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ATTRACTION OF SPHINGID MOTHS (Lepidoptera: Sphingidae) TO 10,12-HEXADECADIENYL ALDEHYDES AND ACETATES: Evidence of Pheromone Components[1]

D.W. REED, E.W. UNDERHILL, and E.M. GIBLIN

National Research Council of Canada
Plant Biotechnology Institute
Saskatoon, Saskatchewan, Canada S7N 0W9

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Abstract—A field survey of 10,12-hexadecadienyl alcohols, acetates, and aldehydes showed attraction to a wide range of sphingid moths. Data are presented showing the attraction of *Smerinthus jamaicensis*, *Smerinthus cerisyi*, *Pachysphinx modesta*, *Hemaris diffinis*, and *Proserpinus flavofasciata* to these compounds. Mass spectral, EAG, and EAD data show evidence for the presence of these dienes in female extracts of *S. cerisyi*, *Hyles gallii*, and *Sphinx drupiferarum*.

Key Words—Attractant, pheromone, 10,12-hexadecadienal, 10,12-hexadecadienyl acetate, *Smerinthus jamaicensis*, *Smerinthus cerisyi*, *Paonias exceactus*, *Paonias myops*, *Pachysphinx modesta*, *Hemaris diffinis*, *Proserpinus flavofasciata*, *Hyles gallii*, *Hyles euphorbiae*, *Sphinx vashti*, *Sphinx drupiferarum*, Lepidoptera, Sphingidae.

INTRODUCTION

Bombykol, *E*10,*Z*12-16:OH[2], was the first pheromone component ever isolated and identified (Butenandt et al., 1959) from the extraction of 500,000 female scent glands of *Bombyx mori* L. Improved isolation and identification techniques have led to the discovery of hundreds of additional pheromones and

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[2] In this paper the chemical nomenclature is abbreviated as follows: *Z* and *E* indicate the *cis* and *trans* configurations of the double bond, the number following *Z* or *E* indicates the position of that double bond, the last number indicates the carbon chain length, the terminal functional groups are :Ac = acetate; :OH = alcohol; :Ald = aldehyde.

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attractants but relatively few involved 10,12-hexadecadienyl alcohols, aldehydes, or acetates (hexadecadienyls). Bombykol was also identified in the female gland extracts of *Bombyx mandarina* by Kuwahara and Yen (1979).

Kasang et al. (1978a) have shown bombykal (*E*10,*Z*12–16:Ald) is a second component of the *B. mori* pheromone and *E*10,*E*12–16:OH is present in its female scent gland (Kasang et al., 1978b). Starratt et al. (1979) identified bombykal as a pheromone component of a sphingid *Manduca sexta* (L.), and Hall et al. (1980) identified *E*10,*E*12–16:Ald as a pheromone component of the noctuid, *Earis insulana* (Boisduval).

Hyles euphorbiae (L.), an Old World sphingid, has been introduced into North America, and most recently to Saskatchewan, as a biological control of leafy spurge (*Euphorbia esula* L.) (Harris, 1984). We are presently investigating the pheromone of *H. euphorbiae* as a potential population monitor, and initial studies have indicated that hexadecadienyl aldehydes are present in female abdominal tip extracts, (Underhill, unpublished). This evidence, as well as the proven pheromone activity of 10,12-hexadecadienyl compounds in several lepidopteran families and previous success in using conjugated dienyl compounds as attractants for a wide range of Lepidoptera (Reed and Chisholm, 1985), led us to synthesize and field test the 10,12-hexadecadienyls. We report here field trapping, electroantennograph (EAG), and coupled gas chromatograph–electroantennographic detector (GC-EAD) data that suggest several members of Sphingidae family use 10,12-hexadecadienyl compounds as pheromone components. We also present mass spectral data that show the presence of one of these compounds in female abdominal tip extracts.

METHODS AND MATERIALS

The chemicals used in this study were synthesized and purified in this laboratory. The *EE*, *EZ*, and *ZE* alcohols were synthesized using methods described by Chisholm et al. (1981). The *E*10,*E*12–16:OH and *E*10,*Z*12–16:OH were recovered from a Wittig condensation reaction of the ylide of butyltriphenylphosphonium bromide with (*E*)-12-tetrahydropyranyloxy-2-dodecenal. *E*10,*E*12–16:OH and *Z*10,*E*12–16:OH were recovered from the Wittig condensation reaction of the ylide of 10-acetoxydecyltriphenylphosphonium bromide with (*E*)-2-hexenal.

