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ELECTROANTENNOGRAM RESPONSES OF *Hyles lineata* (SPHINGIDAE: LEPIDOPTERA) TO VOLATILE COMPOUNDS FROM *Clarkia breweri* (ONAGRACEAE) AND OTHER MOTH-POLLINATED FLOWERS

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Abstract—Electroantennograms (EAGs) from field-collected *Hyles lineata* moths were recorded in response to 10 individual floral volatiles identified from *Clarkia breweri* (Onagraceae), to 22 scent compounds produced by other moth-pollinated flowers and to eight ubiquitous "green leaf volatiles." Females' EAGs were generally 1.5- to 2-fold greater than those observed for male moths. Female : male EAG rank orders were significantly correlated, but marked differences in order were observed for some compounds (e.g., benzyl alcohol, cinnamic aldehyde, geraniol, and linalool). Linalool, benzyl acetate, methyl salicylate, and pyranoid linalool oxide elicited the largest EAG responses (-1.2 to -0.8 mV) among scent compounds from *C. breweri*. EAG responses were significantly lower for monoterpenes as a pooled compound class than for aromatic esters, alcohols and aldehydes, fatty acid derivatives, N-bearing compounds and oxygenated terpenoids. EAG responses to structurally related scent compounds were not significantly different in most

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cases. Both male and female *H. lineata* were sensitive to most *C. breweri* scent compounds at 10^{-2} to 10^{-4} $\mu g/\mu l$ doses, and rank order in potency varied with the dose/concentration tested. *H. lineata's* olfactory sensitivity to diverse volatile compounds across a range of doses/concentrations suggests that a broad array of volatiles could function as floral attractants for foraging hawkmoths.

Key Words—Aromatics, EAG, floral attraction, floral scent, green leaf volatiles, hawkmoths, monoterpenes, nitrogen-bearing compounds, olfaction, semiochemicals, *Hyles lineata*.

INTRODUCTION

The white-lined sphinx moth, *Hyles lineata* L. (Sphingidae: Lepidoptera), is the most widely distributed hawkmoth species in the world; it is present throughout the Americas (subsp. *lineata*), across Europe, Asia, and Africa (subsp. *livornica*) and in Australia (subsp. *livonicoides*; Rothschild and Jordan, 1903; von Knoll, 1925; Hodges, 1971). *H. lineata* moths are avid flower visitors, foraging for nectar at dusk and early evening in most localities (Clements and Long, 1923; Gregory, 1963, 1964; Kislev et al., 1972; Hodges, 1995). Due to its general abundance and broad distribution, *H. lineata* is probably the most important hawkmoth pollinator throughout much of North America (Cruden, 1970; Stockhouse, 1973; Chase and Raven, 1975; Miller, 1978, 1981). The broad acceptance of flower types by *H. lineata* throughout its distribution suggests either that it is attracted to a wide variety of floral scent compounds (or blends thereof) or that it relies on cues other than floral scent (i.e. visual cues) during foraging. One way to test these possibilities is to study the physiological and behavioral responses of *H. lineata* to the scent compounds of the flowers that it visits.

H. lineata moths visit a broad spectrum of plant species bearing flowers of diverse morphological classes (von Knoll, 1925; Fleming, 1970; Kislev et al., 1972). Grant (1983, 1985) cited *H. lineata* as a visitor to flowers of 41 native plant species from 13 angiosperm families in North America. However, hawkmoths are observed to be effective pollinators of a subset of these plants, those with tubular or trumpet-shaped flowers in which the nectar tubes or spurs are as long as or longer than the extended proboscides of the moth. This geometrical relationship increases the probability of head/body contact with floral sex organs, leading to removal and deposit of pollen (Gregory, 1964; Miller, 1978; Grant, 1983; Dafni et al., 1987; Nilsson, 1988; Hodges, 1995; Raguso, 1995). Many hawkmoth pollinated flowers often are pale in coloration, open during the evening, produce copious amounts of nectar, and emit a strong, sweet aroma. This combination of floral traits has evolved repeatedly in most major angiosperm families worldwide and defines the "hawkmoth-pollination syndrome" (Baker,

1961; van der Pijl, 1961; Gregory, 1963; Faegri and van der Pijl, 1979; Miller, 1981; Grant, 1983).

In western North America, *H. lineata* pollinates a number of plant species with pale, tubular, scented flowers, especially within the genera *Aquilegia* (Ranunculaceae), *Mirabilis* (Nyctaginaceae), and *Oenothera* (Onagraceae; reviewed by Grant, 1983, 1985). One plant species typical of this assemblage is *Clarkia breweri* (Gray [Greene]: Onagraceae). *H. lineata* and other hawkmoths are important pollinators of *C. breweri* in the central Coast Range Mountains of California (Raguso, 1995). Genetic and morphological data suggest a recent origin of *C. breweri* from scentless, bee-pollinated ancestors (Lewis and Lewis, 1955; MacSwain et al., 1973; Gottlieb and Weeden, 1979; Raven, 1979). Moth pollination and strong floral scent emission are not observed in any other *Clarkia* species, and are thought to represent a derived condition in the genus (MacSwain et al., 1973; Raven, 1979; Raguso and Pichersky, 1995). The currently accepted model of hawkmoth foraging behavior proposes that floral scent functions as a sign stimulus that "turns on" or releases anemotactic searching flight behavior, followed by visual close-range orientation to flowers (Baerends, 1950; Brantjes, 1973, 1978). Following this model, we propose that floral scent has evolved in *C. breweri* as an adaptive trait modified through selective pressure by hawkmoth pollinators, including *H. lineata* (Raguso and Pichersky, 1995). For this hypothesis to be valid, hawkmoths must be olfactorily sensitive to the array of *C. breweri's* floral scent compounds and able to detect them at concentrations low enough to serve as cues for flower location from a distance.

We tested the olfactory sensitivity of *H. lineata* moths by measuring their electroantennogram (EAG) responses to the floral scent compounds of *C. breweri*. EAGs have been used to assess the olfactory sensitivities of agriculturally important moth species in response to host plant volatiles (Grant, 1971; Adler and Jacobson, 1972; Tichenor and Seigler, 1980; Gabel et al., 1992; Light et al., 1993; Zhu et al., 1993) and to sex pheromones (Schweitzer et al., 1976; Reed et al., 1987). Little is known, however, about the breadth of hawkmoths' olfactory responses to floral volatiles from different chemical structural classes, or to plant vegetative volatiles in general. The purpose of our study was to characterize, through EAG recordings, the peripheral olfactory responses of *Hyles lineata* moths to 40 scent compounds, including ten floral volatiles from *Clarkia breweri*, 22 floral volatiles identified from other moth-pollinated plants and eight "green leaf volatiles" (GLVs) typical of vegetation.

We used EAG response data to address the following questions.

(1) Do *H. lineata* moths respond differently to diverse chemical classes of plant volatiles?

(2) Can *H. lineata* moths distinguish between structurally similar scent compounds bearing slightly different functional groups?

(3) Can *H. lineata* moths detect floral scent compounds at low doses?

Selective differences in olfactory response to specific compounds or structural classes of compounds might predispose *H. lineata* to respond to certain olfactory stimuli through induction of flight orientation and feeding or ovipositional behaviors. By testing *H. lineata's* responses to volatile compounds from different structural classes (terpenoids, aromatics, fatty acid derivatives, and nitrogen-bearing compounds), we can begin to characterize the potential role of olfaction in the foraging behavior of a widespread hawkmoth species.

METHODS AND MATERIALS

Insects

Adult *H. lineata* moths were collected at UV light traps from September 17 to October 4, 1993 in Tucson, Pima Co., AZ and transported live via overnight courier to Albany, CA. Upon arrival, moths were fed a 10% sucrose solution and held in a 1 m^3 screen cage within the laboratory (25°C, 12 L:12 D). Moths had free access to sucrose solution feeding stations, and were fed manually once daily.

Test Compound Justification

Appendix 1 lists the 40 scent compounds tested, their purities, supply sources, and rationale for inclusion in this study. We measured EAG responses to ten of the 12 principal volatiles identified from the floral headspace of *C. breweri*, constituting 97.9% of total volatile emissions analyzed via GC-MS (Raguso and Pichersky, 1995). *C. breweri* floral volatiles fell into two chemical classes, aromatics and oxygenated monoterpenoids, and included benzyl acetate, linalool, and methyl salicylate, three of the most prevalent floral scent components in hawkmoth-pollinated flowers (Brantjes, 1978; Kaiser, 1993; Knudsen and Tollsten, 1993).

