A Sex Attractant for the Green Budworm Moth, *Hedya nubiferana*

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The two major components of the female pheromone blend of *Hedya nubiferana* have been identified as (*E*, *E*)-8,10-dodecadienyl acetate and (*Z*)-8-dodecenyl acetate. Each compound acts on a specialist receptor type in olfactory hair sensilla of the male antenna. In field screening tests, combinations of the two compounds and dodecanyl acetate were highly attractive to *H. nubiferana* males. The (*Z*, *E*)-8,10 isomer attracted males of *Hedya ochroleucana*.

The green budworm moth, *Hedya nubiferana* Haw. (Tortricidae: Olethreutinae) is a member of the leaf roller fauna of apple orchards in Europe [1] and North America [2, 3] where it had been introduced prior to 1913. The univoltine species [1, 3] overwinters as a 3rd instar larva which in spring feeds on the floral parts of a bud. Obviously, a heavy population would have to exist to affect any significant reduction in the bloom and, thereby, in the crop; nevertheless, populations of this size have sometimes been detected in neglected orchards [4, 5].

During field work with synthetic pheromone of the codling moth (*Laspeyresia pomonella* L.), (*E*, *E*)-8,10-dodecadien-1-ol = Codlemone [6], several investigators have observed [7 – 11] attraction of *H. nubiferana* males to this chemical. In these field tests it has further been noted [7] that addition of (*Z*)-8-dodecen-1-ol or of any of (*Z*)-7-, (*Z*)-8-, or (*Z*)-9-dodecenyl acetate to Codlemone abolished the attraction of *H. nubiferana* males, which also were never lured to traps baited with live codling moth females [7]. In field studies in the USA and the Netherlands, males of *Hedya chionosema* Zell. (twin-spotted budworm) were attracted by a combination of (*E*)-8-dodecen-1-ol with the corresponding acetate [12], and *Hedya atropunctana* Zett. by (*Z*)-10-dodecenyl acetate [13]. So far, the composition of the female pheromone has not been published for any species of this genus.

Here we report the partial identification of the female pheromone blend of *H. nubiferana* and the development of a species-specific sex attractant based on its two major components.

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Electrophysiological studies

Electroantennogram (EAG) responses were recorded from *H. nubiferana* male antennae by standard techniques [14, 15] in 1975 – 78. Test compounds included various di-unsaturated analogues in addition to the usual series of mono-unsaturated C_{10} to C_{16} acetates, alcohols, and aldehydes.

In the series of monoenes, quantitative "screening" [14] ended up with (*Z*)-8-dodecenyl acetate (*Z*8-12:Ac) as the most stimulatory structure, followed by the (*E*)-8 and the (*E*)-10 isomer; whereas (*E*, *E*)-8,10-dodecadienyl acetate (*E*8, *E*10–12:Ac) followed by the (*Z*, *E*)-8,10 isomer were the most effective dienes. The two candidate structures, *Z*8–12:Ac and *E*8, *E*10–12:Ac, at the same stimulus amounts elicited about the same EAG response amplitude in the non-adapted male antenna. The corresponding alcohols, *Z*8–12:OH and *E*8, *E*10–12:OH (codlemone), were almost 100 fold less effective.