Synthesis of the *ZZ* isomer, and purification and conversion of all alcohols to aldehydes and acetates was similar to that described by Chisholm et al. (1985). *Z*10,*Z*12–16:OH was synthesized by the catalytic coupling of 10-undecynol with 1-bromopentyne and the subsequent dicyclohexylborane reduction of the diyne to the diene.

Structures were verified by 90-MHz NMR, IR, and mass spectrometry. Isomeric purity was greater than 99% and was checked on a Hewlett Packard

5890A gas chromatograph with flame ionization detector (FID) using a 0.32-mm-ID × 30-m DB-5 fused silica capillary column (J. & W. Scientific, Rancho Cardova, California).

Chemical ionization mass spectral analyses of female tip extracts were done with a Finnigan 3300 GC-MS using a 0.32-mm × 60-m DB-5 fused silica column with hydrogen as carrier gas and methane as reagent gas.

Injections were made at an oven temperature of 40°C, heated to 120°C and temperature programmed 4°/min to 280°C. A multiple-ion detection (MID) method was used to scan for characteristic ions of the 10,12-hexadecadienyls which included ions with *m/z* 169, 219, 221, 235, 237, 239, 253, 265, 279, and 321.

From May 2 to September 10, 1985, field trapping was carried out 100 km northeast of Saskatoon, Saskatchewan, Canada, in a forest area containing spruce, pine, birch, aspen, and poplar trees and a number of herbaceous shrub species. The sticky wing traps (Phero Tech Inc., Vancouver, B.C.) contained chemical lures impregnated into rubber septa (Arthur H. Thomas Co. Philadelphia, Pennsylvania, No. 1780 J07) and protected from oxidation with 100 μl of a solution of 10% buylated hydroxytoluene in acetone. The traps were hung on tree branches 1–2 m from the ground and spaced at least 10 m apart. Inspections of traps and recording of captures were done weekly, and lures were replaced on June 4 and July 12.

The survey consisted of 96 nonreplicated traps containing each of the 10,12-hexadecadienyls as a single component at 500 μg dose and two component lures formulated by adding an additional 50 μg of a compound which differed from the primary component by a single change in functional group or isomeric geometry; also, 500-μg doses of single-component 10-hexadecenyl and 12-hexadecenyl compounds were surveyed.

Light trapping of moths was done using 8-W portable UV light traps (Bioquip Products, Santa Monica, California) in the area of the survey and in an area 10 km south of Saskatoon.

EAG responses of moths to synthetic chemicals were recorded following the method of Chisholm et al. (1975) using light-trapped males of unknown age which were maintained in the laboratory at 5°C and used within 36 hr of capture. EAG data presented are from single male moths which gave reproducible responses. Although the intensity of the absolute responses may have differed from those of other males of the same species, the relative responses were very consistent. EAD responses (Arn et al., 1975) of male antennae to synthetic standards and female extracts were done with light-trapped or hand-caught males and females. The majority of light-trapped females had mated, and extracts showed no EAD activity. Female abdominal tips were excised and extracted with methylene chloride for 0.5 hr. Extracts were concentrated and separated chromatographically using a modified gas chromatograph (Hewlett Packard

5710A) with a 0.32-mm × 30-m DB-5 fused silica capillary column. The effluent was split in a 7:3 ratio to an FID and EAD. Injections of ~1 female equivalent (FE) of extracts or 2- to 5-ng quantities of standards were injected splitless at 40°C for 30 sec and the oven heated to 90°C and temperature programmed 4°/min to 230°C. Good separations of the geometrical isomers of the 10,12-hexadecadienyls were achieved with typical separations being greater than 10 sec and resolutions of better than 1.0.

Replicated experiments were set out when possible to verify captures in the unreplicated survey.

Summed trap captures from randomized, three-times replicated, field tests were transformed $\sqrt{X + 0.5}$, where X is the number of male moths captured in the trap, and then submitted to an analysis of variance, and significantly different means separated by Duncan's multiple-range test.