We tested 22 additional floral volatiles from diverse chemical classes, including aromatics, fatty-acid derivatives, monoterpenes, nitrogen-containing compounds, and a sesquiterpene alcohol. Test compounds in this grouping were chosen for one of three reasons; (1) they frequently occur as floral scent constituents in many other hawkmoth-pollinated flowers (e.g., amyl salicylate, farnesol, geraniol, indole; Morgan and Lyon, 1928; Kaiser 1991, 1993; Knudsen and Tollsten, 1993), (2) they stimulate upwind flight in another family of flower-visiting moths, the Noctuidae (e.g., phenylacetaldehyde, 2-phenylethanol; Jacobson et al., 1976; Haynes et al., 1991; Heath et al., 1992), or (3) they share a common carbon skeleton with one of the above compounds, but differ by one functional group (e.g., methyl anthranylate, methyl-2-methoxybenzoate). Inclusion of these compounds allowed us to determine (1) the chemical breadth

of *H. lineata's* olfactory "vocabulary," (2) whether these moths can detect flowers that are attractive to noctuid moths, and (3) whether they can distinguish between similar odorants bearing subtle chemical differences.

We analyzed EAG responses to eight green leaf volatiles (GLVs). These C_6 and C_8 aliphatic alcohols, aldehydes, and acetates are ubiquitous in plant vegetation and provide strong structural contrast to the aromatic and terpenoid floral volatiles. Female *H. lineata* may respond behaviorally to GLVs during hostplant orientation and oviposition. In addition, these compounds are known to synergize male responses to female sex pheromone in noctuid moths (*Helicoverpa zea*, *Heliothis virescens*; Dickens et al., 1993; Light et al., 1993), and may be important to male sphingids as well.

Olfactory Stimuli

Test compounds were dissolved in HPLC-grade hexane to form 10% volumetric solutions. Compounds poorly soluble in hexane, such as indole and vanillin, were dissolved by weight in diethyl ether. One ul of each solution (approx. 100 ug of test odorant) was pipetted onto filter paper strips, which were allowed to evaporate for ca. 30 sec, then inserted into Pasteur pipettes and stored at 5°C until used. Each test cartridge was loaded 20 min prior to its presentation to the moth's antennae.

EAG Technique

EAG deflections were recorded and measured on a Tektronix 5113 storage oscilloscope as described by Light et al. (1988). Silver-chloride electrodes were prepared in drawn glass capillary tubes, and were electrolytically balanced (Raynauld and Laviolette, 1987). Living moths were mounted on a plexiglass block with a central trough molded with soft paraffin to fit the contours of the moth's ventrum. The terminal five segments of the right antenna (out of 58–61 total segments) were excised and the recording electrode was inserted into the antennal cavity, while the ground electrode was inserted into the head at the base of the antenna. The antenna was bathed continuously by a stream of charcoal-filtered, humidified air at a flow rate of one L/min. A "puff" of test compound was delivered onto the antenna when a three-way solenoid valve was activated, diverting air through the test cartridge for a 1 sec stimulation interval. Each compound stimulation was followed by a minimum 60 sec purge period of filtered air to ensure recovery of antennal receptors.

For each compound tested, EAGs were recorded from five male and seven female *H. lineata* moths. In addition, EAG responses to serial dilutions of eight floral volatiles from *C. breweri* (ranging in dose from ca. 10^{-4} to 10^{2} μg/μl per filter paper) were recorded from three males and six females. "Control" stimuli (1 μl of hexane solvent per filter paper) and "standard" stimuli (1 μl of 1%

linalool in hexane per filter paper) were interspersed about every fifth compound or dose tested. The order of compound presentation was randomized for each individual moth. Experiments ranged from 65–110 min in length; standard stimulus EAG data were plotted vs. time, and no consistent patterns were observed. All experiments involved the testing of single compounds; no blends were used.

Treatment of EAG Data

EAG responses (−mV) to test compounds were adjusted to compensate for solvent and/or mechanoreceptive artifacts by subtracting the accompanying "control" stimulation, yielding "corrected −mV" (see Reed et al., 1987; Gabel et al., 1992). EAG data also were expressed as percent responses of the standard stimulant (linalool, a compound of medium MW and volatility), hereafter written "% of standard" (see Light et al., 1992a, 1992b; van Loon et al., 1992). Due to differences in volatility between the 40 stimulant compounds (MW and boiling point data, Appendix 1), we utilized the standard stimulus as a reference point for comparing EAG responses to different stimulant compounds. Thus, "% of standard" EAG data were used in male-female rank order comparisons and all comparisons involving different chemical classes, isomers and functional groups.

EAG Data Analyses and Statistical Tests

Mean EAG responses (% of standard) to all 40 stimulant compounds (at 10% concentration) were ranked 1–40 in descending order of magnitude for both sexes. We compared male and female EAG rank orders using Spearman's Rank Correlation Coefficient (Sokol and Rohlf, 1981), with which we tested the null hypothesis of no correlation (Rho = 0) between male and female scent compound ranks. If a calculated sample product-moment correlation coefficient (R_s) was found to exceed the upper 95% confidence interval, we rejected the hypothesis of no correlation between male and female EAG rank orders.

We attempted to identify variation in EAGs associated with sex and/or differences between and within chemical classes of olfactory stimulants. EAG data (% of standard) were square-root transformed to compensate for unequal variances and were analyzed using Repeated Measures ANOVA models (Systat 5.2.1; 1992). First, we grouped floral scent and vegetative compounds by chemical class (monoterpenes, oxygenated terpenoids, aromatic esters, aromatic alcohols and aldehydes, fatty acid derivatives, and nitrogen-bearing compounds) and derived a mean response value for each chemical class by averaging the mean EAGs for the individual member compounds. These derived data were analyzed for significant differences related to sex, chemical class, and sex x class interactions using Repeated Measures ANOVA. If significant variation were asso-

ciated with chemical class, specific pairs of stimulant classes were then compared in three *a priori* contrasts: monoterpenes vs. oxygenated terpenoids, aromatic esters vs. aromatic alcohols and aldehydes, and all terpenoids vs. all aromatics. Contrasts were examined by computing Scheffé contrast intervals around the differences between the two compared means (Rothman and Ericson, 1987). We rejected the null hypothesis of equal means if the computed Scheffé intervals did not contain the number zero.

Second, we examined each chemical class separately for potential variation due to differences in structure or functional group, using Repeated Measures ANOVA and tracking sex, compound and sex x compound interactions as factors. These analyses were performed using square root transformed mean EAG data for each compound within chemical classes. For GLVs and aromatic compounds, we performed some follow-up comparisons of mean EAGs for select groups of related compounds using Student's *t*-tests adjusted for multiple comparisons with the sequential Bonferroni method described by Rice (1989), testing null hypotheses of no statistical differences between means. Male and female data were pooled for *t*-tests, because there were no significant ANOVA terms associated with sex (see below). For aromatics, we performed a second series of ANOVA on groups of compounds conforming to the following four classes: benzoic acid methyl esters, *ortho*-hydroxyl-benzoic esters (salicylates), benzaldehydes and phenylpropanoids. Follow-up tests focused on two groups of compounds sharing a common carbon skeleton but differing by functional group or carbon chain length.

We analyzed dose-response EAG data (% of standard) with respect to three specific parameters: threshold dose, maximum EAG response and slope of the dynamic response phase (see Light et al., 1992b). Threshold dose represents the lowest dose at which a stimulant evokes a mean EAG response distinguishable from responses to controls. The dynamic response phase describes the stimulus dose interval during which the greatest change or increase in EAG responsiveness (i.e., slope) is observed.

Antennal Morphological Data

We performed a number of morphological measurements on male and female *H. lineata* moths to determine whether sexual dimorphism in body size is reflected in antennal morphology. We measured dry body mass and forewing length (base to apex; a good surrogate for body size, see Haber and Frankie, 1989) of all experimental moths, and compared these measures using one-tailed Student's *t*-tests, with a null hypothesis of female moths not being significantly larger than males. Then, we measured the mass, length, diameter, and number of segments (annuli) of the left antenna of each moth. Antennal diameter was measured under a dissecting microscope (50X) using a glass slide calibrated to

0.1 mm; all other measurements were made with a ruler calibrated to 1.0 mm. Dry body and antennal masses were measured using a Mettler AE 100 analytical balance calibrated to 0.0001 g. Antennal measurements were adjusted for differences between male and female body size and compared using ANCOVA (Systat 5.2). The ANCOVA model considered antennal mass, diameter, length, and segment number as separate dependent variables, with sex as a treatment and dry body mass and forewing length as covariates.

RESULTS

General Antennal Responsiveness

The mean −mV responses of *H. lineata* male (mean ± SE: −0.18 ± 0.02 mV) and female (−0.24 ± 0.03 mV) antennae to hexane solvent "control" stimulations did not differ significantly ($t = 1.521$, $P = 0.08$), but the responses were generally larger and more variable for females than for males. Similarly, males' (−0.22 ± 0.04 mV) and females' (−0.30 ± 0.07 mV) EAG responses to the 1% linalool standard stimulus did not differ significantly, although neither sex's responses were uniform. All test compounds elicited measurable EAGs that were greater than those for hexane controls in male and female moths.

