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Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male *Helicoverpa zea*

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Abstract Single-cell electrophysiological recordings were obtained from olfactory receptor neurons in antennal trichoid sensilla of male corn earworm, *Helicoverpa zea*. Spontaneous activity of the neuron specific for the major component (*Z*)-11-hexadecenal, the conspecific female-emitted sex pheromone, was not affected by exposure to host plant volatiles. However, stimulations with binary mixtures of a threshold dosage of the pheromone component and increasing dosages of either linalool or (*Z*)-3-hexenol significantly synergized the pheromone-specific neuron's firing rates compared with responses to the major pheromone component alone. Cross-adaptation studies confirmed that the enhanced impulses originated from the pheromone-component-tuned neuron. Because plant volatiles do not stimulate the pheromone-specific neuron when presented alone, the pheromone plus host odor blend would be interpreted as containing more pheromone than it actually does when processed by the pheromone-processing portion of the antennal lobe.

Keywords *Helicoverpa zea* · Host plant volatiles · Pheromone synergists · Single-cell recordings · Antennal neurons

Abbreviations *ORN* olfactory receptor neuron · *Z11–16:Ald* (*Z*)-11-hexadecenal · *Z9–16:Ald* (*Z*)-9-hexadecenal · *Z11–16:Ac* (*Z*)-11-hexadecenyl acetate · *Z11–16:OH* (*Z*)-11-hexadecen-1-ol · *Z3–6:OH* (*Z*)-3-hexen-1-ol · *Z3–6:Ac* (*Z*)-3-hexenyl acetate · *linalool* 3,7-dimethyl-1,6-octadien-3-ol · *SPME* solid phase microextraction · *GC* gas chromatograph

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Introduction

Insects use olfactory cues to orient to mates and host plants. In Lepidoptera, discrimination of sex pheromone blend quality begins with arrays of narrowly and differentially tuned olfactory receptor neurons (ORNs). For discrimination of host plant volatiles, the process involves a combination of both broadly tuned generalist ORNs as well as highly specific ORNs responsive only to a narrow range of odorant molecules (Hansson 1995; Hildebrand 1995). The study of insect olfaction has, in most cases, been limited by the focus placed on gender-specific behaviors, i.e., either how female-emitted sex pheromones influence adult males in locating mates or how host-plant volatiles evoke responses from mated females for locating resources such as food and oviposition sites.

Much effort has been devoted to investigating the potential use of pheromones and other semiochemicals in the control of insect pest species. The corn earworm moth, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), a polyphagous pest of several agricultural crops, is one insect where pheromone communication disruption has been demonstrated (McLaughlin et al. 1981). Female *H. zea* emit a two-component blend of pheromone that attracts conspecific males. The principal pheromone component is (*Z*)-11-hexadecenal (Z11–16:Ald) (Klun et al. 1980; Pope et al. 1984) and constitutes approximately 92% of the volatiles released by calling females. The only other known pheromone component is (Z)-9-hexadecenal (Z9–16:Ald) (Klun et al. 1980; Pope et al. 1984), which constitutes ca. 1.5% of the emitted blend (Pope et al. 1984) and significantly increases upwind flight of males when blended with Z11–16:Ald (Vetter and Baker 1984). Pheromone production by female *H. zea* is increased by the presence of host plants (Raina et al. 1992), but as in other moth species, plants or plant volatiles are not necessary for females to produce or emit pheromone, or to attract males. Male *H. zea* have been shown to fly upwind readily to a pheromone source in

the wind tunnel that emits only the two-component pheromone blend in the absence of other volatiles (Vetter and Baker 1984; Vickers et al. 1991).

Under natural field conditions, however, airborne pheromone plumes are transported in air masses comprised of complex mixtures of other volatile chemicals, including those originating from surrounding vegetation that may also include *H. zea* larval host plants. Plant volatiles are known to influence the behavior of phytophagous insects in many ways, including influencing courtship behavior of male and female moths (Birch et al. 1990; Landolt and Philips 1997). The question of how mixtures of pheromone components or plant volatiles are reported by ORNs to higher centers is of increasing importance to understanding how insects discriminate odor qualities and respond behaviorally to natural chemical signals. Physiological investigations at the peripheral receptor level have generally concentrated in studying how the ORNs respond to single odorants (Todd and Baker 1999). Little attention has been paid to the possibility of mixture interactions occurring at the ORN level, even with regard to their responses to combinations of sex pheromone components (O'Connell et al. 1986; Akers and O'Connell 1988). Even fewer studies have involved ORN responses to mixtures of pheromones and plant volatiles (Den Otter et al. 1978; Van der Pers et al. 1980), probably because little behavioral evidence exists for the effects of such mixtures on moth behavior. A few field-trapping studies have demonstrated that certain groups of plant volatiles increase the capture of male moths in pheromone traps (Dickens et al. 1990, 1993; Light et al. 1993). However, it is not clear whether the enhancement of male attraction to the traps is due to separate responses of male moths to either the sex pheromone or the plant volatile portion, or to a response to the total blend that may in its entirety represent a unique odor quality.

Here we report that the responses of ORNs tuned only to the major pheromone component of *H. zea*, Z11–16:Ald, is synergized when certain plant volatiles are presented as a mixture along with the pheromone component. These same plant volatiles evoked no activity whatsoever in the Z11–16:Ald-specific ORNs when they are presented in the absence of the pheromone. These results suggest that enhanced male behavioral responses to mixtures of pheromone plus plant volatiles are due at least in part to enhancement of pheromone specific pathways.