RESULTS AND DISCUSSION

The 1985 survey attracted five sphingid species: *Smerinthus jamaicensis* (Drury), *Smerinthus cerisyi* Kirby, *Pachysphinx modesta* (Harris), *Hemaris diffinis* (Boisduval), and *Proserpinus flavofasciata* (Walker) (Tables 1 and 2) as well as at least eight other lepidopteran species (unpublished data). Although the survey traps were not replicated, male captures were replicated over time, and attractive lure components were clearly evident for a number of sphingids. The best example is that of *S. cerisyi*, where all baits containing *Z*10,*E*12-16:Ald at the 500-μg dose (Table 2) except where *Z*10,*E*12-16:Ac or *E*10,*E*12-*Z*10,*E*12-16:Ac (500 μg) + *Z*10,*E*12-16:Ald (50 μg) caught more than six times as many moths as any other trap. In a replicated test (Table 3), this two-component mixture at 550 μg was a significantly better attractant than the mixture at 110 μg or the acetate alone at 500 μg. *Z*10,*E*12-16:Ald alone or in combination with any other hexadecadienyl compound as well as the corresponding hexadecenyl compounds failed to attract *S. cerisyi*. EAGs done on male *S. cerisyi* (Figure 1) showed strong responses to its attractant components.

Smerinthus jamaicensis males were caught in traps containing *Z*10,*E*12-16:Ald at the 500-μg dose (Table 2) except where *Z*10,*E*12-16:Ac or *E*10,*E*12-16:Ald was a second component. EAG data (Figure 1) for *S. jamaicensis* showed a strong response to its attractant, *Z*10,*E*12-16:Ald, as well as relatively large responses to *E*10,*E*12-16:Ald, and to *Z*10,*E*12-16:Ac, which suggests they may have inhibited trap capture in the survey. Corresponding hexadecenyl compounds were not attractive in the field, and EAG analysis showed only weak responses.

Pachysphinx modesta males were captured along with *S. cerisyi* in traps containing *Z*10,*E*12-16:Ac at 500 μg except in traps where *Z*10,*E*12-16:Ald was a second component (Tables 2 and 3). EAG data for *P. modesta* (Figure

TABLE 1. SUMMARY OF SPHINGIDS ATTRACTED TO FIELD TRAPS DURING 1985

Sphingidae		Attractant (μg)	Total number trapped	Trapping period
Subfamily: Sphinginae				
Tribe: Smerinthini	*Smerinthus jamaicensis*	*Z*10, *E*12–16 : Ald (500)	37	May 23–July 23
	Smerinthus cerisyi	*Z*10, *E*12–16 : Ac (500) +*Z*10, *E*12–16 : Ald (50)	258	May 17–July 29
	Pachysphinx modesta	*Z*10, *E*12–16 : Ac (500)	42	June 10–Sept. 10
Subfamily: Macroglassinae				
Tribe: Dilophontini	*Hemaris diffinis*	*Z*10, *E*12–16 : Ald (500)	12	June 10–July 2
Tribe: Macroglossini	*Proserpinus flavofasciata*	*E*10, *E*12–16 : Ald (500)	5	June 4

TABLE 2. CAPTURES OF MALE MOTHS IN SURVEY TRAPS (NONREPLICATED, MAY 10 to SEPTEMBER 10, 1985)

