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# ISOLATION, IDENTIFICATION AND SYNTHESIS OF SEX PHEROMONE COMPONENTS OF THE CAROB MOTH, *ECTOMYELOIS CERATONIAE*

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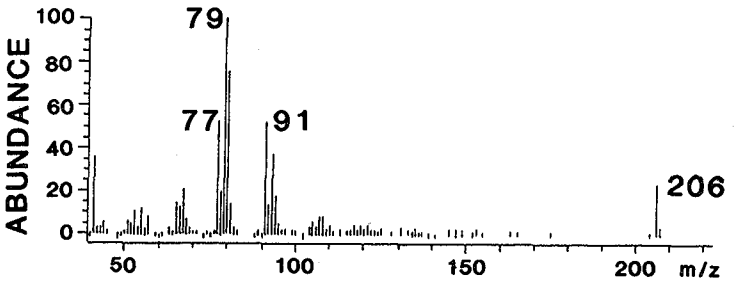
*Summary*: The sex pheromone of females of the carob moth, *Ectomyelois ceratoniae*, was identified to be a mixture of (*Z,E*)-9,11,13-tetradecatrienal, (*Z,E*)-9,11-tetradecadienal and (*Z*)-9-tetradecenal in the ratio of 10:1:1. A synthetic blend proved to be attractive.

The carob moth, *Ectomyelois ceratoniae* (Zeller)(Lepidoptera: Pyralidae), is a widespread pyralid moth pest of nuts and fruits, including carobs, almonds, and dates. A sensitive means of monitoring and detecting new populations, such as is offered by synthetic sex pheromone traps, is urgently needed.

Sex pheromone glands of 2- to 5-day-old virgin female moths were excised during the period of pheromone emission and extracted for 30 min in $CS_2$. Analysis of the concentrated extract by combined GC/EAG[1)], using DB-1 or DB-wax[2)] as the stationary phases, revealed three peaks with strong EAG activity eluting on both columns in the ratio of 1:1:10.

Upon comparison with an authentic sample, GC/MS analysis[3)] proved the first eluting minor EAG-active compounds ($M^+$ = 210) to be (*Z*)-9-tetradecenal. The mass spectrum of the other minor EAG-active peak ($M^+$ = 208) showed a base peak at *m/z* 67 and an abundant molecular ion[4)], which suggested a tetradecadienal with conjugated double bonds[5)]. The major EAG-active compound ($M^+$ = 206) had a mass spectrum, which corresponded to a tetradecatrienal with at least one pair of conjugated double bonds. Since microozonolysis produced 8-formyloctanal, the first double bond was in the nine position, and, excluding allenes and enynes, additional double bonds would have to be in the 11 and 13 positions.

EAG tests with a series of monounsaturated straight-chain acetates and alcohols showing 12 to 16 carbon atoms revealed particularly high activity of the (*Z*)-9- and (*E*)-11-isomers in the 14-carbon series. On the basis of the identification of (*Z*)-tetradecenal it was suggestive that the other two compounds were (*Z,E*)-9,11-tetradecadienal and (*Z,E*)-9,11,13-tetradecatrienal respectively.




Mass spectrum of the major active compound of *E.ceratoniae*, Hewlett Packard 5970

(*Z,E*)-9,11-Tetradecadienal was prepared from commercially available (*Z,E*)-9,11-tetradecadienol (SIGMA) by oxidation with PDC/molecular sieve[6]. Mass spectrum and GC retention times of the synthetic sample were identical to those of the natural product.

The synthesis of (*Z,E*)-9,11,13-tetradecatrienal was carried out as a $C_9$-$C_5$-sequence: 9-Decen-1-ol (ALDRICH) was silylated with tert. butyldimethylsilylchloride[7] (TBDMSCl, ALDRICH)[8], which after ozonolysis yielded the TBDMS derivative of 9-hydroxynonanal (1)[8]. Ethyl-(*E*)-2,4-pentadienoate (ALDRICH) was converted to 1-bromo-(*E*)-2,4-pentadiene (2) through reduction with $LiAlH_4$ and subsequent treatment of the resulting alcohol with triphenylphosphonium dibromide[9]. Refluxing 2 and triphenylphosphane in benzene furnished the respective Wittig salt (3)[10]. Wittig reaction of 1 with 3 followed by cleavage of the silyl ether group with tetrabutyl ammonium fluoride[11], previously neutralized with acetic acid, gave a mixture of geometrical isomers of 9,(*E*)-11,13-tetradecatrienol which could be separated by reversed-phase HPLC[12]. The first eluting compound ($M^+$ = 208) showed the NMR data given below ($J_{H9,10}$ = 11,2 Hz: cis- and $J_{H11,12}$ = 14.8 Hz: trans-) and thus proved to be the (*Z,E*)-isomer; the data are in good accord with respective values for (*Z,E,E*)- and (*Z,E,Z*)-10,12,14-hexadecatrienyl acetate[13]. The alcohol could be oxidized[6] to the very sensitive (*Z,E*)-9,11,13-tetradecatrienal, which was identical to the natural product.

