relation to moth olfactory cellular signalling

Transduction in insect olfactory neurones is mediated via an IP3 (rather than cAMP) second-messenger cascade (Boekhoff *et al.*, 1990, 1993); otherwise, the process has

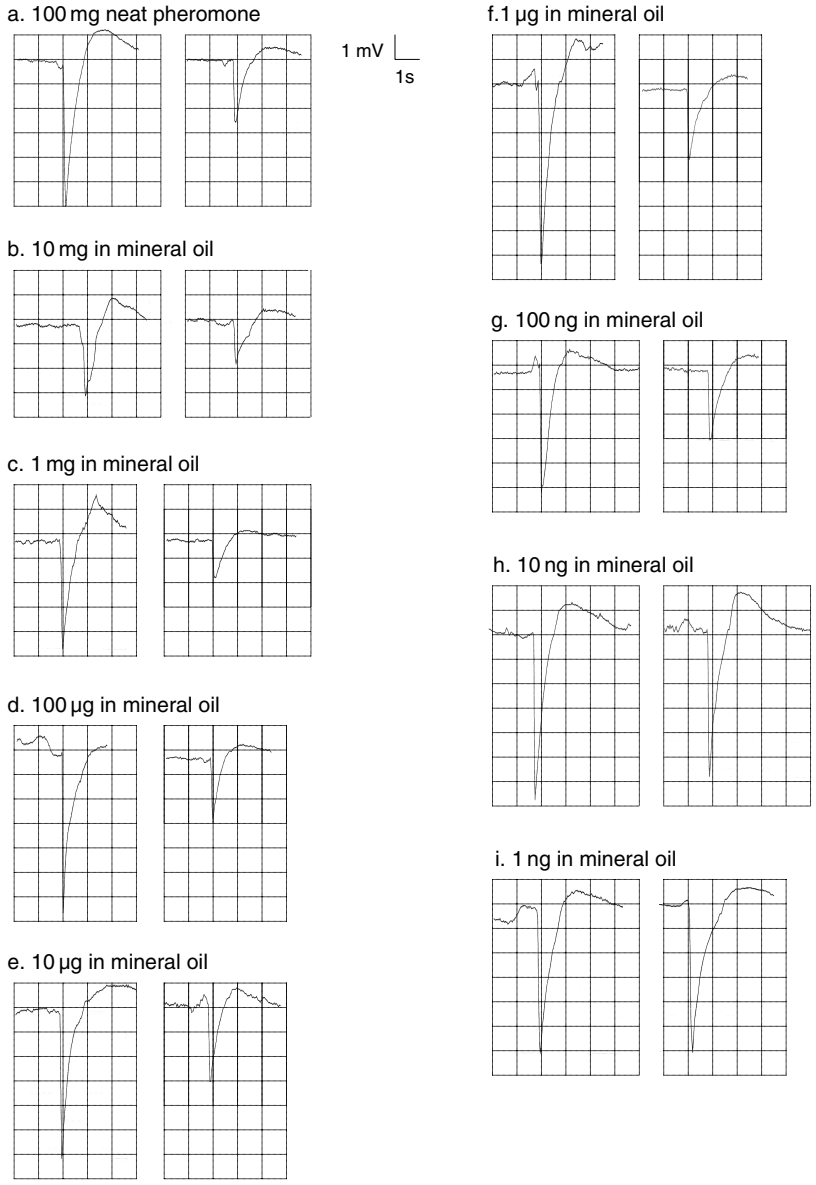

Fig. 3. Representative EAG tracings of *Choristoneura rosaceana* responding to 1-mL puffs from pheromone cartridges loaded with 200 µg of pheromone. Tracings on the left within each column are responses of *C. rosaceana* prior to exposure in adaptation chambers. Tracings on the right side within each column are responses of the same individuals after 60 min of exposure within adaptation chambers containing the above-mentioned loading dosages of pheromone. Each horizontal tick mark represents 1 s and each vertical tick mark represents 1 mV.

many similarities to that of vertebrates (Stengl *et al*., 1992). In *Heliothis virescens*, the concentration of IP_3 peaks 50 ms after nanomolar applications of pheromone (Boekhoff *et al*., 1993). Applications of larger dosages of pheromone yield rapid and transient increases in IP_3 followed by a rise in cGMP sustained over 10 s. Based on these results, Boekhoff *et al*. (1993) postulated that cGMP is involved in adaptation. Consistent with this interpretation, exogenously applied cGMP abolished the phasic but not tonic component of the pheromone-stimulated IP_3 signal in *H. virescens* (Figure 5b in Boekhoff *et al*., 1993). Importantly, there was a good match between the time-course of this phasic to tonic shift and the kinetics of the biochemical interaction between IP_3 and cGMP. Sustained pheromonal stimulation is also known to induce cGMP signals in *Antheraea polyphemus* as well as *Bombyx mori* (Ziegelberger *et al*., 1990), and pheromone-activated cation channels sensitive to cGMP have been found in insect olfactory cilia (Zufall & Hatt, 1991). Thus, as proposed by Zufall & Leinders-Zufall (2000), there is good reason to believe that

