

account for the evolution of such a system, I would suggest a 'common skill' hypothesis: If a behavioral capacity can be used in a variety of motivational contexts that are sometimes concurrent, there will be selective pressures for the development of a system to order these contexts. In the present case, object retrieval was the common skill (or a common skill) that served as a foundation for the evolution of a generalized value system. This system allows these, and perhaps other rodent species<sup>5-13</sup>, to choose between objects that are related to different motivational systems, but that can be treated in a similar manner with respect to transportation. Object retrieval may therefore stand somewhere between response systems dedicated to a single goal, that are often designated as species-typical behavior, and those that are largely instrumental in character. The latter by implication may have arisen in the same fashion as retrieval.

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## Sex pheromone blend of the codling moth, *Cydia pomonella*: Evidence for a behavioral role of dodecan-1-ol<sup>1</sup>

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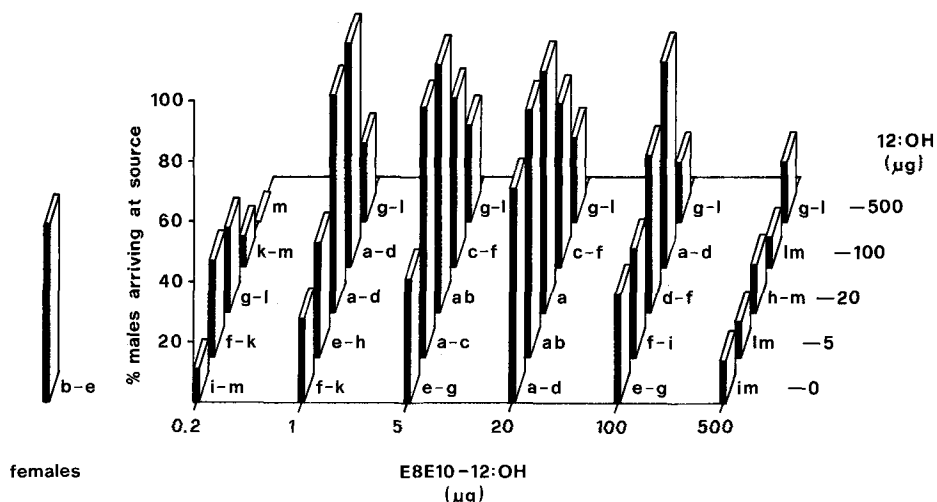
**Summary.** Pheromone glands and effluvia of the codling moth female contain *E*-8, *E*-10-dodecadien-1-ol as main component, accompanied by its geometric isomers, the corresponding acetate and aldehyde (both in gland extracts only), *E*-9-dodecen-1-ol and saturated alcohols of 10 to 18 carbons. Dodecan-1-ol as the most abundant secondary component (about 30% of the female blend) acts in the wind tunnel by widening the dose range over which codling moth males are optimally attracted to *E*-8, *E*-10-dodecadien-1-ol.

**Key words.** Codling moth; *Cydia pomonella*; sex pheromone; male attraction; *E*-8, *E*-10-dodecadien-1-ol; dodecan-1-ol; synergism; wind tunnel; chemical analysis.

*Cydia pomonella* L. (Lepidoptera: Tortricidae, Olethreutini) is a worldwide pest on apple, peach and walnut<sup>2</sup>. *E*-8, *E*-10-Dodecadien-1-ol (*E*8*E*10-12:OH; other short forms see table) was established as a male attractant for this species, based on electroantennographic (EAG) data and synthesis<sup>3</sup>, and subsequently identified in female extracts<sup>4,5</sup>. Although this compound is extremely effective, behavioral observations suggest that other female products are involved in courtship of *C. pomonella*<sup>6,7</sup>. Additional components related to *E*8*E*10-12:OH were identified in female glands<sup>7,8</sup>; however, evidence for the behavioral role of any of these has been lacking, with the exception of an inhibitory effect observed for *E*8*E*10-12:Ac<sup>9,10</sup>. Here we present results of analysis of *C. pomonella* female extract and effluvia, and first evidence for a role of 12:OH in the sexual activity of males.