Materials and methods

Insects

H. zea larvae were reared on a modified pinto-bean diet (Shorey and Hale 1965). After pupation the moths were separated according to sex and held in a 450-ml cage placed in an environmental chamber on a 14:10 h L:D cycle at 25°C and 55 ± 5% relative humidity. Emerging adults were supplied with a 10% sugar solution.

Test compounds

The female-produced sex attractant pheromone of *H. zea*, Z11–16:Ald, purity >98% (confirmed by gas chromatography) was obtained from Bedoukian Research, Danbury, Conn., USA. The plant volatiles (purity >98%) were kindly provided by Dr. J. Tumlinson, USDA, Gainesville, Fla., USA. These included linalool (3,7-dimethyl-1,6-octadien-3-ol), (*Z*)-3-hexen-1-ol (Z3–6:OH) and *β*-ocimene. Serial dilutions of the compounds were made in HPLC-grade hexane. Stimuli were applied as 10-µl aliquots on a 7×30 mm piece of Whatman No. 1 filter paper that was inserted into a Pasteur pipette (15 cm long). The plant volatiles were tested in decade doses (0.1–100 µg), beginning with the lowest dose. Each binary blend consisted of 0.1 µg of Z11–16:Ald plus an increasing dose of the corresponding plant volatile. Filter papers containing 10 µl of the solvent were used as controls. For all cartridges, solvent was allowed to evaporate from the impregnated filter papers in a fume hood before inserting the papers into the pipettes, and then allowing several more minutes to elapse before sealing the wide end of the pipette with aluminum foil. Sealed stimulus cartridges were stored at –20°C when not in use, and brought out to room temperature prior to a recording session.

Single-cell recordings

Electrophysiological recordings were obtained from adult males, 1–3 days after emergence. An *H. zea* male was placed inside a disposable Eppendorf pipette tip with the narrow end cut to allow the head to pass through. The head was immobilized with dental wax and one of the exposed antennae fastened by a tungsten hook onto a soft wax stage formed around the head. The preparation was mounted on a Syntech Portable Recording Unit, type INR-2 (Syntech, Hilversum, The Netherlands). Recordings from single receptor neurons were performed using tungsten microelectrodes (0.2 mm diameter) that were electrolytically sharpened with KNO_2 solution to a tip diameter of about 0.1 µm. The recording electrode tip was inserted into the base of a sensilla trichodeum until extracellular activity was displayed, whereas the indifferent electrode was inserted into the insect abdomen. The a.c. signal from the recording electrode was connected to the built-in amplifier of the portable recording unit and the a.c. output fed into a computer. The neuronal activity was monitored by a loudspeaker and displayed simultaneously on the computer. We processed the data with Syntech AutoSpike 4.0 software.

A stream of purified and humidified air continuously blew over the antenna (10 ml s^{-1}), and was directed through a 14-cm-long stainless steel tube (8 mm i.d.) whose outlet was positioned 2 cm from the antenna. Recordings were made from randomly selected sensilla from the mid- to proximal section of the antenna. Once a stable contact was established with a receptor neuron specific to Z11–16:Ald, we used a stimulus dosage of 0.1 µg that had been determined to be the threshold dose for male *H. zea*. With a stimulus flow-controller device (Syntech), a 0.02-s air pulse at a 40 ml s^{-1} flow rate was injected through the odor cartridge and into the air stream. Each test series consisted of a total of 32 stimuli in the following order: control stimulus, pheromone alone, individual plant volatile stimuli in random order beginning with the lowest dose (0.1 µg), followed by the corresponding cartridges containing binary blends of Z11–16:Ald (0.1 µg) plus increasing doses of plant volatiles, in random order with respect to type of the plant volatile. An interval of at least 30 s was maintained between stimulations. We counted the number of spikes generated 500 ms period after stimulus onset. An analysis of variance (ANOVA) was used to test for differences in response patterns among subjects across the eight different treatments, followed by Tukey's Studentized Range (HSD) test at $P < 0.05$ levels to determine which groups differed from one another.

Cross-adaptation studies

Previous studies indicated that Z11–16:Ald-specific receptor neurons in heliothine moths reside in sensilla trichodea type "A"

(Almaas et al 1991; Cossé et al 1998). Because transmission electron microscopic studies showed that only ~10% of the sensilla trichodea on *H. zea* antenna contain just one ORN (S.A. Ochieng et al. unpublished data), the majority of sensilla of this type must contain a second neuron for which no specific odorant has been identified. To rule out the possibility that blends somehow activate this silent neuron that is co-localized with the Z11–16:Ald-specific neuron when a stimulus blend of the pheromone and plant odorant was applied, we conducted cross-adaptation studies. Using the stimulus flow controller, a single 50-ms puff was generated followed by an interstimulus interval of either 0.3 s or 1 s, and then a second 50-ms stimulus from a second cartridge was generated. The stimulus regime consisted of the following pairs of odorants from cartridges 1 and 2 presented alternately at both interstimulus intervals: (1) Z11–16:Ald followed by a blank control (Z11–16:Ald/Control); (2) Control/Z11–16:Ald; (3) Z11–16:Ald/Z11–16:Ald; (4) Z11–16:Ald/linalool; (5) linalool/Z11–16:Ald; (6) linalool/blend; (7) blend/linalool; (8) Z11–16:Ald/blend; (9) blend/Z11–16:Ald; (10) linalool/control; and (11) blend/blend. More than 30 s was allowed to elapse between each stimulus pair application.