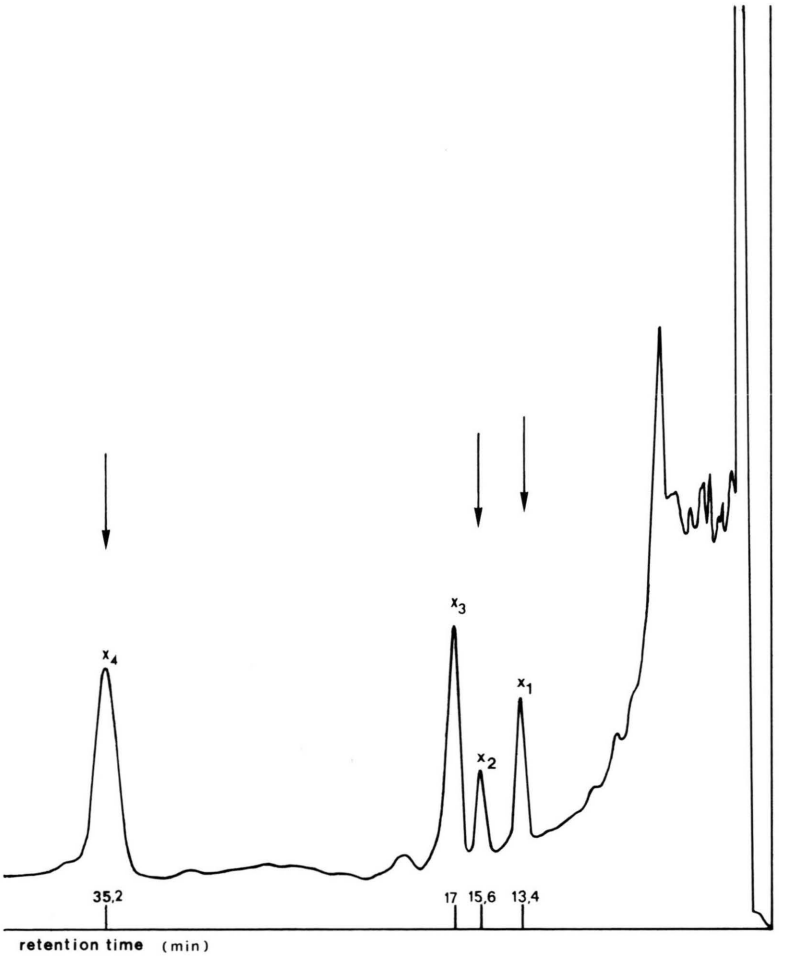

Fig. 1. Gas chromatographic trace (Perkin-Elmer 3920 with solid sampler MS-41, column 3 m inox 1/8″, ECNSS-M, GCQ, 130 °C, gas vector M_2 10 m/min) of the sex pheromone glands of 15 virgin females of *Hedya nubiferana*, indicating 4 components (x_1 to x_4) at 13,4 min, 15,6 min, 17 min, and 35,2 min. Arrows denote retention times for dodecyl acetate (13.2 min), (*E*)-8-dodecenyl acetate (15.8 min), and (*E*,*E*)-8,10-dodecadienyl acetate (35.1 min).

In 1978, in addition to EAGs the nerve impulse responses of single receptor cells of male olfactory hair sensilla (*S. trichodea*) were evaluated using the tip recording technique [16, 17]. In these measurements, one type of receptor cell (type A, largest spike amplitude) responded maximally to *E*8, *E*10−12:Ac and another (type B) to *Z*8−12:Ac. No evidence for receptors specific to further (acetate, alcohol) components was obtained during these studies. Contrasting the EAG spectrum, the A and B cells showed a high degree of response specificity, reciprocal effects of *E*8, *E*10−12:Ac and *Z*8−12:Ac on the other receptor type requiring a > 100 fold increase of stimulus amount. The two response maxima in the *H. nubiferana* male EAG spectrum (see above) can thus be referred to the activities of two prevailing types of receptor cells summing up in the EAG response [18].

Chemical analysis

In 1978, larvae of *H. nubiferana* were collected in the field, reared to the adult stage on an artificial diet, and GC analyses of the female sex pheromone glands were made.

The GC trace obtained from 15 virgin females (4 days old and dissected during the calling period; solid sample injection of total gland effluents) is shown in Fig. 1. It indicates the presence of four components (**1** to **4**) in a 16.3 : 10.1 : 30.0 : 43.6 percent ratio. Components **3** (30.0%) and **4** (43.6%) have tentatively been identified by their retention times on various GC columns and their mass spectra as respectively *Z*8−12 : Ac and *E*8, *E*10−12 : Ac, confirming the electrophysiological results. The rentention time of component **1** corresponded to dodecyl acetate (12 : Ac) (which in the field acts as an attraction

Table I. Chemical composition of formulations **1** to **9** used in the field trapping tests specified in Tables II and III.