**Materials and methods.** Insects were reared on an artificial diet<sup>11</sup>. Extraction of sex glands of 3-day-old virgin females (made in the early scotophase), and analyses by gas chromatography-mass spectrometry (GC-MS) and GC with EAG detection (GC-EAD) on Silar 10c and SP-1000 were done as described for *Adoxophyes orana* F.v.R.<sup>12</sup>. For determination of double bond positions in monoenic alcohols, the extracts were reacted with trifluoroacetic anhydride and then with dimethyl disulfide (DMDS)<sup>13,14</sup>. Preliminary experiments aimed at collection of effluvia indicated that recovery from charcoal (Grob filters, Brechbühler AG, Schlieren, Switzerland) of *E*8*E*10-12:OH and aldehydes was very poor (circa 10%) in contrast to saturated and monounsaturated hydrocarbons, alcohols and acetates (circa 60%). On the other hand, nearly 50% of *E*8*E*10-12:OH released from a cover

Response of codling moth males in the wind tunnel to live females and various dosages of *E*-8, *E*-10-dodecadien-1-ol and dodecan-1-ol applied to a rubber cap. Values followed by the same letter are not significantly different as indicated by ANOVA and Duncan's multiple range test ( $P = 0.05$ ).



slip in a 20 ml glass bulb with an air flow of 10 ml/min for 20 min was recovered from the walls and almost nothing from a glass tube placed at the exit. Glass was thus chosen for collection of female volatiles.

A 0.5 l Pyrex bottle was washed with RBS detergent as prescribed (Fluka AG, Buchs, Switzerland), rinsed with tap water and dried at 200°C for 1 h. Forty 4-day-old females held in the wind tunnel room were placed in the bottle 5 h before scotophase. Calling behavior commenced circa 0.5 h from lights out and continued for the rest of the dark period. A metal bellows regulator set the air flow at 20 ml/min. When calling terminated, the females were removed and the interior of the bottle washed down with 3 × 250 µl hexane (Merck, for residue analysis). The eluate was filtered and concentrated to 75 µl at room temperature.

Wind tunnel tests were carried out as previously described for *Eupoecilia ambiguella* Hb.<sup>15</sup> Conditions were 24°C, 65% relative humidity and 3000–6000 lux during the photophase and 20°C, 75% relative humidity and 15–20 lux during the scotophase. Tests were performed with 2–3-day-old males from 0.5 to 2.5 h after lights out during which time activity was optimal. Individuals (50 per data point, 70–160 with *E8E10-12:OH* alone) were released and activation, takeoff, upwind flight and landing at the source recorded.

**Results.** GC-MS analysis of 50 female equivalents (FE) confirmed the presence of circa 2 ng *E8E10-12:OH* per sex gland, accompanied by smaller amounts of related products (table). The *E, Z* isomer of the main component was present at 5%. The proportion of *Z, E* detected with the antenna of *Pammene rheadella* Cl.<sup>16</sup> was at least 0.5% though in some chromatograms of effluvia and fresh extracts the peak was higher. Female effluvium of one calling period contained twice the amount of *E8E10-12:OH* found in the sex gland, indicating that pheromone biosynthesis takes place during calling.

Small amounts of the acetate and aldehyde derivatives of the main attractant were present in sex gland extracts. *E8E10-12:Al* was found in trace amounts (0.25%) during GC-MS analysis of pure *E8E10-12:OH*, probably formed in the injector; however, the proportion in the sex gland extracts was 4 times higher. *E8E10-12:Ac* was detected in several extracts by GC-EAD with *Hedya nubilifera* Hw. male antennae<sup>8</sup> at circa 10 pg/FE.

Presence of *E9-12:OH* was established at about 10% of *E8E10-12:OH* in both sex glands and effluvia. Its structure was confirmed by GC-EAD with a *Sparganothis pilleriana* D. & S. antenna<sup>8</sup> and, after trifluoroacetylation and reaction with DMDS, by GC-MS of the thioglycol ether ( $M^+$ ,  $m/z$  374;  $A^+$ ,  $m/z$  89;  $B^+$ ,  $m/z$  285). Evidence for the presence of a second compound with MS characteristics of a dodecenol and the retention time of the *E-8* isomer was found in the effluvial analyses at a level of less

than 10% of *E9-12:OH*. Saturated alcohols of 10–18 carbons were detected in both sex glands and effluvia whereas octadecyl and eicosyl acetates, n-alkanes from 21 to 29 carbons and palmitic acid were present in sex gland extracts only.