Release rate of *Z*11–16:Ald and linalool

In order to examine the actual amount of compounds coming out of the Pasteur pipette stimulus cartridges, release rates of *Z*11–16:Ald and linalool, one of the synergistic compounds, were analyzed using two different techniques: solid phase microextraction (SPME) and solvent extraction with Tenax minitrap. SPME samples were prepared by exposing SPME fiber (PDMS field sampler, Supelco, USA) perpendicularly at approximately 1 mm from the main airflow outlet, and introducing given number of stimulus puffs in the main airflow. The SPME samples prepared were subsequently analyzed by gas chromatograph (GC, HP-5890, Hewlett Packard, USA) for quantification. Solvent extraction was made by using microglass (200 µl, Drummond Scientific, USA) minitrap filled with 25 mg of Tenax (60/80, Alltech, USA). The Tenax minitrap was connected directly to the narrow end of Pasteur pipette stimulus cartridge via tight-seal rubber connector, and volatile sampling was made by introducing given number of stimulus puffs. 500 ng of tridecane was added in the minitrap as internal standard, and the minitrap was rinsed twice with 100 µl of hexane. The hexane extract was subsequently analyzed by GC for quantification. Airflow rate, stimulus injection puffing and stimulus cartridge preparation were carried out using same protocol as for the electrophysiological studies.

Results

We limited our test series to the ORNs specific to the major sex pheromone component of *H. zea*, Z11–16:Ald, which have been shown to comprise about 71% of the male antennal olfactory receptor neurons (Cossé et al. 1998). Single-cell recordings were obtained from 24 receptor neurons from 12 male moths. Complete series of 32 stimuli each were obtained from 10 receptor neurons that revealed consistent response to repeated stimulations with blank control and the pheromone alone. The mean responses of these receptor neurons were computed to obtain the dose-response curves (Fig. 3).

In all the series, there was no increase in activity due to stimulation with either the control cartridge or any of the plant volatiles alone, even at the highest tested doses (Figs. 1, trace f; 3a–c), and subsequent ANOVA tests showed no significant difference between control and the plant volatiles (Fig. 3a–c). In general, stimulation with Z11–16:Ald (0.1 µg) resulted in increased firing of about 20 impulses s^{-1} (Figs. 1, trace b; 2, trace a) compared with the background spontaneous activities of about 3–5 impulses s^{-1} (Fig. 1a). The addition of increasing doses of plant volatiles (linalool or Z3–6:OH) (Figs. 1, trace d; 2, traces b–e, g–j; 3a, b) to 0.1 µg of the pheromone significantly increased the firing rate of the sex pheromone ORNs ($P < 0.05$). However, addition of increasing doses of β-ocimene to Z11–16:Ald (0.1 µg) did not increase the responses of the ORNs over the responses to the pheromone alone (Fig. 3c). It appeared that the blend of linalool and Z11–16:Ald (Fig. 2, traces b–e) more markedly and reliably increased the receptor neuron responses than did the blend of Z3–6:OH plus Z11–16:Ald (Fig. 2, traces g–j).

Cross-adaptation studies

Cross-adaptation studies showed that the increased firing in response to blends of plant volatiles plus Z11–16:Ald was due to the activity of the one ORN known to be active in this type of hair, that is, the ORN tuned to Z11–16:Ald. The single-cell responses showed that this ORN could be partially adapted by Z11–16:Ald/Z11–16:Ald pair, with a 0.3-s interstimulus interval (Figs. 4, trace b; 5, histogram g). At an increased interstimulus interval of 1 s, this neuron became more markedly disadapted to Z11–16:Ald/Z11–16:Ald pair (Figs. 4, trace f; 6, histogram g). This ORN's ability to respond to subsequent puffs of Z11–16:Ald was impaired even more by pre-exposure to the blend of linalool plus Z11–16:Ald compared to Z11–16:Ald alone at both 0.3-s (Figs. 4, trace c; 5, histogram f) and 1-s (Figs. 4, trace g; 6, histogram f) stimulus intervals.

The enhanced adaptation of the Z11–16:Ald-tuned ORN by the blend of Z11–16:Ald plus linalool is consistent with the blend acting only on the Z11–16:Ald-tuned neuron. Pre-exposure to linalool had no effect on the firing rate observed in response to puffs of either the Z11–16:Ald plus linalool blend, or the Z11–16:Ald alone (Figs. 5, histograms b, d; 6, histograms b, d). These results indicate that no ORN firing activity in response to the blend was being contributed by any other ORN apart from the Z11–16:Ald-tuned neuron.

Release rate of *Z*11–16:Ald and linalool

Release rate of *Z*11–16:Ald from Pasteur pipette odor cartridge was two to three times greater when only *Z*11–16:Ald was present than when a mixture of *Z*11–16:Ald and linalool was present in the cartridge (Table 1). In contrast, there was no difference in the release rate of linalool between single-compound- and mixture-containing cartridges. Release rate of linalool was 500–1500 times higher than that of *Z*11–16:Ald when 10 µg was loaded. Absolute release rates of *Z*11–16:Ald and linalool in a mixture at 10 µg each were 0.044 ng/puff and 51.29 ng/puff, respectively.