(*Z,E*)-9,11,13-Tetradecatrienol: $^1$H-NMR ($C_6D_6$); chem. shifts/coupling const. Data of the double bond system of the aldehyde are the same.

| C-$H_x$ | 1 | 2-7 | 8 | 9 | 10 | 11 | 12 | 13 | | 14,14' |
|---|---|---|---|---|---|---|---|---|---|---|
| δ | 3.36 | 1.15<br>-1.45 | 2.15 | 5.46 | 6.09 | 6.58 | 6.18 | 6.37 | | 5.00<br>5.13 |
| $H_x/H_y$ | | | 8/9 8'/9 | 9/10 | 10/11 | 11/12 | 12/13 | 13/14 | 13/14' | 14/14' |
| J[Hz] | | | 8,0 8,0 | 11,2 | 11,2 | 14,8 | 10,2 | 10,4 | 16,8 | 1,0 |

Synthetic (*Z*)-9-tetradecenal, (*Z,E*)-9,11-tetradecadienal and (*Z,E*)-9,11,13-tetradecatrienal were EAG-active. Behaviour tests showed high attractivity of the trienal; the other aldehydes, per se behaviourally inactive, show a slightly synergistic effect: in a wind tunnel[14], 0.6 ng of a synthetic blend of the three compounds in naturally occuring proportions reached 80 % of the attractivity of 5 female equivalents.


1) H. Arn, E. Städler, S. Rauscher, Z. Naturforsch. **30c** 722 (1975).
2) GC conditions: 30 m x 0.25 mm i.d. fused silica; inj. temp. 250°C; program: 2 min/80°C then 10°/min to 230°C; FID temp 250°C; $H_2$ carrier gas flow 1,5 ml/min.
3) Hewlett-Packard 5970 coupled with a Hewlett-Packard 5890 gas chromatograph.
4) Mass spectrum: m/z (%) = 67 (100), 41 (55), 95 (42), 82 (40), 81 (36), 55 (36), 68 (30), 79 (28), 93 (14), 53 (14), 96 (13), 77 (13), 208 (13).
5) C. Löfstedt, G. Odham, Biomed. Mass Spec., **11** 106 (1984).
6) J. Herscovici, M. J. Egron, K. Antonakis, J. Chem. Soc., Perkin I 1967 (1982).
7) E.J. Corey, A. Venkatiswarlu, J. Am. Chem. Soc. **94** 6190 (1972).
8) All NMR-spectra were obtained with a Bruker WM 400 at 400 MHz. $^1$H-NMR ($CDCl_3$): δ = 0.05 (s, 6 H); 0.86 (s, 9 H); 1.26-1.4 (m, 8 H); 1.5 (m, 2 H); 1.6 (m, 2 H); 2.4 (m, 2 H); 3.57 (t, 2 H); 9.72 (1 H). B.p. 120°C/0.5 mm.
9) G.A. Wiley, R. Hershkowitz, B.M. Rein, B.G. Chung, J. Am. Chem. Soc. **86** 964 (1964).
10) $^1$H-NMR ($CDCl_3$) δ = 4.90 (m, 2 H); 5.13 (m, 2 H); 5.5 (m, 1 H); 6.18 (m, 1 H); 6.45 (m, 1 H); 7.68 (m, 6 H); 7.75-7.95 (m, 9H). M.p. 207-208°C.
11) E.J. Corey, B.B. Snider, J. Am. Chem. Soc. **94** 2549 (1972).
12) B.p. 108-110°C/0.1 mm. HPLC conditions: RP-18; low pressure gradient; methanol/water.
13) T. Ando, Y. Ogura, M. Koyama, M. Jurane, M. Uchima, K.Y. Seol, Agric. Biol. Chem. **52** 2459 (1988).
14) N = 100; Upwind flight at 23°C; 0.3 lux; wind velocity 50 cm/sec.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# Diet related courtship success in the Oriental fruit moth, Grapholita molesta (Tortricidae)