Name of compound (and abbreviation used)	Steric purity [%]	Amount (microgram) of compound in formulation no.								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
(*E,E*)-8,10-dodecadienyl acetate (*E*8,*E*10 – 12 : Ac)	>99	480	480	–	–	–	500	480	480	440
(*Z,E*)-8,10-dodecadienyl acetate (*Z*8,*E*10 – 12 : Ac)	96	–	–	1000	500	500	–	–	–	–
(*Z*)-8-dodecenyl acetate (*Z*8 – 12 : Ac)	97	320	320	–	500	500	–	320	–	300
(*E*)-8-dodecenyl acetate (*E*8 – 12 : Ac)	>99	–	–	–	–	–	–	–	–	100
(*E*)-10-dodecenyl acetate (*E*10 – 12 : Ac)	>99	–	–	–	–	–	–	–	320	–
dodecyl acetate (12 : Ac)	>99	1000	1000	–	–	1000	–	–	1000	160
(*E*)-8-dodecen-1-ol (*E*8 – 12 : OH)	>99	–	10	–	–	–	–	–	–	–

Table II. Numbers of males of 11 Tortricidae spp. (O, Olethreutinae; T, Tortricinae) attracted to synthetic formulations **1** to **9** (specified in Table 1) in an apple orchard at St. Rémy l'Honoré, June 2 to July 21, 1978.

	1	**2**	**3**	**4**	**5**	**6** *	**7** *	**8** *	**9** **
Hedya nubiferana Haw. (O)	64	61	–	–	2	–	21	–	11
Hedya ochroleucana Frön. (O)	–	–	4	1	–	–	–	–	–
Eucosma cana Haw. (O)	7	2	–	–	4	–	5	–	–
Eucosma cumulana Guin. (O)	–	–	–	–	–	8	–	–	–
Epiblema sp. (O)	–	5	2	–	–	–	–	–	–
Rhyacionia pinicolana Dbld. (O)	–	–	1	4	–	–	–	–	–
Grapholitha funebrana Tr. (O)	–	–	–	1	2	–	–	–	–
Laspeyresia medicaginis Kuzn. (O)	–	–	–	–	–	8	–	–	–
Batodes angustioranus Haw. (T)	–	–	–	–	–	2	–	–	–
Cnephasia pumicana Zell. (T)	–	–	–	–	–	1	–	–	–
Cnephasia alternella Den. (T)	–	–	–	–	–	–	–	1	–

Formulations **6** to **9** were set up respectively: * June 13 or ** July 12.

Table III. Numbers of males of 12 Tortricidae spp. attracted to synthetic formulations **1** to **9** in a deciduous forest at St. Hilarion, June 2 to August 7, 1978.

	1	**2**	**3**	**4**	**5**	**6** *	**7** *	**8** *	**9** **
Hedya nubiferana Haw. (O)	40	62	1	–	–	–	22	–	21
Hedya ochroleucana Frön. (O)	–	–	1	1	–	–	–	–	–
Eucosma cana Haw. (O)	6	3	–	–	–	–	–	–	–
Eucosma cumulana Guin. (O)	–	–	–	–	–	1	–	–	–
Zeiraphera isertana F. (O)	2	–	–	–	–	–	–	–	–
Notocelia rosaecolana Dbld. (O)	–	–	–	–	–	–	1	–	–
Notocelia sp. (O)	–	–	–	–	–	–	5	–	–
Grapholitha funebrana Tr. (O)	–	1	–	–	–	–	–	–	–
Laspeyresia adenocarpi Rag. (O)	–	1	–	–	–	–	–	–	–
Batodes angustioranus Haw. (T)	–	–	–	–	–	1	–	–	–
Clepsis unifasciana Dup. (T)	–	–	–	–	–	–	1	–	–
Tortrix viridana L. (T)	–	1	–	–	–	–	–	–	–

Formulations **6** to **9** were set up respectively: * June 13 or ** July 12.

synergist, as shown below), and of component **2**, to several dodecenyl acetates including *E*8−12 : Ac.