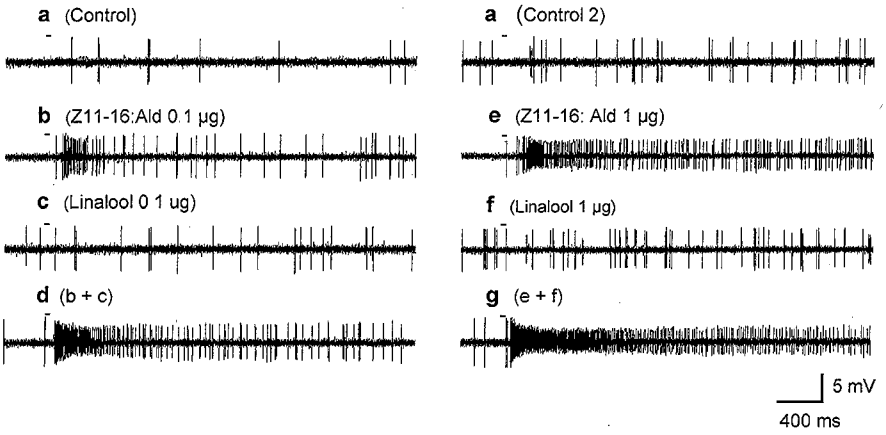

Fig. 1. Typical neural records from an olfactory receptor neuron specific to (*Z*)-11-hexadecenal (Z11–16:Ald). Stimulation with blank control (*trace a*), 0.1 µg and 1 µg linalool (*traces c and f*, respectively) did not affect the spontaneous neural activities. Stimulation with 0.1 µg of Z11–16:Ald (*trace b*) evoked a phasic impulse frequency whereas a mixture of Z11–16:Ald (0.1 µg) and 3,7-dimethyl-1,6-octadien-3-ol (linalool; 0.1 µg) (*trace d*), resulted in enhanced responses greater than the response to the pheromone alone. Similarly, stimulation with blend of Z11–16:Ald (1 µg) and linalool (1 µg) (*trace g*) resulted in enhanced impulse frequencies that were higher than the response to the pheromone alone (*tracee*). *Horizontal bar above each trace* represents stimulus duration (20 ms)

Discussion

These results demonstrate that certain plant volatiles, when presented as a blend with Z11–16:Ald, the major sex pheromone component of *H. zea*, significantly increase in synergistic fashion the firing rate of the ORN that is tuned to Z11–16:Ald compared with the response to Z11–16:Ald alone. To our knowledge, this is the first neurophysiological demonstration at the peripheral olfactory receptor level of a pheromone component-tuned neuron's activity being increased by a blend of plant volatiles plus pheromone. Particularly striking is the fact that when presented alone, neither of the two synergistic plant volatiles tested (linalool or Z3–6:OH) caused any significant firing from this or any other ORN in this hair at any tested concentrations (Fig. 3a, b). Therefore, there are many unresolved and interesting questions to be answered about the perireceptor and receptor-related mechanisms involved in the synergism evoked in this ORN by blends of such different chemical structure. The relationships of these compounds to binding protein affinities within this type of hair, as well as the relationships of the binding proteins to the membrane-bound pheromone receptors or to possible co-docking sites near the receptor on this one type of ORN, remain to be investigated. Apparently, the synergistic interaction by the pheromone and plant-odor-blend was not an artifact of greater total emission of compounds when the two compounds were released from the same cartridge

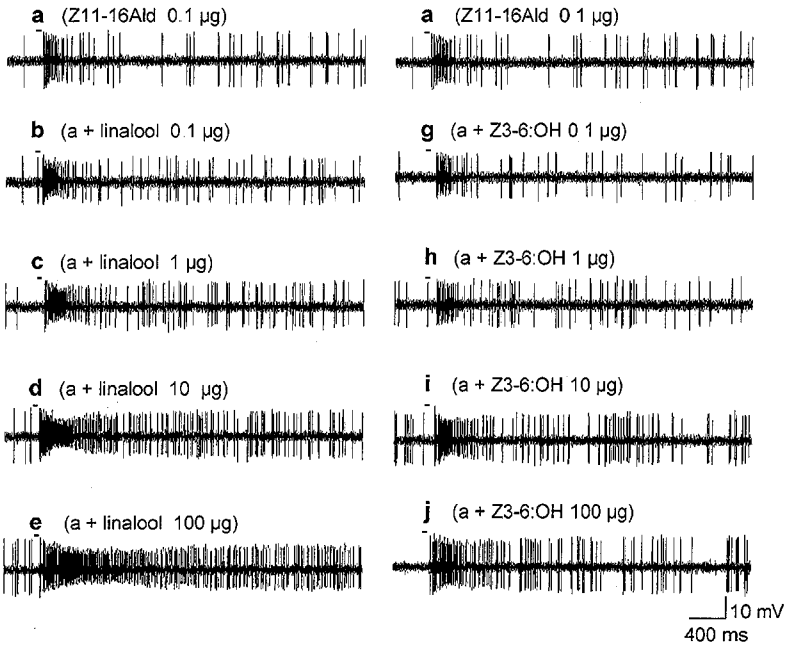

Fig. 2. Dose-response characteristics of an olfactory RN specific to Z11–16:Ald. Traces illustrate temporal pattern of firing in response to Z11–16:Ald (0.1 µg) alone (*trace a*) plus binary mixtures containing Z11–16:Ald (0.1 µg) and varying amounts of linalool (*traces b–e*) and Z-3-hexen-1-ol (Z3–6:OH; *traces g–j*), respectively. *Horizontal bar above each trace* represents stimulus duration (20 ms)