Responses to Clarkia breweri Floral Volatiles

Each of the 10 *C. breweri* floral volatiles elicited measurable EAG responses, but the largest EAGs in both sexes (females > −0.8 mV; males > −0.45 mV) were observed in response to benzyl acetate, methyl salicylate and linalool (Figure 1A). Female EAGs were larger than those of males for all of the *C. breweri* compounds, nearly two-fold larger in the cases of eugenol, methyl salicylate and pyranoid linalool oxide (Figure 1A). Benzyl benzoate elicited EAGs from both sexes that were similar in magnitude to those for eugenol and methylisoeugenol, but benzyl benzoate should be regarded as the most potent stimulant of the three, due to its large size (MW 212) and low volatility (b.p. 323°C; see Appendix 1). Male and female EAG responses to methylisoeugenol, vanillin, and veratraldehyde were the lowest among this group of compounds, less than or equal in magnitude to those for the standard stimulus.

Responses to Other Floral Volatiles

Mean male and female EAG responses to the 22 additional floral scent compounds ranged in magnitude from very high (e.g., 2-methyl-butyraldoxime, < −0.8 mV; Figure 1B) to barely detectable (e.g., myrcene, ≤ −0.15 mV). Female EAGs were greater than those of males for all compounds except *allo-*

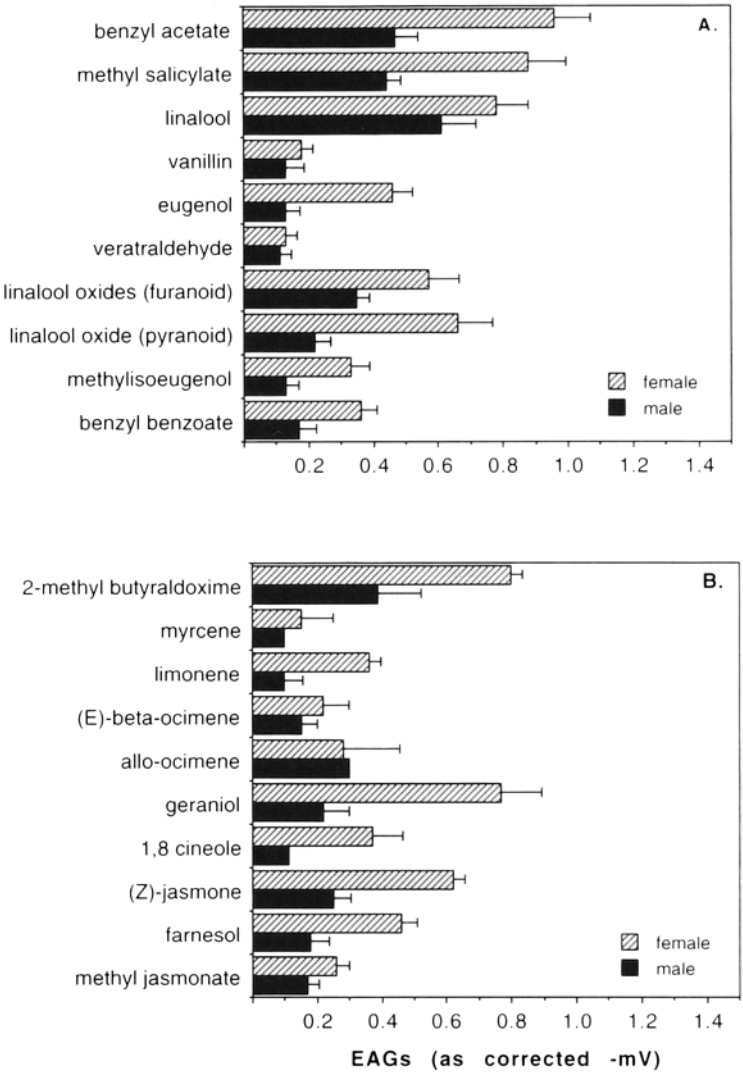


FIG. 1. Female and male *H. lineata* EAG responses to individual scent compounds, expressed as mean corrected $-$mV $\pm$ SE (see text). A. Floral headspace scent compounds from *Clarkia breweri*. B, C. Floral scent compounds from other moth-pollinated plant species; B. Terpenoids and fatty acid derivatives and C. Aromatic or benzenoid compounds. D. "Green Leaf Volatiles."

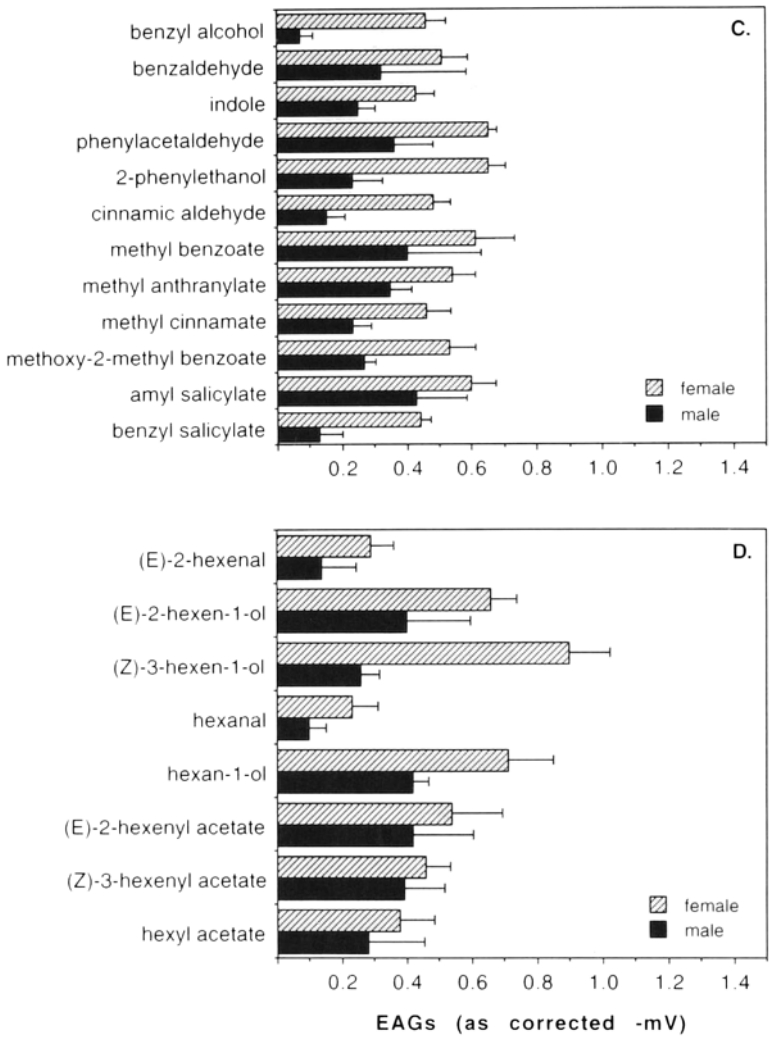


FIG. 1. Continued.

ocimene, especially so in the cases of benzyl alcohol, cinnamic aldehyde, and geraniol (Figures 1B, C). When *H. lineata* EAG magnitudes are scaled to differences in relative volatility, as related to molecular weight and boiling point (Appendix 1), stimulant compounds such as 2-methyl-butyraldoxime (MW 102) and farnesol (MW 222) are comparable in potency, while large compounds (MW

> 200) evoking strong EAG responses, such as amyl salicylate and (*Z*)-jasmone (Figures 1B, C), must be considered among the most highly potent antennal stimulants tested.

Responses to Green Leaf Volatiles (GLVs)

EAG responses to aliphatic alcohols and acetates were moderate to large in magnitude (> -0.4 mV) in both males and females, but EAG responses to the aldehydes hexanal and (E)-2-hexenal were relatively small (Figure 1D). Females' and males' EAG responses to most compounds were similar in magnitude (Figure 1D). The rank order of GLV response potency was: C_6 alcohols $\geq$ C_8 acetates $\geq$ C_6 aldehydes.

Rank Orders of EAG Responses

The rank orders of male and female EAG responsiveness (% of standard) to stimulant compounds at ca. 100 ug dosage are given in Table 1. Male and female ranks were significantly correlated (Spearman's Rank Correlation Coefficient = 0.715, $P < 0.001$), but marked differences in rank were observed for a number of the compounds, particularly within the top ten ranks (see Table 1). Because female EAGs were greater than those of males for most compounds, some differences in rank order were due to high male responsiveness to compounds that were relatively poor female stimulants (e.g., amyl salicylate, *allo*-ocimene, (*E*)-2- and (*Z*)-3-hexenyl acetate, hexyl acetate, linalool; see Table 1). The remaining differences in rank order were attributable to lower male responsiveness to compounds that were strong olfactory stimulants to females (e.g., benzyl alcohol, geraniol, (*Z*)-3-hexen-1-ol, pyranoid linalool oxide, 2-phenylethanol).