Wind tunnel tests initiated to examine the effects of the secondary sex pheromone components on male behavior indicate that 12:OH, the second most important component in both the gland and effluvium, acts as a synergist (fig.). With *E8E10-12:OH* alone, best attraction in terms of males arriving at the source is obtained at 20 µg, doses 4–5 times higher and lower being only half as attractive. While the saturated alcohol has no effect at the optimum dose of *E8E10-12:OH*, it widens the response window: in the presence of 20–100 µg 12:OH optimal attraction is achieved over the range of 1–100 µg *E8E10-12:OH*. This effect of 12:OH can already be observed at the onset of upwind flight but not for excitation or takeoff, indicating that this compound aids orientation. Attraction obtained with the optimal blends (68–82% males reaching the source) is as good as or better than the response to females (59%).

Despite these findings, systematic field tests have so far failed to reveal an involvement of 12:OH or any of the other secondary components in attraction of codling moth males to *E8E10-12:OH*. A reason for this may have been the high variability in moth catch encountered even with *E8E10-12:OH* alone.

**Discussion.** The multicomponent blend produced by *C. pomonella* females follows a pattern found in many Lepidoptera. With the exception of *Z8E10-12:OH* (detected by GC-EAD only), 10:OH, 18:OH, 18:Ac and 20:Ac, the products reported here have been identified in *C. pomonella* in an independent study<sup>7</sup>. Most secondary components show structural similarities to the

Compounds identified in *Cydia pomonella* sex pheromone analysis

Component	Short form	nanogram per female sex gland	effluvium
Decan-1-ol	10:OH	0.005	0.15
Dodecan-1-ol	12:OH	1	3.5
<i>E-9-Dodecen-1-ol</i>	<i>E9-12:OH</i>	0.2	0.8
<i>E-8, E-10-Dodecadienal</i>	<i>E8E10-12:Al</i>	0.02	n.d. <sup>a</sup>
<i>E-8, E-10-Dodecadienyl acetate</i>	<i>E8E10-12:Ac</i>	0.005 <sup>b</sup>	n.d.
<i>E-8, E-10-Dodecadien-1-ol</i>	<i>E8E10-12:OH</i>	2.1	5.4
<i>Z-8, E-10-Dodecadien-1-ol</i>	<i>Z8E10-12:OH</i>	0.01 <sup>b</sup>	present
<i>E-8, Z-10-Dodecadien-1-ol</i>	<i>E8Z10-12:OH</i>	0.08	0.3
Tetradecan-1-ol	14:OH	0.2	0.5
Hexadecan-1-ol	16:OH	0.04	1
Octadecan-1-ol	18:OH	0.08	1.2
Octadecyl acetate	18:Ac	present	n.d.
Eicosyl acetate	20:Ac	present	n.d.

<sup>a</sup> not detected by GC-MS; <sup>b</sup> detected by GC-EAD only.

established pheromone *E8E10-12:OH*. The occurrence of *E9-12:OH* in the blend seems at first unusual. A compound of matching retention range and amount was found in earlier analyses<sup>5</sup>. Biosynthesis of lepidopteran pheromones has been studied quite extensively, but no evidence for the pathways leading to the *E-8,E-10* double bond system found in the codling moth has been presented. *E9-12:OH* could be the key compound for this pathway; in an analogous case, the 10,12-dienic system of bombykol was suggested to result from dehydrogenation of a *Δ*-11 monoene<sup>17</sup>.

In our wind tunnel tests, 12:OH restores biological activity of overdoses of *E8E10-12:OH*, as observed for dodecyl acetate in *E. ambigua*<sup>15</sup>. At low attractant doses it enhances biological activity, shifting the response curve by a factor of 4–20. The saturated alcohol could thus account for the 10-fold difference in attractiveness observed between *E8E10-12:OH* and an equivalent amount of female extract<sup>7</sup>, but not for the factor of 1000 obtained when observing male excitation in another study<sup>6</sup>. This may indicate a role for some of the other female components.