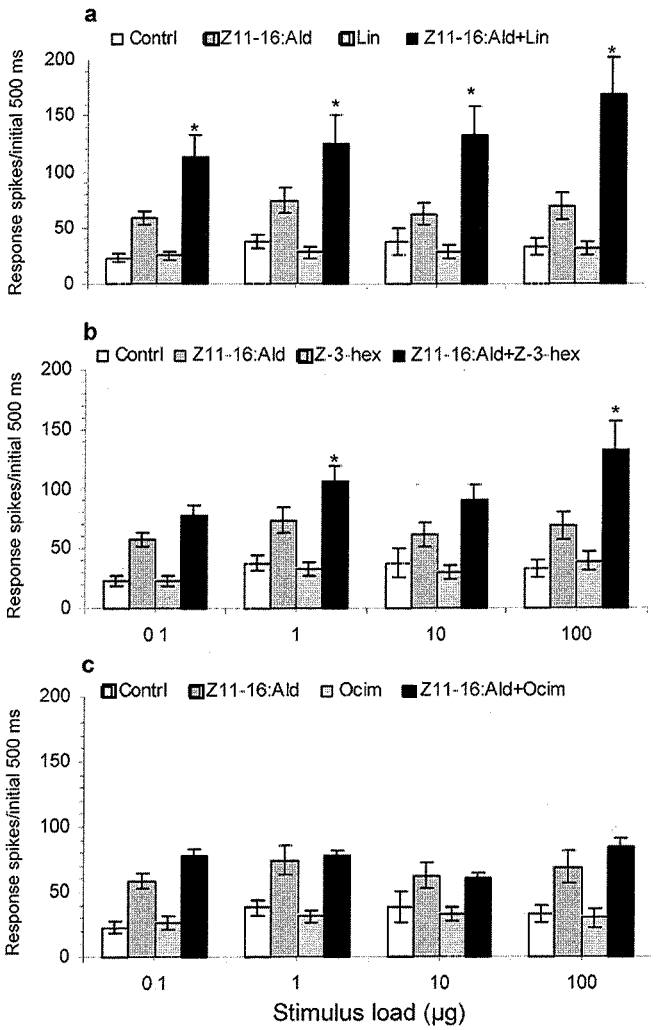

Fig. 3. Mean (±SEM) number of spikes per initial 500 ms during stimulations with control, Z11–16:Ald (0.1 µg), graded doses (0.1–100 µg) of plant volatiles linalool (**a**), Z3–6:OH (**b**), *β*-ocimene (**c**), and the mixtures of Z11–16:Ald (0.1 µg) and the respective graded plant volatile concentrations. Results are derived from ten olfactory receptor neurons (ORNs), each from a different animal. *Histogram with asterisk* is significantly different (Tukey's Studentized Range test; $P < 0.05$) from bars in the same stimulus load

(Table 1). If anything, the neuronal activity should be reduced when presented with a mixture because the amount of pheromone coming out was one-half to one-third of that from a single cartridge; however, the activity was greatly enhanced, despite the lower pheromone concentration.

In addition, there are other questions concerning the relationship of the timing of the arrival of the two odorants and the heightened firing of this ORN. Our results show that initially exposing the ORN to linalool did not increase its subsequent response to a puff of Z11–16:Ald presented only 0.3 s later (Fig. 5, histogram b), compared with the puff to the blend (Fig. 5, histogram d). Evidently, the timing of the arrival of the linalool with the pheromone needs to be more synchronous in order to achieve a synergistic increase in firing. Fadamiro and Baker (1997) and Todd and Baker (1999) have previously suggested that on-site processing of blends by neurons that are co-compartmentalized within the same sensory hairs on moth antennae is the most heuristic explanation for how perfectly admixed blends in a plume emanating from a single emission source can be discriminated by moths compared with two imperfectly mixed plumes comprised of those same compounds emanating from two separate sources. We do not know yet how synchronous the mixtures of plant volatiles plus pheromone need to be to evoke this synergism. Behaviorally, the antagonism caused by the presence of (*Z*)-11-exadecenyl acetate (Z11–16:Ac), a behavioral antagonist of *H. zea*, in the pheromone blend was significantly poorer when Z11–16:Ac was not perfectly admixed with the pheromone in every filament (Fadamiro and Baker 1997).