Chemical Class Comparisons

When EAG responses to the individual compounds were combined as pooled, averaged and derived indexes for structural chemical classes (Figure 2, Table 2), variation between these classes was significant ($P < 0.001$, Repeated Measures ANOVA). However, the effects of sex and sex x class interactions were not significant (see Table 2A). Monoterpenes elicited the smallest mean EAG magnitudes as a pooled compound class, and these responses were significantly smaller than those elicited by oxygenated terpenoids (Scheffé interval contrast, see Table 2B). However, grand mean EAG responses to aromatic esters were not significantly larger than those to aromatic alcohols and aldehydes, nor were mean responses to aromatic volatiles as a whole statistically separable from those to all grouped terpenoids (Table 2B).

TABLE 1. RANK ORDERS OF *Hyles lineata* EAG RESPONSES TO SCENT COMPOUNDS, EXPRESSED AS % OF STANDARD: FEMALES VS. MALES

Chemical class compound	Female rank	Male rank
Aromatics		
amyl salicylate	14	14
benzaldehyde	18	25
benzyl acetate	2	5
benzyl alcohol	16	39
benzyl benzoate	32	32
benzyl salicylate	33	29
(*E*)-cinnamic aldehyde	20	30
eugenol	27	27
indole	11	21
methoxy-2-methylbenzoate	19	12
methyl anthranylate	15	13
methyl benzoate	21	18
methyl cinnamate	29	17
methylisoeugenol	35	28
methyl salicylate	3	4
phenylacetaldehyde	12	9
2-phenylethanol	7	16
vanillin	38	37
veratraldehyde	39	34
Fatty-acid derivatives		
hexanal	34	33
hexan-1-ol	9	2
(*E*)-2-hexenal	28	36
(*E*)-2-hexen-1-ol	5	7
(*Z*)-3-hexen-1-ol	1	8
(*E*)-2-hexenyl acetate	17	6
(*Z*)-3-hexenyl acetate	24	3
hexyl acetate	22	26
(*Z*)-jasmone	10	23
2-methylbutyraldoxime	4	11
methyl jasmonate	31	22
Terpenoids		
1,8 cineole	23	38
farnesol	26	20
geraniol	6	24
limonene	30	31
linalool	8	1
linalool oxide (Z-pyranoid)	13	15
linalool oxides (*Z*/*E* furanoid)	25	10
myrcene	40	40
allo-ocimene	37	19
(*E*)-*beta*-ocimene	36	35

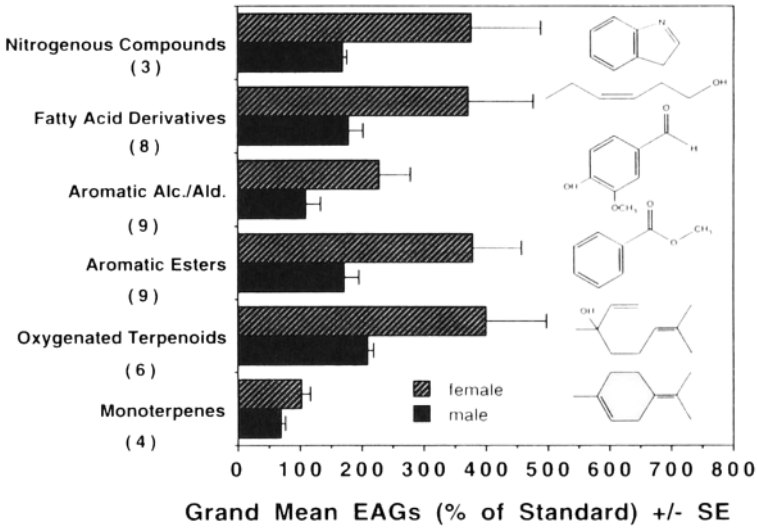


FIG. 2. Summary of *Hyles lineata* moths' EAG responsiveness to different chemical classes, as derived from pooling and averaging the mean EAG responses to each individual test compound belonging to a given chemical class (see Appendix 1). Relative responses are expressed as % of response to standard stimulus (1% v/v linalool in hexane). Number of compounds pooled for each chemical class is given in parentheses. Representative chemical structures are given for each class.

Variation Within Chemical Classes

Significant levels of variation were identified within the aromatic ester, aromatic aldehyde/alcohol, aliphatic GLV, monoterpene, and oxygenated terpene chemical classes (Repeated Measures ANOVA, Table 3). The effects of sex and sex x compound interactions were not found to be significant within most compound classes, with the exception of monoterpenes. This result is most likely due to the small number of compounds in this group (four) and especially strong sex differences in response to limonene (Figure 1B). Among aromatic sub-classes, significant compound-level variance was detected for the salicylates and benzoic acid methyl esters, but not for the benzaldehyde- and phenylpropanoid-related compounds (Table 4).

Follow-up tests of GLV compounds included comparisons of mean EAG responses to compounds varying in the chain position and configuration of C=C double bounds (e.g., (*Z*)-3-hexen-1-ol vs. (*E*)-2-hexen-1-ol) and to C_6 and C_8 GLV compounds sharing the same basic carbon skeleton but differing in degree of saturation, such as hexanal and (*E*)-2-hexenal (Student's *t*-tests, $P \leq 0.005$

TABLE 2. COMPARISON AMONG POOLED, DERIVED, SQUARE-ROOT TRANSFORMED MEAN EAG RESPONSES (% OF STANDARD STIMULUS)

A. Repeated Measures ANOVA

Category	Factor	SS	DF	MS	F	P
Between subjects	sex	227.43	1	227.43	3.07	0.13
	error	444.51	6	74.09		
Within subjects	class	322.44	5	64.49	14.35	<0.001
	class × sex	21.72	5	4.34	0.97	0.45
	error	134.86	30	4.50		

B. *A Priori* Scheffé Contrasts

Comparison	Grand means (transformed)	Scheffé[a] interval
Monoterpenes vs oxyg. terpenoids	9.33 17.54	8.17 ± 3.17
Aromatic esters vs arom. aldehydes and alcohols	16.74 13.03	3.70 ± 3.71
Aromatics vs terpenoids	14.89 13.46	1.43 ± 2.63

[a]Null hypothesis of equal means is rejected if Scheffé interval does not contain the number zero.

for multiple comparisons; Table 5A). None of the five comparisons presented in Table 5A resulted in significantly different mean EAG responses.

Although we observed significant variation in EAG responses to aromatic esters (ANOVA, $P = 0.02$, Table 4), individual differences between methyl benzoate and its *ortho*-substituted derivatives were not statistically significant in pairwise *t*-test comparisons (Table 5B). We did not perform other possible pairwise comparisons within this subclass. We also observed significant variation in EAG responses among salicylates (ANOVA, $P < 0.001$, Table 4). EAG responses to methyl salicylate (with a methyl ester) were significantly greater than those to the larger benzyl salicylate (with a benzyl ester), but not significantly different from those for amyl salicylate (Table 5B).

Dose Responses to Clarkia breweri Volatiles

Dose-response EAG curves for male and female responses to test compounds were similar in shape but varied in their threshold, slope, and maximum -mV response (Table 6). Threshold doses for both sexes were on the order of 10^{-4} $\mu g/\mu l$ for linalool, pyranoid linalool oxide, and methyl salicylate (also

TABLE 3. VARIATION WITHIN COMPOUND CLASSES, REPEATED MEASURES ANOVA[a]

Compound class	Factor	SS	*df*	MS	*F*	*P*
Aromatic esters (N=9)	sex	497.56	1	497.56	3.26	0.12
	error	916.91	6	152.82		
	compound	789.39	8	98.67	7.79	**<0.001**
	cmpd. × sex	100.70	8	12.59	0.99	0.45
	error	607.73	48	12.66		
Aromatic aldehydes & alcohols (N=9)	sex	481.22	1	481.22	3.52	0.11
	error	830.68	6	136.78		
	compound	595.65	8	74.46	5.40	**0.004**
	cmpd. × sex	254.60	8	31.83	2.31	0.08
	error	661.47	48	13.78		
Aliphatic GLV's (N=8)	sex	284.76	1	284.76	1.79	0.23
	error	956.33	6	159.39		
	compound	876.28	7	125.18	7.79	**<0.001**
	cmpd. × sex	174.00	7	24.86	1.55	0.18
	error	675.24	42	16.08		
Nitrogen-bearing compounds (N=3)	sex	185.08	1	185.08	2.15	0.19
	error	516.40	6	86.07		
	compound	49.96	2	24.98	2.98	0.09
	cmpd. × sex	13.14	2	6.57	0.78	0.48
	error	100.56	12	8.38		
Monoterpenes (N=4)	sex	13.21	1	13.21	245.18	**0.004**
	error	0.11	2	0.05		
	compound	140.80	3	46.93	18.89	**0.002**
	cmpd. × sex	45.28	3	15.10	6.08	**0.03**
	error	14.91	6	2.48		
Oxygenated terpenoids (N=6)	sex	238.51	1	238.51	2.11	0.20
	error	677.62	6	112.94		
	compound	312.47	6	62.49	2.96	**0.03**
	cmpd. × sex	72.44	5	14.49	0.69	0.64
	error	634.07	30	21.14		