- 1 This research was supported by the Swiss National Science Foundation. We thank W. Riggenschach for supplying moths and T. Wildbolz, P. Charmillot and M. Tóth for conducting field trials.
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## The identification of spiroacetals in the volatile secretions of two species of fruit fly (*Dacus dorsalis*, *Dacus cucurbitae*)

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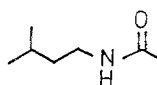
**Summary.** Aeration extracts from female *Dacus cucurbitae* and female *Dacus dorsalis* have been shown to contain a variety of 2,8-dialkyl-1,7-dioxaspiro [5.5] undecanes together with N-3-methylbutylacetamide.

**Key words.** Fruit fly; *Dacus dorsalis*; 2,8-dialkyl-1,7-dioxaspiro [5.5] undecanes; N-3-methylbutylacetamide.

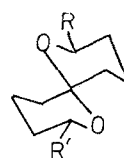
Both the melon fly, *Dacus cucurbitae* Coquillett, and the oriental fruit fly, *Dacus dorsalis* Hendel, are distributed throughout East Africa, India, Southeast Asia and Hawaii<sup>1</sup>, and are serious pests of melon and other Cucurbitaceae. Control is mainly by the use of insecticides. A synthetic attractant for male *D. cucurbitae*, 4-(p-acetoxyphenyl)-2-butanone (cuelure) is used for monitoring populations<sup>2</sup>, while methyleugenol is used as a synthetic attractant for *D. dorsalis*<sup>3</sup>. Previous work from this laboratory<sup>4</sup> has shown that the rectal gland secretion of male *D. cucurbitae* contains 2-methoxy-N-3-methylbutyl acetamide together with two other amides, three pyrazine derivatives and 2-ethoxybenzoic acid. This work has now been extended to investigate the volatile secretions produced by adult female flies, *D. cucurbitae* and *D. dorsalis*.

*D. dorsalis* and *D. cucurbitae* were taken from a laboratory culture originally obtained from USDA, Honolulu. The sexes were segregated within 1 day of emergence and kept at 25 ± 1°C, 70 ± 5% relative humidity and a light intensity of circa 1000 lx maintained on a 13 h light: 11 h dark cycle. The volatile secretions produced by sexually mature (10-day-old) female fruit flies (50) were obtained by passing filtered air (25 ml min<sup>-1</sup>) through a glass chamber containing live female flies, and absorbing the volatile compounds on an activated charcoal filter<sup>5</sup> (15 cm × 1 cm). After seven days the emitted volatiles were extracted from the charcoal filter by elution with dichloromethane (10 ml). The aeration extract was concentrated (100 µl) by distillation at atmospheric pressure and analyzed by gas chromatography (GC)

and/or gas chromatography-mass spectrometry (GCMS). Typically, the extract was analyzed by GC (5% Carbowax 20M on 100–120 Diatomite AAW-DMCS, 5% OV101 on Chromosorb W-HP) and subsequently by GCMS (Kratos MS 30, EI 70 ev). The major component of the aeration extract from both species of fruit flies was N-3-methylbutyl acetamide (**1**) (1 µg/insect per day). The mass spectrum<sup>6</sup> and gas chromatographic properties were found to be identical to an authentic sample, prepared by unambiguous synthesis. The aeration extract from female *D. cucurbitae* gave two further major components which were identified as 2,8-dimethyl-1,7-dioxaspiro [5.5] undecanes on the basis of their mass spectra. Particularly characteristic were the fragment ions at m/z 112, 115<sup>7</sup>. (E,E)-2,8-Dimethyl-1,7-dioxaspiro [5.5] undecane (**2**) and (Z,E)-2,8-dimethyl-1,7-dioxaspiro [5.5] undecane (**3**) were synthesized by the method of Francke et al.<sup>8</sup>



(1)



(2) R=R'=CH<sub>3</sub>  
(4) R=CH<sub>3</sub>CH<sub>2</sub>, R'=CH<sub>3</sub>  
(5) R=CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>, R'=CH<sub>3</sub>



(3) R=R'=CH<sub>3</sub>