In our study, increasing amounts of linalool in the blend caused a relative increase in the Z11–16:Ald-tuned ORN's firing rate as if the amount of Z11–16:Ald had increased. The heightened activity of this ORN response to this blend would be reported to the "cumulus" glomerulus within the pheromone-processing portion of the male's antennal lobe, called the macroglomerular complex (MGC), as if there was more Z11–16:Ald pre-

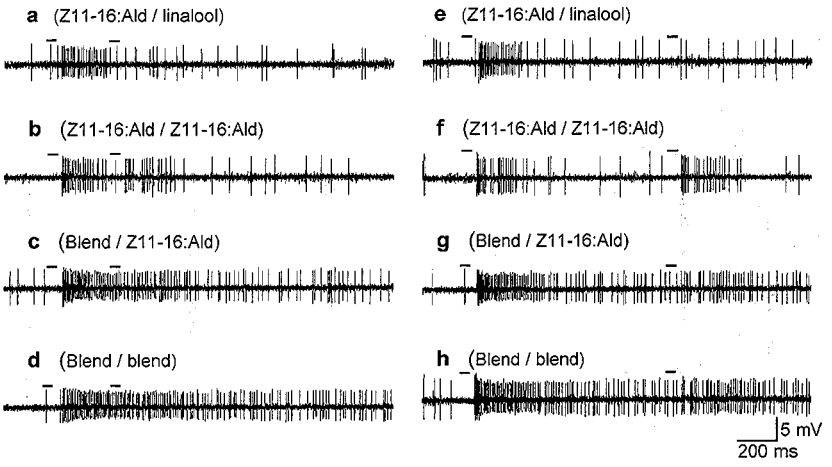

Fig. 4. Typical cross-adaptation responses of Z11–16:Ald-specific neuron to stimulus pairs. In each pair, the first stimulus is followed by the second stimulus, for instance, Z11–16:Ald/linalool is a stimulation with Z11–16:Ald followed by linalool stimulus after 0.3 s or 1 s. Each trace shows two 50-ms stimulations (*black horizontal bars*) with an interstimulus interval of 0.3 s (*traces a–d*) and 1 s (*traces e–h*). Stimulus dose was 0.1 µg Z11–16:Ald, 1 µg linalool, and a blend of the two

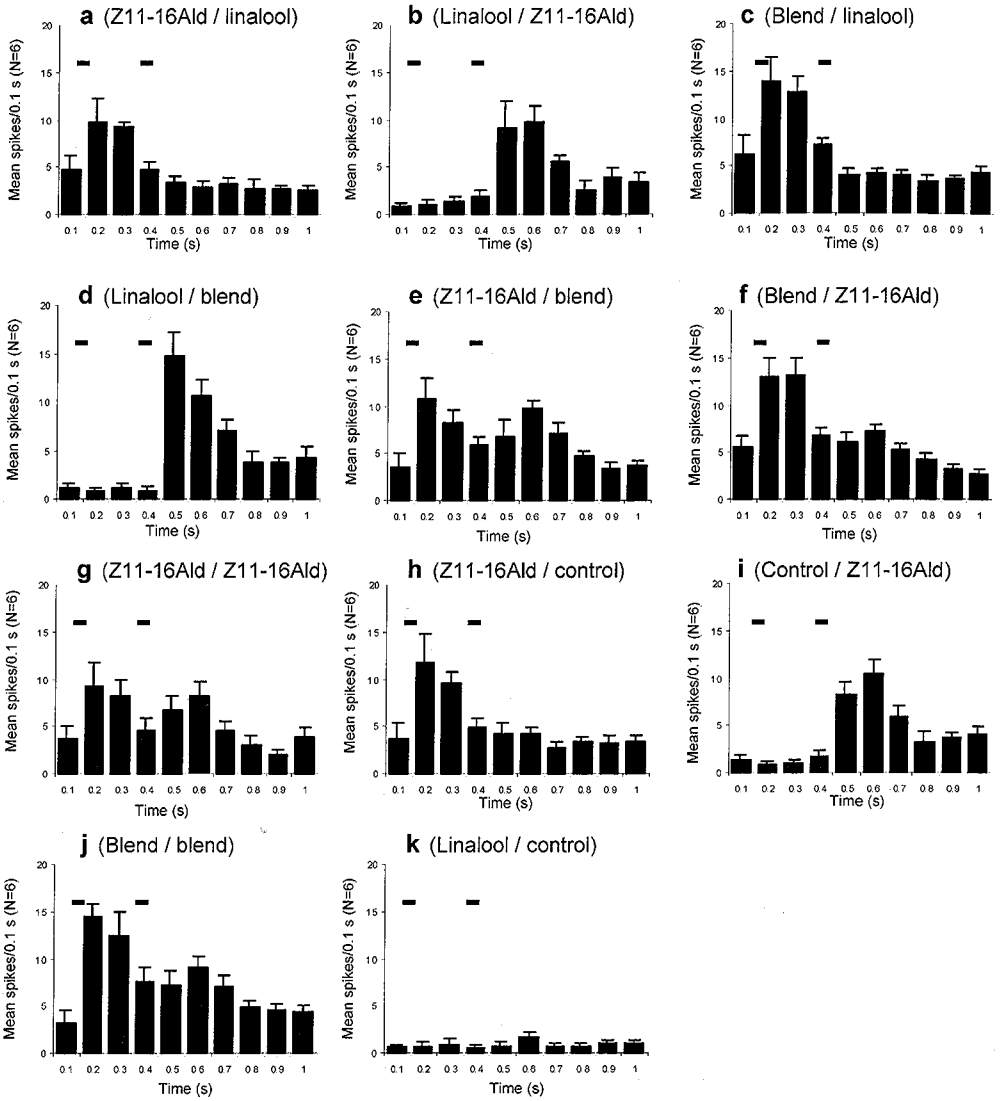

Fig. 5. Histograms (*a*–k)showing the mean (±SEM) spike frequencies ($n=6$) of Z11–16Ald-specific neuron in response to 11 stimulus pairs. Each histogram shows two 50-ms stimulations (*black horizontal bars*) with an interstimulus interval of 0.3 s. Stimulus dose was 0.1 µg Z11–16Ald, 1 µg linalool, and a blend of the two. Control stimulus was 10 µl of the solvent