[a]Abbreviations: SS = sum of squares, *df* = degrees of freedom, MS = mean square. Significant *p* values are given in bold face.

benzyl acetate for females), 10^{-3} for methylisoeugenol, 10^{-2} for benzyl benzoate, and 10^{-1} for eugenol (Figure 3A–C). Males were sensitive to lower threshold doses of furanoid linalool oxides (10^{-2} $\mu g/\mu l$) than were females (threshold = 10^{0} $\mu g/\mu l$), while females had lower thresholds (10^{-4} $\mu g/\mu l$) to benzyl acetate than did males ($>10^{-3}$ $\mu g/\mu l$). The highest EAG response max-

TABLE 4. VARIATION WITHIN AROMATIC SUB-CLASSES, REPEATED MEASURES ANOVA[a]

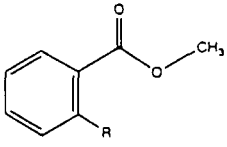
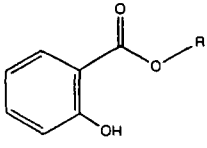
Compound class	Factor	SS	*df*	MS	*F*	*P*
Salicylates (N=3)	sex	253.84	1	253.84	4.97	0.07
	error	306.20	6	51.03		
	compound	411.33	2	205.67	18.14	**<0.00**
	cmpd. × sex	7.30	2	3.68	0.33	0.73
	error	136.09	12	11.34		
Benzoic acid esters (N=4)	sex	160.73	1	160.73	1.83	0.23
	error	527.72	6	87.95		
	compound	248.98	3	83.99	5.46	**0.02**
	cmpd. × sex	17.35	3	5.78	0.38	0.69
	error	273.86	18	15.22		
Benzal-dehydes (N=3)	sex	46.41	1	46.41	1.78	0.23
	error	156.45	6	26.07		
	compound	159.49	2	79.74	4.35	0.06
	cmpd. × sex	78.78	2	39.39	2.15	0.18
	error	220.15	12	18.35		
Phenyl-propanoids (N=4)	sex	143.90	1	143.90	2.56	0.16
	error	336.71	6	56.12		
	compound	53.45	3	17.82	2.42	0.11
	cmpd. × sex	53.17	3	17.73	2.41	0.11
	error	132.30	18	7.35		

[a]Abbreviations: SS = sum of squares, *df* = degrees of freedom, MS = mean square. Significant *p* values are given in bold face.

ima for both sexes were observed for benzyl acetate, linalool, and methyl salicylate. These compounds evoked the steepest slopes of EAG response curves over the broadest dynamic response ranges (10^{-1} to 10^{2} $\mu g/\mu l$ concentrations; Table 6).

Male and female EAG rank orders for eight *C. breweri* compounds at doses from 10^{2} down to 10^{-4} $\mu g/\mu l$ are listed in Table 7. Mean rank orders over all doses were comparable to those listed for doses of 10% (Table 1), with some inconsistencies resulting from different threshold doses among stimulants (e.g., furanoid linalool oxides). Compounds such as linalool and methyl salicylate were consistently strong antennal stimulants throughout the range of doses tested. Benzyl acetate was a relatively poor stimulant at dosages below 10^{-2} $\mu g/\mu l$, while pyranoid linalool oxide was a relatively strong antennal stimulant at doses below 10^{0} $\mu g/\mu l$.

TABLE 5. COMPARISONS OF ISOMERS AND FUNCTIONAL GROUPS WITHIN CHEMICAL CLASSES[a]

Comparison	Student's *t*	*P*
A. GREEN LEAF VOLATILES		
$C_6H_{12}O$		
(*Z*)-3-hexen-ol vs	−0.49	0.64
(*E*)-2-hexen-1-ol		
$C_8H_{14}O$		
(*Z*)-3-hexenyl acetate vs	0.09	0.93
(*E*)-2-hexenyl acetate		
C_6		
hexan-1-ol vs	0.23	0.82
(*E*)-2-hexen-1-ol		
hexanal vs	−1.20	0.25
(*E*)-2-hexenal		
C_8		
hexyl acetate vs	−1.02	0.32
(*E*)-2-hexenyl acetate		
B. AROMATIC ESTERS		
1. *ortho*-substituted methyl esters of Benzoic Acid		
		
methyl benzoate (R = H) vs	−0.37	0.72
methyl anthranylate (R = NH_2)		
methyl benzoate vs	−2.63	0.02
methyl salicylate (R = OH)		
methyl benzoate vs	−0.26	0.80
methoxy-2-methyl benzoate (R = OCH_3)		
2. Salicylates.		
		
methyl salicylate (R = CH_3) vs	−2.13	0.05
amyl salicylate (R = $CH_2CH_2CH\ CH_3$)		
CH_3		
methyl salicylate vs		
benzyl salicylate (R = $-CH_2-$ )	−3.40	**0.004**

[a]Comparisons are two-tailed Student's *t*-tests comparing square-root transformed mean EAGs (% of standard). Experiment-wide significance level is $P \leq 0.005$, after Bonferroni adjustment for multiple comparisons. Significant *p*-values given in bold print.

TABLE 6. DOSE-RESPONSE PARAMETERS, *Hyles lineata* EAG SENSITIVITY (% OF STANDARD) TO *Clarkia breweri* FLORAL VOLATILES (A = FEMALE, B = MALE)

Stimulatory compound	% of *C. breweri* floral scent[a]		Log threshold dosage[b]	Max. response	Dynamic response phase	
					slope	interval
linalool	27.63	A.	< -4	428 %	109	10^{-1}–10^{2}
		B.	< -4	448 %	133	10^{-1}–10^{2}
linalool	0.77	A.	0	160	80	10^{0}–10^{2}
oxides (furanoid)		B.	< -2	180	52	10^{-1}–10^{2}
linalool	10.72	A.	< -4	117	73	10^{0}–10^{1}
oxide (pyranoid)		B.	> -4	125	117	10^{0}–10^{1}
benzyl acetate	42.15	A.	-4	718	214	10^{-1}–10^{2}
		B.	> -3	296	123	10^{0}–10^{2}
benzyl benzoate	3.93	A.	-2	109	45	10^{0}–10^{2}
		B.	> -2	75	25	10^{1}–10^{2}
methyl	3.32	A.	< -4	598	166	10^{-1}–10^{2}
salicylate		B.	< -4	375	117	10^{-1}–10^{2}
eugenol	1.32	A.	> -1	109	55	10^{-1}–10^{1}
		B.	> -1	100	51	10^{-1}–10^{1}
methylisoeugenol	1.44	A.	< -3	92	35	10^{-1}–10^{1}
		B.	< -3	62	21	10^{-1}–10^{1}

[a]Percent of floral headspace composition, 24 hr scent collection over Tenax TA/charcoal, GC-MS analysis: Raguso and Pichersky, 1995. Vanillin and veratraldehyde were not used in dose-response experiments.
[b]Dosage of compound in hexane solution ($\mu g/\mu l$) as applied to filter papers in odor delivery cartridges.

Morphological Measurements

Female *H. lineata* moths were significantly larger than males, both in terms of dry body mass ($P = 0.007$) and forewing length ($P = 0.002$; one-tailed *t*-test, Table 8A). However, this inequality in body size was not reflected in antennal morphology. After scaling for body size differences using ANCOVA, female and male antennal diameter, antennal mass, and antennal segment number were not significantly different, but male antennae were significantly longer than those of females, relative to body size ($P = 0.014$, Table 8b).

DISCUSSION

Sexual Dimorphism and Rank Order

Antennae of adult male and female *H. lineata* responded in a sensitive and dynamic manner to the presentation of chemically diverse olfactory stimulants.