Lure composition (μg)	*P. modesta*	*S. jamaicensis*	*S. cerisyi*	*H. diffinis*
*E*10,*E*12-16:Ac (500)	0	0	0	0
*E*10,*Z*12-16:Ac (500)	1	0	0	0
*E*10,*Z*12-16:Ac (500) + *Z*10,*E*12-16:Ac (50)	1	0	0	0
*Z*10,*E*12-16:Ac (500)	2	0	10	0
*Z*10,*E*12-16:Ac (500) + *E*10,*E*12-16:Ac (50)	12	0	8	0
*Z*10,*E*12-16:Ac (500) + *E*10,*Z*12-16:Ac (50)	10	0	8	0
*Z*10,*E*12-16:Ac (500) + *Z*10,*Z*12-16:Ac (50)	3	0	1	0
*Z*10,*E*12-16:Ac (500) + *Z*10,*E*12-16:Ald (50)	0	0	62	2
*Z*10,*E*12-16:Ac (500) + *Z*10,*E*12-16:OH (50)	4	0	8	0
*Z*10,*Z*12-16:Ac (500)	0	0	0	0
*Z*10,*E*12-16:Ald (500)	0	6	0	0
*Z*10,*E*12-16:Ald (500) + *Z*10,*E*12-16:Ac (50)	0	0	0	2
*Z*10,*E*12-16:Ald (500) + *E*10,*E*12-16:Ald (50)	0	0	0	0
*Z*10,*E*12-16:Ald (500) + *E*10,*Z*12-16:Ald (50)	0	4	0	0
*Z*10,*E*12-16:Ald (500) + *Z*10,*Z*12-16:Ald (50)	0	12	0	2
*Z*10,*E*12-16:Ald (500) + *Z*10,*E*12-16:OH (50)	0	3	0	0
*Z*10,*E*12-16:OH (500)	0	0	0	1
*Z*10,*E*12-16:OH (500) + *Z*10,*E*12-16:Ald (50)	0	0	0	2

TABLE 3. CAPTURES OF *S. cerisyi* AND *P. modesta* IN BAITED TRAPS CONTAINING *Z*10,*E*12-HEXADECADIENYL ACETATE AND ALDEHYDE[a]

Lure composition (μg)	Mean No. males captured/trap (±SD)	
	S. cerisyi	*P. modesta*
*Z*10,*E*12-16:Ac (500)	5.0 (±1.7)*b*	3.3 (±1.1)*c*
*Z*10,*E*12-16:Ac (100) + *Z*10,*E*12-16:Ald (10)	5.7 (±5.0)*b*	0*d*
*Z*10,*E*12-16:Ac (500) + *Z*10,*E*12-16:Ald (50)	23.7 (±8.5)*a*	0*d*

[a]Three replications May 23–July 15, 1985. Means followed by the same letter are not different ($P = 0.05$).

1) shows strong responses for its attractant *Z*10,*E*12-16:Ac and also *E*10,*Z*12-16:Ac and *Z*10,*E*12-16:Ald. The presence of this aldehyde in *S. cerisyi* female extracts (see below) may indicate that, as well as being an attractant component of *S. cerisyi*, it serves to inhibit the attraction of *P. modesta* and thereby aids in species isolation.

From field observations it was noticed that the day flyer *Hemaris diffinis* was readily attracted to lures containing *Z*10,*E*12-16:Ald but few of these agile flyers landed in traps. Specimens for EAG analysis were literally "hand caught" when males were attracted to contaminated hands after handling lures containing 10,12-hexadecadienals. The EAG profile shows strong responses to all the hexadecadienals (Figure 2). Again, the corresponding hexadecenyl aldehydes were not attractive in the field nor were they EAG active.

Proserpinus flavofasciata was similar to *H. diffinis* in that very few of this agile, bee mimic were captured (five males) but all were taken in traps containing *Z*10,*E*12-16:Ald as a major component. No suitable specimens were obtained for EAG or EAD analysis.

A few specimens of each of the two *Paonias* species were light trapped, and EAG profiles showed strong responses for only the 10,12-hexadecadienyl acetates (Figure 2). One *Paonias exceactus* was captured in a trap containing *E*10,*Z*12-16:Ac (500 μg) + *E*10,*Z*12-16:Ald (50 μg).

Data were obtained supporting the occurrence of 10,12-hexadecadienyl acetates and aldehydes in female abdominal tip extracts of *S. cerisyi*, *Sphinx drupiferarum* (J.E. Smith), and *Hyles gallii* (Rottenburg).

Female abdominal tip extracts were obtained from light-trapped *S. cerisyi*, and GC-EAD analysis with conspecific male antennae to 1 FE showed responses corresponding to retention times of *Z*10,*E*12-16:Ac (140 μV signal) and *Z*10,*E*12-16:Ald (50 μV signal) (Figure 3). This evidence, along with trapping and EAG data, suggests strongly that these are actual pheromone components.