sent than there actually is (Christensen et al. 1991; Vickers et al. 1998). The two plant volatiles that were effective in evoking this response were both alcohols; β-ocimene was ineffective in this regard, even though it is a monoterpene similar in structure to linalool. However, one difference between the blends containing Z11–16:Ald plus plant-volatiles compared with the blends containing the pheromone antagonists (S.A. Ochieng et al., unpublished data) is that the plant volatiles will also be stimulating ORNs tuned to the plant volatiles that send their projections to their own (ordinary) glomeruli in the antennal lobe. The ORNs tuned to the pheromone antagonists send their projections to a separate glomerulus within the MGC (Vickers et al. 1998; Hansson et al. 1995). Thus, blends that increase attraction that are comprised of Z11–16:Ald plus plant volatiles will be discriminable, from blends of Z11–16:Ald plus pheromone antagonists by the different pattern of activity they evoke in the antennal lobe glomeruli. These different blends are thus able to cause very different behavioral outcomes.

For moth pheromone systems, the possibility of blend-related mixture interactions occurring at the primary sensory neuron level has only rarely been considered, and these studies involved mostly pheromone-component blends (Akers and O'Connell 1988; O'Connell et al. 1986). O'Connell et al. (1986) did find significant mixture enhancement in response to certain binary blends of pheromone components in the ORNs on male *Trichoplusia ni* antennae. They also found instances of mixture suppression as well. There have also been a few reports of within-sensillum subthreshold hyperpolarizations in response to puffs of otherwise inactive single odorants that have indicated the potential for mixture interactions to be occurring within such sensilla (Hansson et al. 1990; Kaissling et al. 1989; Todd et al. 1992). Some of these hyperpolarizations involved plant volatiles interacting with ORNs contained in trichoid hairs known to house only pheromone-sensitive ORNs (Hansson et al. 1990; Kaissling et al. 1989). In studies of other arthropods, mixture interactions in response to odors have been documented in

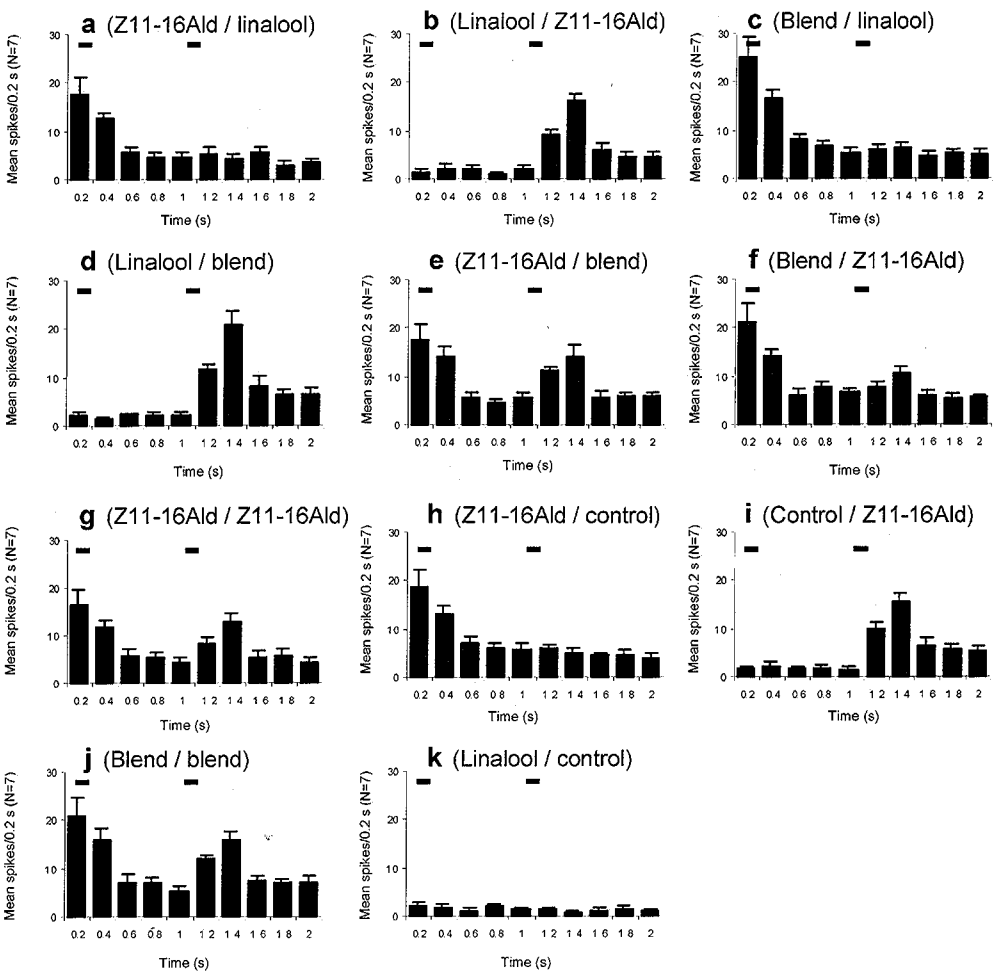

Fig. 6. Histograms (*a–k*) showing the mean (±SEM) spike frequencies ($n=7$) of Z11–16Ald-specific neuron in response to 11 stimulus pairs. Each histogram shows two 50-ms stimulations (*black horizontal bars*) with an interstimulus interval of 1 s. Stimulus dose was 0.1 µg Z11–16Ald, 1 µg linalool, and a blend of the two. Control stimulus was 10 µl of the solvent