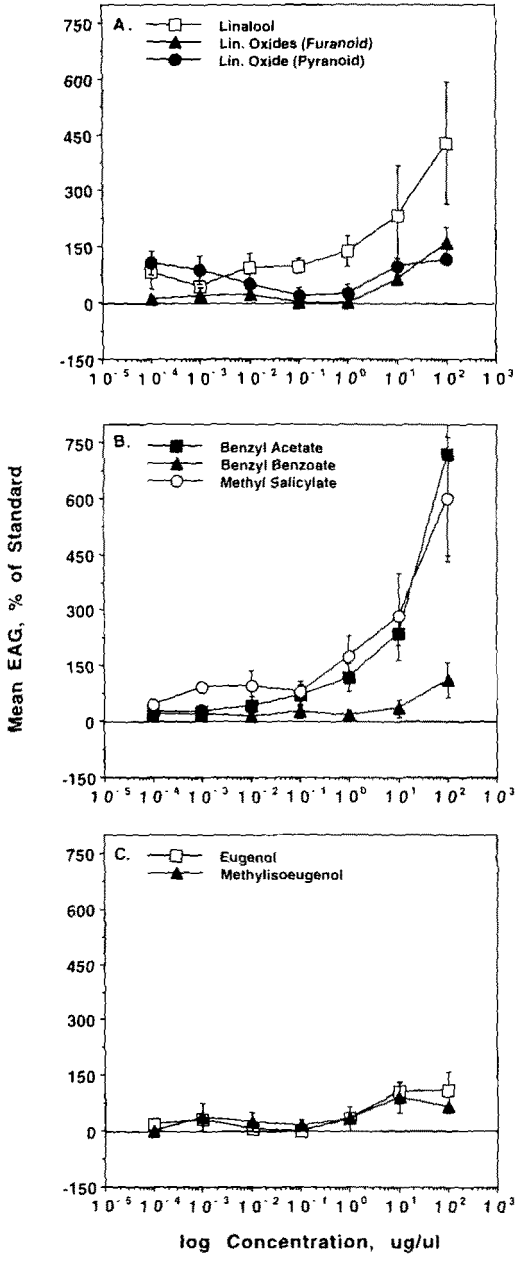


FIG. 3. Dose-response curves of female (A–C) and male (D–F) *H. lineata*, showing EAG responses to *C. breweri* floral volatiles at different v/v concentrations (as applied to filter paper wicks), expressed as mean % of standard ± SE vs log μg/μl.

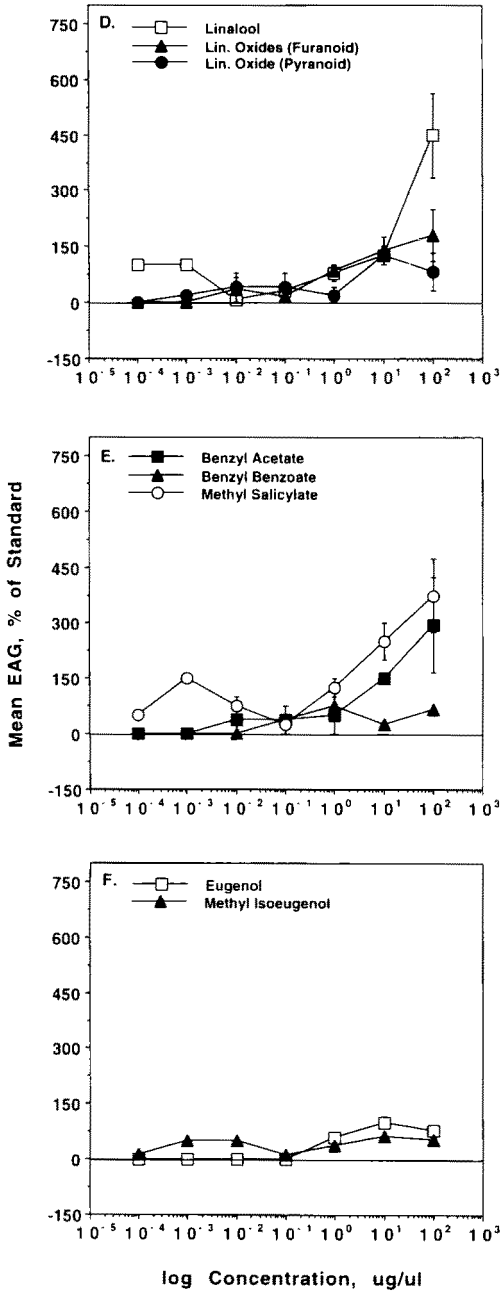


FIG. 3. Continued.

TABLE 7. RANK ORDERS OF EAG RESPONSES (% OF STANDARD) TO DIFFERENT CONCENTRATIONS OF *Clarkia breweri* FLORAL SCENT COMPOUNDS[a]

Stimulatory compound	Log concentration ($\mu g/\mu l$) per filter paper														$\bar{X}$	
	10^2		10^1		10^0		10^{-1}		10^{-2}		10^{-3}		10^{-4}			
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
benzyl acetate	3	1	2	2	6	3	2	3	4	4	-	6	-	4	3	3
benzyl benzoate	7	6	8	8	4	7	3	4	-	6	-	-	-	-	6	7
eugenol	6	7	6	4	5	5	8	-	-	-	-	-	-	-	8	5
linalool	1	3	4	3	3	2	4	1	6	2	2	3	1	2	2	2
linalool oxides (furanoid)	4	4	3	7	2	8	6	-	5	-	-	-	-	-	4	8
linalool oxide (pyranoid)	5	5	5	5	8	6	1	5	3	3	4	2	-	1	5	4
methylisoeugenol	8	8	7	6	7	4	7	6	2	5	3	4	-	-	7	6
methyl salicylate	2	2	1	1	1	1	5	2	1	1	1	1	2	3	1	1

Note: Dashes indicate that compound could not be ranked at that dosage because it was below the threshold of detection.

[a] M = male (N = 3), F = female (N = 6).

Female EAGs were greater than those of males for most compounds, sometimes dramatically so (Figures 1A–D), yet sex was not a significant factor in most Repeated Measures ANOVA between and within chemical classes (Tables 2–4). The female-biased disparity in EAG responses to our experimental volatiles was not a scaling artifact due to the overall larger body size of females, because female antennae were actually smaller than those of males relative to body size (Table 8). However, variation within sexes was large in general (see error bars, Figures 1A-D), probably due to the sample sizes and differences in the moths' ages and experiences in the wild; thus, sex was not a significant factor in most of our ANOVA tests. Male and female EAG rank orders were significantly correlated (Table 1), and the shapes, thresholds, and slopes of the dose/response curves for the *Clarkia breweri* floral volatiles were fundamentally the same for both sexes, with responses of females being slightly larger in magnitude (Figures 3A–F, Tables 6, 7). These results suggest that male and female antennae are endowed with homologous types of olfactory receptors tuned to a wide range of "floral" and "vegetative" odorants. The few EAG rank order discrepancies observed among males and females (e.g., higher male responses to *allo*-ocimene; higher female responses to geraniol) may reflect (1) specific sex-related quantitative differences in antennal receptor neuron populations, (2) qualitative differences in olfactory physiology, or (3) altered EAG receptivities resulting from adult moth experiences prior to capture in the wild

TABLE 8. COMPARISON OF MALE AND FEMALE *H. lineata* BODY SIZE AND ANTENNAL MORPHOLOGY[a]

A. Sexual dimorphism in body size (one-tailed Student's *t* test)

Category (units)	Mean measurement (± SE)			
	Males	Females	*t*	*P*
Dry body mass (g)	0.250 (± 0.019)	0.340 (± 0.021)	3.14	**0.007**
Forewing length (mm)	32.3 (± 0.9)	37.4 (± 1.0)	3.76	**0.002**

B. Comparison of antennal morphological measurements using ANCOVA

Treatment	SS	*df*	MS	*F*	*P*	Adjusted Measures	
						sex	LSM ± SE
1. Antennal diameter (mm)							
Sex	0.007	1	0.007	2.226	0.170	female	0.75 ± 0.03
Dry mass	0.000	1	0.000	0.083	0.780	male	0.82 ± 0.03
Forewing	0.000	1	0.000	0.015	0.905		
Error	0.028	9	0.003				
2. Segment number							
Sex	10.378	1	10.378	0.307	0.593	female	60.72 ± 2.79
Dry mass	13.788	1	13.788	0.408	0.539	male	58.00 ± 3.11
Forewing	1.490	1	1.490	0.044	0.808		
Error	304.256	9	33.806				
3. Antennal dry mass (g)							
Sex	0.005	1	0.005	2.896	0.123	female	0.0013 ± 0.0002
Dry mass	0.002	1	0.002	1.094	0.323	male	0.0018 ± 0.0002
Forewing	0.000	1	0.000	0.139	0.718		
Error	0.015	9	0.002				
4. Antennal length (mm)							
Sex	3.031	1	3.031	9.326	**0.014**	female	12.24 ± 0.27
Dry mass	1.475	1	1.475	4.538	0.062	male	13.72 ± 0.30
Forewing	0.383	1	0.383	1.179	0.306		
Error	2.925	9	0.325				

Note: Numbers in bold face are statistically significant at $P \leq 0.05$. Sample sizes: females, N = 7; males, N = 6.