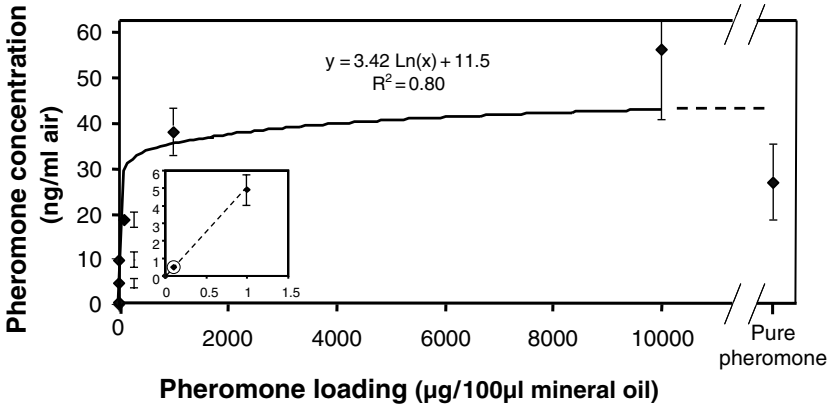

Fig. 4. GLC-quantified pheromone concentration in adaptation chambers in relation to loading dosage of pheromone in planchettes. The circled datum within the box expansion is an estimation of the threshold concentration causing long-lasting adaptation in *Choristoneura rosaceana*. Error bars indicate standard error of the mean.

insects and vertebrates share parallel mechanisms yielding long-lasting adaptation.

Some, but not all, electrophysiological recordings from pheromone-sensitive single-sensilla of insects clearly reveal long-lasting adaptation. As recorded by Kaissling (1986), *A. polyphemus* pheromonal sensilla showed a transition from phasic bursts of action potentials to a much-reduced and more steady-state tonic response within the first 100 ms of stimulation at 10^{-2} µg of bombykol. The normal phasic response returned only after a 10- and 30-min resting interval following 10 s and 10 min stimulation, respectively.

Our data on *C. rosaceana* appear to be consistent with cGMP-mediated long-term adaptation. To date, we have measured this adaptation only by EAG; nevertheless, we believe this effect may be correlated with a phasic to tonic shift in action potential output if measured at the single-sensillum level. Such shifts are well documented in the insect pheromonal literature, e.g. *A. polyphemus*, *A. pernyi* (Strausfeld & Kaissling, 1986), *Grapholita molesta*, *A. segetum* (Baker *et al*., 1988), *Trichoplusia ni* (Borroni & O'Connell, 1992; Grant *et al*., 1997), and *H. virescens* (Almaas & Mustaparta, 1991). However, long-lasting vs. short-term variants of adaptation have not yet been fully differentiated.

Possible significance of long-lasting adaptation under pheromone disruption regimes in the field

Currently, hand-applied rope dispensers at *c*. one per tree are the dominant method of dispensing pheromone for mating disruption of moth pests in orchards (Nagata, 1989; Agnello *et al*., 1996; Knight *et al*., 1998; Knight & Turner, 1999). The release rate for ropes marketed for leafroller moths averages *c*. 11 ng s^{-1} (Knight *et al*., 1998; Knight & Turner, 1999). Moths within the treated crop can be exposed to various pheromone concentrations: a 'cloud' of pheromone resulting from a coalescence of plumes emanating from the many dispensers; a localized plume downwind of a nearby dispenser; or, at the highest level, a moth could be attracted onto a dispenser. In the current field tests, *C. rosaceana* did exhibit long-lasting adaptation upon exposure to pheromone ropes, but only when held within a few centimetres of the dispenser. Nevertheless, these results demonstrate that this phenomenon can occur under field conditions. Use of low-density, high-release dispensers like puffers (Shorey & Gerber, 1996) or Microsprayers (Isaacs *et al*., 1999) offers even greater opportunity for male moths to be exposed to extraordinary concentrations of pheromone; the pheromone solution emitted in an aerosol spray falls onto foliage and droplets of pure pheromone accumulate over time on the source tree. Moreover, large and highly concentrated plumes are thought to waft great distances downwind of the source trees.