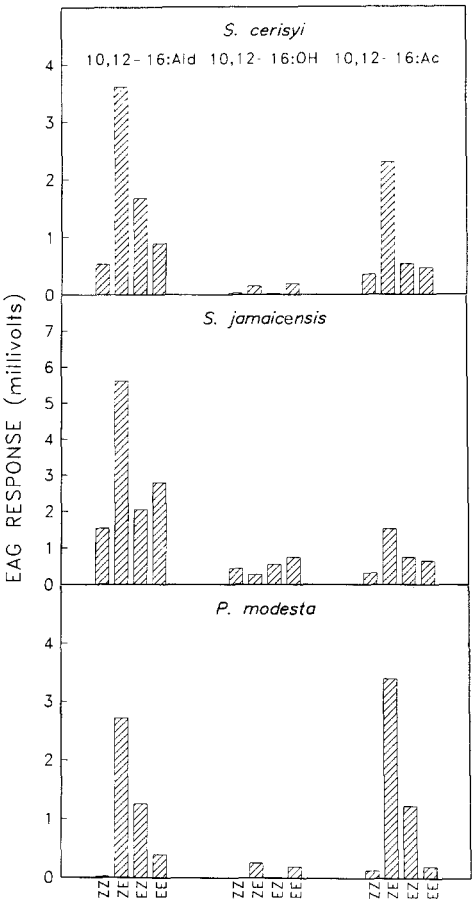

FIG. 1. EAG responses of males to synthetic 10,12-hexadecadienyls at 1-μg doses for *S. cerisyi* and *S. jamaicensis* and 10-μg doses for *P. modesta*.

Light-trapped *S. drupiferarum* females were collected and abdominal tip extracts were prepared and analyzed by GC-EAD using the antennae from light-trapped *S. vashti*. Analysis showed responses corresponding to the retention times of *Z*10,*E*12–16:Ald (80 μV signal), *E*10,*Z*12–16:Ald (430 μV signal), and *E*10,*E*12–16:Ald (80 μV signal) (Figure 3). GC-MS analysis using the MID method for *S. drupiferarum* tip extracts showed a peak at the same retention time of synthetic *E*10,*Z*12–16:Ald with characteristic ions: $(M + 29)^+$, *m/z* 265; $(M + 1)^+$, *m/z* 237; $(M - 1)^+$, *m/z* 235; and $[(M + 1) - 18]^+$, *m/z* 219 in the same relative ratios as synthetic *E*10,*Z*12–16:Ald.

Both MS and GC data showed the *E*10,*Z*12–16:Ald present in amounts greater than 30 ng/female. The *ZE* and *EE* may be present in picogram quan-

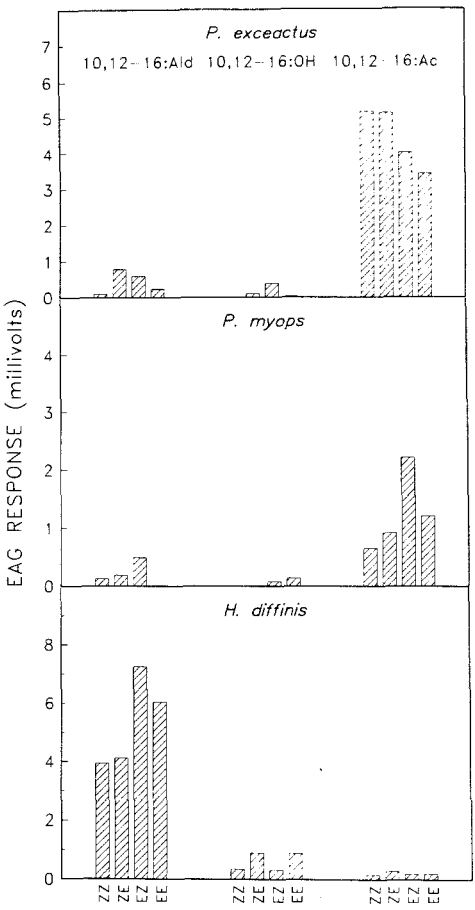

FIG. 2. EAG responses of males to synthetic 10,12-hexadecadienyls at 10-μg doses.