[a] Abbreviations: ANCOVA = analysis of covariance, SS = sum of squares, *df* = degrees of freedom, MS = mean square, LSM = least squares mean, SE = standard error.

(see Vet et al., 1990; De Jong and Pham-Delegue, 1991). We have no data bearing on this question at present.

Previous observations of sex-bias in pheromone response (Schweitzer et al., 1976; Reed et al., 1987; Christensen et al., 1989), floral visitation (Kislev et al., 1972; Knudsen and Tollsten, 1993) and host plant orientation and ovi-

position (Yamamoto et al., 1969; Tichenor and Seigler, 1980) in *Manduca sexta* (L.) and other hawkmoth species suggest that complex sexual dimorphism might exist for scent compound receptors in the antennae of nectar-feeding hawkmoths. For example, while both sexes of *M. sexta* visit flowers, females respond to solanaceous host plant volatiles during oviposition behavior (Tichenor and Seigler, 1980), while males respond to female sex pheromone (Schweitzer et al., 1976; Hildebrand et al., 1992), detected by the specific *sensilla trichoidea* receptors that physically and numerically dominate their antennae (up to 69% of all receptors per antennal segment; Lee and Strausfeld, 1990). Thus, greater female EAG magnitudes in *H. lineata* might reflect either larger populations of antennal receptors tuned to plant volatiles (see Mayer et al. 1984), or greater chemosensory efficiency in females, either due to the reduced size of trichoid *sensilla* on their antennae (Lee and Strausfeld, 1990) or to differences in aerodynamic flow of scent-laden air over the differently shaped antennae (M. Willis, pers. comm.). Kislev et al. (1972) suggest that male-bias in floral visitation by *H. lineata* in Israel is due to differences in male and female responses to certain floral scent compounds. Our results, however, support Knudsen and Tollsten's (1993) contention that this bias is not explainable by innate differences in peripheral olfactory reception of floral volatiles. If anything, female *H. lineata* antennae appear to be slightly more receptive to most floral scent compounds than are those of males.

EAG Responses and Chemical Classes

We observed strong EAG responses to 10% volumetric concentrations of a wide variety of scent compounds emitted by moth-pollinated flowers, including aromatic esters, aldehydes, and alcohols, oxygenated monoterpenoids, a sesquiterpenol, fatty-acid derivatives and assorted aliphatic and aromatic nitrogen-bearing compounds. Similarly, Brantjes (1973) recorded large EAGs using two other hawkmoth species (*Deilephila elpenor* L. and *M. sexta*), in response to 13 individual floral compounds (mostly terpenoids and aromatics) and to floral scent extracts from nine plant species, but his data were not presented quantitatively. Collectively, these results demonstrate that nectar-feeding hawkmoths are capable of detecting a wide range of floral and vegetative volatiles from diverse chemical classes. This broad olfactory vocabulary includes floral compounds (e.g., 2-phenylethanol and phenylacetaldehyde) that typify noctuid moth-pollinated flowers such as *Silene vulgaris* (Caryophyllaceae; Pettersson, 1991; Knudsen and Tollsten, 1993) and *Abelia grandiflora* (Caprifoliaceae; Haynes et al., 1991). Indeed, these and other "noctuid" flowers are often visited by sphingids (Nilsson, 1983; Pettersson, 1991; Wasserthal 1993).

We observed strong EAG responses to eight aliphatic GLV compounds, particularly to C_6 alcohols and C_8 acetates. Many of these compounds also are

potent EAG stimulants for *M. sexta* (Schweitzer et al., 1976; Light unpubl. data) and *Pieris* butterflies (van Loon et al., 1992). However, the GLVs are smaller and more volatile molecules than most of the aromatic and terpenoid test compounds; thus, the number of GLV molecules delivered during a stimulatory puff would be proportionally higher than that of larger, less volatile odorants. Although vegetative/leaf volatiles are clearly important to ovipositional decision-making in female *M. sexta* (Yamamoto et al., 1969; Ramaswamy, 1988), the role of GLVs as potential olfactory stimulants during *Hyles* oviposition bouts has not yet been examined. *H. lineata* is extremely polyphagous throughout its range, utilizing plants from the Nyctaginaceae, Onagraceae, Polygonaceae, Portulacaceae, Rosaceae, Scrophulariacaea, and Vitaceae as ovipositional sites and larval hosts (Wiltshire, 1957; Hodges, 1971; Common, 1990). Host species or genus-specific vegetative volatiles appear to function as important olfactory cues to females of *M. sexta*, which oviposit only on Solanaceae (Tichenor and Seigler, 1980), but host choice by gravid *Hyles* females may depend more on general cues, such as the ubiquitous GLVs and terpenes such as caryophyllene, myrcene, and pinene (Visser et al., 1979; Light et al., 1992a, 1992b, 1993; van Loon et al., 1992).

Functional Group Variation

Significant differences in antennal responsiveness correlated with structural variation in pheromone or plant volatile chemistry have been observed in hawkmoths (Reed et al., 1987), butterflies (van Loon et al., 1992) and other phytophagous insects (see Light et al., 1988, 1992a, 1992b). In our experiments, the importance of specific functional groups in determining EAG response potency depended strongly on the particular carbon skeleton geometry and/or chemical class. For example, oxygenated monoterpenoids elicited significantly higher EAG responses than did monoterpenes (Figures 1A, B, Table 2), the chief difference among them being the presence of a hydroxyl group (e.g., linalool vs. myrcene). Among the aliphatic GLVs, our results document a clear superiority in EAG potency of aliphatic alcohols over aldehydes. However, differences in levels of carbon skeleton saturation and double bond (C=C) geometry were not associated with statistically significant EAG differences.

We detected significant amounts of variation in EAG responses to diverse aromatic esters, aldehydes, and alcohols (Table 3), especially to benzoic acid esters and salicylates (Table 4), yet few specific functional group comparisons resulted in significantly different EAG responses. Benzaldehyde was a more potent antennal stimulant than either of the substituted benzaldehydes, vanillin and veratraldehyde, especially for female moths (Figs. 1A, C), but these differences did not translate into significant within-class variance (ANOVA, Table 4). Similarly, we did not detect significant variance associated with differences

in *para*-position oxidation among such compounds as eugenol, methylisoeugenol, vanillin, and veratraldehyde (ANOVA, Table 4).

Pairs of aromatic compounds that varied in the presence or absence of *ortho*-substituted hydroxyl-groups, such as methyl benzoate vs. methyl salicylate and benzyl benzoate vs. benzyl salicylate did not differ significantly. Methyl anthranylate, methyl salicylate and methyl-2-methoxybenzoate share a common skeleton but substitute amino-, hydroxyl- and methoxyl- groups, respectively, for the *ortho*-position hydrogen of methyl benzoate. However, mean EAG responses to these compounds did not differ significantly from those to methyl benzoate itself. Among salicylates, methyl salicylate elicited significantly higher EAGs than did benzyl salicylate, but this result could be an artifact of benzyl salicylate's higher molecular weight (40% larger) and lower volatility (Appendix 1). From these data, we are unable to infer whether *H. lineata* moths possess separate antennal receptors for chemically similar plant volatiles such as methyl salicylate and methyl anthranylate, which are quite distinct to the human palate (oil of wintergreen and concord grape, respectively), or whether functional group differences bear any physiological or behavioral importance to these insects.

Dose-Response Sensitivities

Foraging hawkmoths undoubtedly encounter a rich and broad spectrum of plant odors while in flight and during floral visitation. The dose-response data suggest that *H. lineata* moths are sensitive to most *C. breweri* floral compounds at a dose of ca. 1 ng on filter paper. This keen receptivity supports the possibility that scent compounds function as long-distance attractants to foraging hawkmoths (Tinbergen, 1958; Bratjes, 1973). Our results mirror those of Van Loon et al. (1992; *Pieris* butterflies) in showing that EAG rank orders may change with different stimulant dosages (e.g., eugenol and methylisoeugenol; Figure 3C, F, Table 6), suggesting that certain plant volatiles may have different behavioral or physiological activities at different concentrations. In the absence of behavioral bioassays or choice experiments, however, it is difficult to determine physiologically relevant odorant concentrations as emitted from a flower. The identification of floral volatiles using dynamic headspace sorption is usually used as a qualitative analytical procedure (Bergström et al., 1980; Bicchi and Joulain, 1990), but at best provides quantitative data only on cumulative floral odor emission per unit fresh weight over time (usually 12–24 hours; Raguso and Pichersky, 1995). These studies provide little if any inference (and usually an overestimation) of the actual concentrations of floral scent compounds encountered by foraging moths in a wind-borne odor plume (Murlis et al., 1992). Subtle changes in scent production or concentration, similar to changes in color, may convey information on nectar reward or physiological condition to a foraging insect (Weiss, 1991; Dobson, 1994). Thus, antennal sensitivity to floral scent

compounds across a range of concentrations might be as important to a moth during a floral visit as it is to a moth flying upwind in search of a flower.