C. rosaceana, a long-lasting adaptor, has the reputation of a pest moth that is difficult to disrupt (Novak *et al*., 1978; Reissig *et al*., 1978; Roelofs & Novak, 1981; Deland *et al*., 1994; Agnello *et al*., 1996; Lawson *et al*., 1996; J. R. Miller *et al*., unpublished data) relative to the closely related red-banded leafroller, *A. velutinana*, which does not exhibit long-lasting adaptation (Stelinski *et al*., 2003). In certain cases, populations of *C. rosaceana* from Western Canada, 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 (Evenden *et al*., 1999a, b). We speculate that under pheromone mating-disruption regimes, moths capable of long-lasting adaptation like that of *C. rosaceana* may be advantaged relative to those who cannot. For example, perhaps moths experiencing long-lasting adaptation might sufficiently suppress overt sexual responses so as to allow them to depart extraordinarily high-dosage pheromone sites where the likelihood of find-

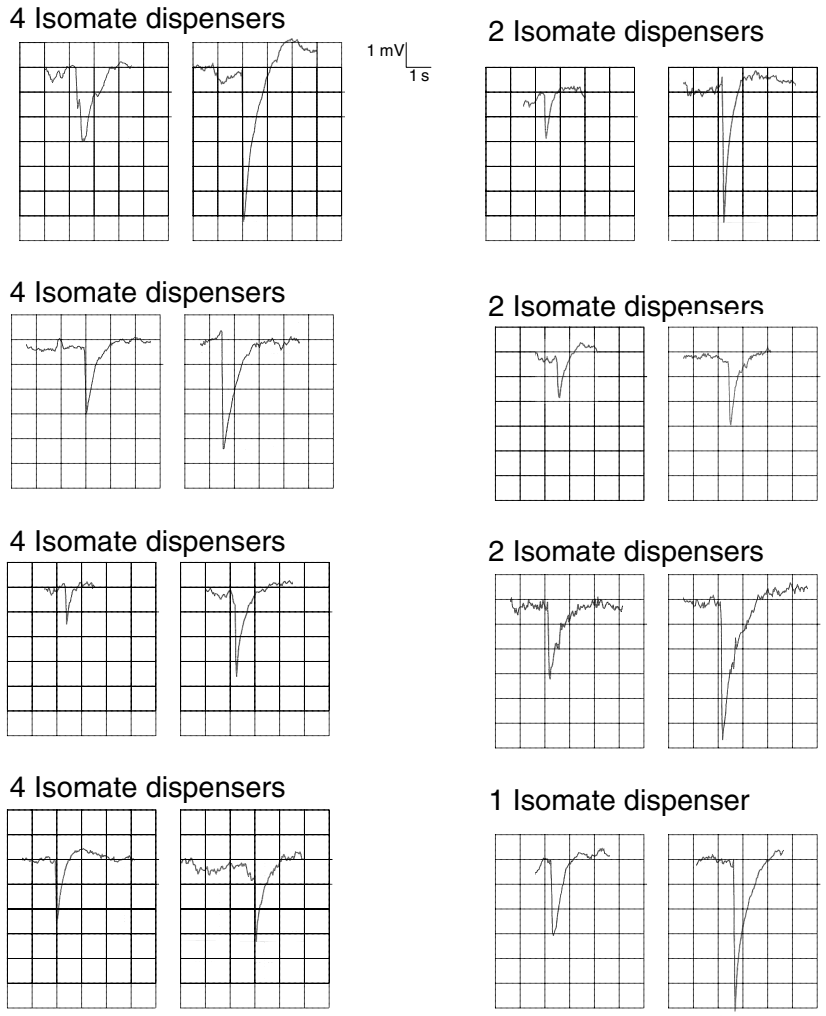

Fig. 5. Representative EAG tracings of adapted *Choristoneura rosaceana* responding to 1-mL puffs from cartridges loaded with 200 μg of pheromone. Tracings on the left within each column are responses of *C. rosaceana* 1 min after 24 h of field exposure under various pheromone exposure treatments. Tracings on the right side within each column are responses of the same individuals after 10 min of recovery in pheromone-free air.

ing and mating with a female is nil. If they then happen to arrive in a location of low pheromone, disadaptation would occur within 10 min and their ability to discriminate and orientate to a natural pheromone plume would be restored, provided the possible effects of central nervous system habituation were shielded (Bartell & Lawrence, 1977; Kuenen & Baker, 1981). Alternatively, long-lasting adaptation might preclude normal orientation and act to arrest flight so that the responder is not attracted to an abnormally high pheromone dosage. Notably, the work of Grant *et al.* (1997) supports the idea that pheromone receptors need to be capable of phasic responses for normal plume-following behaviour. When shifts in wind direction bring pockets of pheromone-free air (Cardé & Minks, 1995), a long-lasting adaptor would soon escape that location, possibly shielded from CNS fatigue.

Testing the hypothesis that absence of long-lasting adaptation is correlated with ease in pheromone disruption across moth taxa is the next step in this study. Another intriguing puzzle is why long-lasting adaptation to sex attractant pheromones exists when it is difficult to envisage a natural context in which selection would have rewarded it. Perhaps this phenomenon is a generalized physiological response to high dosages of chemostimuli set in place (and broadly retained) to handle high dosages of other natural products, e.g. plant volatiles. This idea begs cross-adaptivity tests spanning broad classes of compounds. Answers to such questions, generated by these early characterizations of long-lasting olfactory adaptation in insects, may reveal fundamental principles of insect physiology and behaviour that can be exploited for improved management of important pests.

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