tities, but they were not verified by MS analysis. No *S. drupiferarum* were captured in baited traps, but the EAD shows the presence of at least one and possibly three 10,12-hexadecadienals in the extract and a multicomponent mixture may be necessary for attraction. *Hyles gallii* was not found in the trapping survey area (light trap or survey traps), but males and females were obtained from the light trap south of Saskatoon (no survey traps). Female abdominal tip extracts showed EAD responses corresponding to the retention time and presence of *E*10,*E*12–16: Ald (130 μV signal) when using a male *H. gallii* antenna. *Hyles euphorbiae* male antennae (obtained from a lab culture) responded to female *H. gallii* extracts, indicating the presence of *E*10,*Z*12–16: Ald (50 μV signal). GC-EAD analysis of *H. gallii* to standard solutions of hexadecadienyls showed strong responses to the *EE* and *EZ* aldehydes, with *EE* being the strongest.

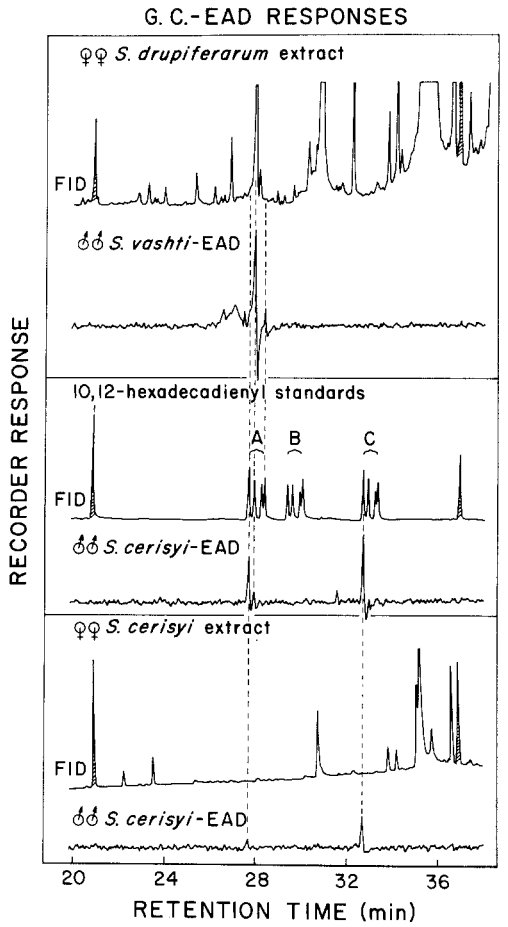

FIG. 3. Representative EAD and associated FID responses of male sphingids to female extracts (FID 0.7 FE : EAD 0.3 FE) and synthetic standards (FID 2.8 ng : EAD 1.2 ng). The elution order of the 10,12-hexadecadienyl isomers was *ZE*, *EZ*, *ZZ*, and *EE* for the aldehydes (A), alcohols (B), and acetates (C).

On several occasions the presence of only scales in the sticky traps indicated a lack of moth retention, particularly for the larger moth species. When the large *P. modesta* was captured in the sticky traps, the traps became saturated when they contained only three or four moths, and results may have been more conclusive if a retentive, nonsaturating trap had been used.

A comparison was made of captures of *S. cerisyi* and *S. jamaicensis* in sticky wing traps and large (9-liter) double inverted cone-orifice traps with varying orifice diameters (Steck and Bailey, 1978). An opening of 2 cm was necessary to allow the *Smerinthus* species to enter but these were found to be no

better than the sticky wing traps. None of the other sphingids attracted to wing traps could be trapped using the inverted cone-orifice trap.

A different problem arose with the more agile flyers such as *Hemaris diffinis*, *Proserpinus flavofasciata*, and possibly the *Sphinx* and *Hyles* species. These hovering, nectar feeders very rarely landed in the sticky traps even though some were obviously attracted to the lures.

In North America there are two subfamilies of Sphingidae, containing five tribes. Our data show attraction to and/or the presence of 10,12-hexadecadienyl acetates or aldehydes in species representing four tribes. A chemotaxonomic approach using hexadecadienyl compounds could be used for any other sphingid species whose pheromones need to be identified.

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