EAGs and the C. breweri-H. lineata Interaction

In our study of the *C. breweri-H. lineata* pollination interaction, we have identified aromatic esters (benzyl acetate and methyl salicylate) and oxygenated monoterpenoids (linalool, linalool oxides) as the principal floral volatiles (>80% of GC-MS peak area; Raguso and Pichersky, 1995) and the most potent antennal chemostimulants. Aromatic esters and oxygenated terpenoids are among the most common scent components identified from hawkmoth-pollinated flowers spanning diverse plant families (Loughrin et al., 1990; Kaiser, 1991, 1993; Knudsen and Tollsten, 1993). Aromatic esters, in particular, are suspected to function as floral attractants of many hawkmoth species (Morgan and Lyon, 1928; Hodges, 1971; Nilsson, 1983), but they are not restricted to the aromas of night-blooming flowers (Knutsen and Tollsten, 1993). The presence of the uncommon N-bearing oxime compounds and oxygenated sesquiterpenes (e.g., farnesol and nerolidol) in many temperate and tropical hawkmoth-pollinated flowers has led many authors to suggest that these compounds function as hawkmoth attractants (Nilsson et al., 1985; Kaiser, 1993; Knudsen and Tollsten, 1993). Our data do not suggest anything unusual about these compounds at the level of antennal detection (see Figures 1B, C).

The EAG is believed to represent the sum of receptor potentials elicited in all sensory neurons by stimuli presented to the antennae (Boeckh et al., 1965; Schweitzer et al., 1976; Mayer et al. 1984). In essence, a discernible EAG response greater than the control stimulation indicates that a moth's antenna is sensitive to that odorant at the trial dosage. However, the behavioral activities of compounds can only be identified through controlled behavioral bioassays conducted in field or laboratory settings (Dodson et al., 1969; Jacobson et al., 1976; Tichenor and Seigler, 1980; Haynes et al., 1991).

CONCLUSIONS

Our EAG studies of *Hyles lineata* olfaction firmly establish that these moths can detect all of the floral scent compounds of *Clarkia breweri* at physiologically relevant concentrations. These results are consistent with the hypothesis that hawkmoths can detect the floral scent of this plant from a distance. In addition, male and female moths demonstrated a broad olfactory vocabulary, showing strong EAG responses to a variety of aromatic, aliphatic, terpenoid and nitrogen-bearing scent compounds found in the floral and vegetative tissues of many plants utilized by hawkmoths as nectar and oviposition resources. Considering

these findings, the behavioral responses of hawkmoths to diverse floral odors are not likely to result from gross differences in antennal receptor physiology, but rather from differences in individual experiences and/or central nervous system organization. The hypothesis that the floral scent of *C. breweri* could function as a long-distance attractant cannot be rejected by these findings, but EAG data are not sufficient to demonstrate behavioral attraction, arrestment, or repulsion, but only the capacity for olfactory detection. Behavioral experiments using live hawkmoths in wind tunnels or flight cages will be required to test this hypothesis.

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APPENDIX 1. TEST COMPOUNDS, PURITIES, SOURCES, AND JUSTIFICATION FOR INCLUSION IN STUDY

Compound (Class[1])	Formula	MW	B.P. °C	Purity	Source	Justification[2]
Aromatic Compounds						
benzyl alcohol (1)	C_6H_6O	94	205	92%	Eastman/White	3,4
benzaldehyde (1)	C_7H_6O	106	178	99%	Aldrich	3,4
indole (6)	C_8H_7N	107	254	95%	Eastman	3
phenylacetaldehyde (1)	C_8H_8O	120	194	–	Aldrich	4
2-phenylethanol (1)	$C_8H_{10}O$	122	205	–	Eastman/White	4
(*E*)-cinnamic aldehyde (1)	C_9H_8O	132	248	95%	R. Flath	3,5
methyl benzoate (2)	$C_8H_8O_2$	136	200	98%	Fritzche	3
benzyl acetate (2)	$C_9H_{10}O_2$	150	214	99+%	Aldrich	1,3,4
methyl (2,6) anthranylate	$C_8H_9O_2N$	151	*	–	CPL	5
methyl salicylate (2)	$C_8H_8O_3$	152	224	98%	Aldrich	1,3
vanillin (1)	$C_8H_8O_3$	152	285	99%	Aldrich	1
methyl cinnamate (2)	$C_{10}H_{10}O_2$	162	262	99%	Aldrich	5
eugenol (1)	$C_{10}H_{12}O_2$	164	255	99%	Aldrich	1

APPENDIX 1. Continued

Compound (Class[a])	Formula	MW	B.P. °C	Purity	Source	Justification
veratraldehyde (1)	$C_9H_{10}O_3$	166	281	99%	Aldrich	1
methoxy-2-methyl benzoate (2)	$C_9H_{11}O_3$	167	245	-	R. Flath	3,5
methyl-isoeugenol (1)		178	*	99%	Aldrich	1
amyl salicylate (2)	$C_{12}H_{16}O_3$	208	*	75%	TCI	3,5
benzyl benzoate (2)	$C_{14}H_{12}O_3$	212	323	-	Sigma	1,3
benzyl salicylate (2)	$C_{14}H_{12}O_3$	228	*	99%	ICN	3,5
Fatty-Acid Derivatives						
(*E*)-2-hexenal (3)	$C_6H_{10}O$	98	*	99%	Aldrich	2
(*E*)-2-hexen-1-ol (3)	$C_6H_{12}O$	100	159	97%	Aldrich	2
(*Z*)-3-hexen-1-ol (3)	$C_6H_{12}O$	100	157	98%	Aldrich	2
hexanal (3)	$C_6H_{12}O$	100	131	99%	Aldrich	2
hexan-1-ol (3)	$C_6H_{14}O$	102	157	98%	Aldrich	2
2-methyl-butyraldoxime (3,6)	$C_5H_{12}ON$	102	*	-	R. Flath	3,4
(*E*)-2-hexenyl acetate (3)	$C_8H_{14}O_2$	142	*	98%	Aldrich	2
(*Z*)-3-hexenyl acetate (3)	$C_8H_{14}O_2$	142	*	98%	Aldrich	2
hexyl acetate (3)	$C_8H_{16}O_2$	144	169	98%	Aldrich	2
(*Z*)-jasmone (3)	$C_{11}H_{16}O$	164	258	70%	ICN	3
methyl jasmonate (3)	$C_{13}H_{20}O_3$	224	*	-	R. Flath	5
Terpenoids						
limonene (4)	$C_{10}H_{16}$	136	176	99%	Aldrich	3
myrcene (4)	$C_{10}H_{16}$	136	167	98%	Aldrich	3,5
(*E*)-*beta*-ocimene (4)	$C_{10}H_{16}$	136	178	97%	R. Flath	3
allo-ocimene (4)	$C_{10}H_{16}$	136	*	86%	Fluka	5
linalool (5)	$C_{10}H_{18}O$	154	198	97%	Sigma	1,3
1,8 cineole (5)	$C_{10}H_{18}O$	154	177	99%	Aldrich	3
geraniol (5)	$C_{10}H_{18}O$	154	230	98%	Aldrich	1,3
linalool oxides (*E*/*Z*-furanoid) (5)	$C_{10}H_{18}O_2$	170	*	99%	Aldrich	1,3
linalool oxide (*Z*-pyranoid) (5)	$C_{10}H_{18}O_2$	170	*	99%	KLS	1,3
farnesol (*E*/*Z* mix) (5)	$C_{15}H_{26}O$	222	*	95%	Aldrich	3

[a]Chemical classes assigned as follows:
1 = aromatic alcohols and aldehydes
2 = aromatic esters
3 = fatty-acid derived compounds
4 = monoterpenes
5 = oxygenated terpenoids
6 = N-bearing compounds (aromatic and aliphatic)

APPENDIX 1. Continued

[a] Justification for inclusion in study:
1. present in floral headspace GC-MS analysis of *Clarkia breweri* (Raguso and Pichersky, 1995).
2. "green leaf volatiles", ubiquitous in vegetation.
3. present in many hawmoth-pollinated, night-blooming flowers (Knutsen and Tollsten, 1993).
4. present in many noctuid moth-pollinated flowers (Haynes et al., 1991; Knutsen and Tollsten, 1993).
5. similar to other test compounds but varying in one functional group (see text).

[b] Boiling point data at atmospheric pressure not available in Merck Index, CRC Handbook of Chemistry and Physics, Sigma/Aldrich Handbook of Chemical Safety.

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