In an independent study of *H. nubiferana* in the New York area, W. L. Roelofs has recently established in female extracts the presence of 12:Ac, *E*8−12:Ac, *Z*8−12:Ac, and *E*8, *E*10−12:Ac in a 9 : 5 : 31 : 55 percent ratio [19].

Compounds **3** and **4** seem to represent the first case of a monoenic and a dienic C_{12} component combined in the pheromone of the same tortricid species. *Z*8−12:Ac is a common pheromone structure known from various Olethreutinae spp.; *E*8, *E*10−12:Ac has been reported as a pheromone component or synthetic attractant, respectively, for three members of this subfamily, *Rhyacionia rigidana* Fern. [20], *Laspeyresia* (*Cydia*) *nigricana* Steph. [21], and *L. medicaginis* Kuzn. [22].

Field attraction tests, 1977

Pilot studies with candidate chemicals were conducted in 1977 in apple orchards in France (Voisins-le-Bretonneux, June 29 to August 8) and Sweden (Solna, July 22 to August 12).

At Voisins-le-Bretonneux, 1000 µg *Z*8−12:Ac (3% *E*), 1000 µg *E*10−12:Ac (> 99% *E*) and a 500 µg + 500 µg combination of the two compounds were tested, each as a single trap, using INRA pheromone traps (Ets Leglise, Bordeaux) with rubber septa dispensers (Ets Leune, Paris). In this test series, the only tortricid attracted was a single male *Notocelia suffusana* Dup. responding to the 1 : 1 mixture. However, the trap loaded with 1000 µg *E*10−12:Ac caught approx. 2,000 *Lithocolletis blancardella* F. (Gracillariidae) during the 5 weeks period, confirming earlier reports [23, 24] of strong attractancy of *E*10−12:Ac on this leaf miner species.

At Solna, four traps baited each with 1000 µg *Z*8, *E*10−12:Ac (96% *Z*, *E*) attracted 13 males *Hedya ochroleucana* Fröl., a species feeding on rose and sometimes found in apple orchards. The same four traps caught 4 male *Latronympha strigana* F., 4 male *Eucosma cana* Haw. and 1 male *Spilonota ocellana* F., all Olethreutinae, and also 4 males of an unidentified sp. of Tineidae.

Field attraction tests, 1978

Nine synthetic formulations (Table I), suggested by results of the chemical and electrophysiological analyses, have been tested in parallel at two localities in Northwestern France over an 8 weeks period. Test areas were an apple orchard at St Rémy l'Honoré and a deciduous forest at St Hilarion. In both series each formulation was represented by a single trap of the type as in 1977. Keeping trap distances at 100 m, trap positions were exchanged every week to reduce possible effects of preferential location.

The trapping results are summarized in Tables II and III. As can be seen, males of *H. nubiferana* comprise more than two thirds of the total catch. In both series the largest numbers of this species were attracted to formulations containing *E*8, *E*10−12:Ac plus *Z*8−12:Ac in a percent ratio similar to the female secretion (see above). A synergistic effect of the saturated analogue, dodecyl acetate (12 : Ac), is apparent from the trapping results, whereas addition of small amounts of *E*8−12:Ac (the candidate structure for minor component **2**, see above) or *E*8−12:OH did not seem to increase markedly the catch rate (Tables II and III).

Confirming the results of field tests of 1977, formulations containing *Z*8, *E*10−12:Ac (96% *Z*, *E*) again attracted *Hedya ochroleucana* males (Tables II and III). This chemical is unknown so far as a lepidopteran pheromone and has to our knowledge not previously been employed in field screening programs.

In these two series, 15 additional Tortricidae spp. were attracted in low numbers of 1 to 18 individuals (Tables II and III). The actual pheromone structures have so far been reported for two of these, *Cnephasia pumicana* Zell. [25] and *Grapholitha funebrana* Tr. [26, 27].

Although the composition of the female pheromone blend of *Hedya nubiferana* has not been fully elucidated, proper combination of its two major components, *E*8, *E*10−12:Ac and *Z*8−12:Ac has been shown here to provide an effective and specific attractant which can now be used for monitoring populations of this species in apple orchards.

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