Table 1. Comparison of release rates of (*Z*)-11-hexadecenal (*Z*11–16:Ald) and 3,7-dimethyl-1,6-octadien-3-ol (linalool) between single-compound-containing dispensers and mixture-containing dispensers (*SPME* solid phase microextraction)

Sampling method	Compound	Release rate (ng/puff)	
		Single[a]	Mixture[b]
SPME[c]	*Z*11–16:Ald	0.0026 ± 0.0005	0.001 ± 0.0009
	Linalool	1.50 ± 0.254	1.73 ± 0.444
Tenax[d]	*Z*11–16:Ald	–	0.044 ± 0.0049
	Linalool	–	51.29 ± 11.282

[a] Each dispenser was loaded with 10 µg of each compound
[b] Mixture of two compounds, 10 µg of *Z*11–16:Ald and 10 µg of linalool, was loaded in a dispenser
[c] Amount of compounds collected with SPME sampler by 5 puffs for linalool and 1024 puffs for *Z*11–16:Ald (mean ± SE; $n=3$–9)
[d] Amount of compound collected with Tenax minitrap by 5 puffs for linalool and 256 puffs for *Z*11–16:Ald (mean ± SE; $n=3$–14)

the ORNs of the American lobster (Atema et al. 1989) and the spiny lobster, *Panulirus argus* (Steullet and Derby 1997). Mixture suppressions rather than enhancements were reported, involving reduced impulse frequencies in response to blends of food-related odorants compared with the responses to the same odorants presented alone.

In recordings from the output (projection) interneurons in the antennal lobe, pheromone component "blend-sensitive" interneurons have been found in four of the five noctuid moth species sampled thus far, including *H. zea* (Christensen et al. 1991; Anton and Hansson 1995, 1999; Wu et al. 1996; Berg et al. 1998). Based on our findings, it is possible that some of the blend enhancement recorded from projection interneurons that has been attributed to post-synaptic antennal lobe integration of blend quality, may actually already have occurred upstream at the level of the ORNs. In such instances, the antennal lobe projection interneurons may be serving as mere relays of blend interactions that have already taken place on-site during transduction at the receptor neuron level. We do not propose that all such interactions recorded in the CNS will have occurred only at the receptor neuron level, or that mixture interactions involving mixtures of plant volatiles and pheromone odors will occur in all moth species. Obviously, this phenomenon deserves further investigation in a wider range of species and of course needs to be interpreted in the context of the species' natural behavior. In a follow-up study, we will conduct a more systematic experiment over a wide range of intervals to link the electrophysiological data with behavioral effects.

Clearly, extensive behavioral experiments need to be performed to help interpret the biological significance of this synergistic ORN response to blends of plant volatiles plus Z11–16:Ald. It is possible that the previously reported increased trap captures of *H. zea* males in the field (Light et al. 1993) in response to blends of *H. zea* pheromone plus the green-leaf volatile, (*Z*)-3-hexenyl acetate (Z3–6:Ac), were caused by an increased firing of this ORN in response to this plant-volatile-plus-pheromone-component blend. A similar increase in male trap capture was reported for *Heliothis virescens* in response to blends of Z3–6:Ac plus the *H. virescens* pheromone (Dickens et al. 1990). However, only Z3–6:Ac and not Z3–6:OH blended with the *H. zea* pheromone resulted in significantly increased captures compared with pheromone alone (Light et al. 1993). There was a trend, not significant, for Z3–6:OH plus pheromone to increase capture. We did not test Z3–6:Ac in these initial studies of this ORN's response to pheromone plus plant volatile mixtures, and likewise, blends containing linalool were not tested in the field by Light et al. (1993). Preliminary results from wind-tunnel flight bioassays (Jeremy Heath, personal communication), show that linalool blended with the *H. zea* pheromone is capable at certain dosages of increasing the number of upwind flights and source contacts by male *H. zea* compared with the number responding to the pheromone alone. Thus, enhanced activity in pheromone olfactory pathways may be one plausible explanation for the increased attraction and capture of male *H. zea* when certain plant volatiles are blended with the *H. zea* sex pheromone (Dickens et al. 1990; Light et al. 1993).

This plant-pheromone synergism represents a convergence of plant- and sex pheromone-odor processing in moths that have not been documented neurophysiologically before. Our results may have practical significance in that perhaps pheromone mating disruption dispensers for some moth species can be manufactured with less of the expensive pheromone active ingredients while retaining or even increasing disruption efficacy by adding small amounts of selected inexpensive plant volatiles to the pheromone blend.

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