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Gas chromatographic analysis of hairpencil extracts from male *Grapholita molesta* (Busck) reared on a natural diet of green thinning apples, showed low titres of mellein and methyl jasmonate in newly emerged males. Methyl epi-jasmonate occured in one-day-old, and ethyl *trans*-cinnamate in two-day-old males. Maximal titre was reached for all four components on day two to four after emergence. Males reared on artificial diet as larvae or starved as adults contained lower amounts of the behaviourally most active courtship pheromone component ethyl *trans*-cinnamate, but no difference in mating success between apple-reared and diet-reared males could be demonstrated. Sequestration of ethyl *trans*-cinnamate into the hairpencils increased mating success over control males. A greater proportion of displays made by ethyl *trans*-cinnamate-treated males to calling females resulted in female movement towards the male. Females attracted to ethyl *trans*-cinnamate-treated males moved significantly faster than females attracted to control males.

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## Introduction

The choice of a conspecific male that a female makes in order to mate is one that may influence the fitness of her offspring and, if the selection factor is genetically based, will also affect the reproductive success of her progeny. Fisher (1958) explained the existence of female preference as the outcome of an evolutionary process in which both preference and preferred character evolve together, and O'Donald (1967) and Lande (1981) constructed mathematical models that supported Fishers' findings. Many case studies demonstrate sexual selection through *male competition* and this theory has become widely accepted. However, only a few experiments, such as Andersson's work (1982) on the long-tailed widowbird, have been performed where the theory of sexual selection by *female choice* has been experimentally supported (see Majerus 1986, for review).

In the Oriental fruit moth (OFM), *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), the male is attracted to the female by a sex pheromone released by her at dusk. After location of the female, usually by flight, the male performs a series of courtship behaviours: the male moves past the calling female and, at a distance of a few centimetres, rhythmically extrudes and retracts two sets of hairpencils, which are normally bundled into special pouches on the 7th and 8th abdominal segments (Baker and Cardé 1979). Volatile chemicals present on the hairpencils are propelled towards the female by the male fanning his wings.

The chemicals present on the hairpencils and the wind generated by wing vibration have been shown to be the main factors attracting the female to the male

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(Baker and Cardé 1979). Vision cannot be omitted as a channel of sensory input leading to a female response, but blind females are relatively unhindered in their mating attempts (Baker and Cardé 1979). The female response results in her walking towards the male and touching his abdominal tip with her head. This evokes a copulatory attempt from the male and if successful, the couple link end-to-end.

Without the female signalling her acceptance of the male by moving to and touching the end of his abdomen, coupling, and hence copulation, cannot occur. Thus, the female can select a mate by moving only towards the display that she finds attractive. As many as 79% of wild *G. molesta* females only mate once (Dustan 1964) and so it would seem essential that the female chooses a conspecific male likely to enhance the fitness of her offspring. Males, on the other hand, confined in cages with an excess of virgin females are capable of mating an average of 1.2 times $d^{-1}$ during their first week of adulthood (Dustan 1964) and so their choice of female is less exacting.

Baker et al. (1981) identified the components of the male courtship pheromone as: ethyl *trans*-cinnamate, R-(-)-mellein, methyl jasmonate, and methyl 2-epijasmonate. In behavioural bioassay tests of female attraction from 2 cm away ethyl *trans*-cinnamate evoked the greatest response, whereas the other three compounds evoked only a slight response. However, methyl 2-epijasmonate acted with ethyl *trans*-cinnamate to further elevate the female response to 80% (Nishida et al. 1985).

It has been suggested on a couple of occasions that the larval/adult diet may affect the compounds present in the courtship pheromone (Baker et al. 1981, Nishida et al. 1985) and that this may have repercussions upon the mating success of an individual raised on a particular diet. In the present study we were interested in determining the amount of variation in courtship pheromone titre due to diet, and whether such variation would indeed affect the males' ability to attract females and succeed in mating.

## Methods and materials

*Insects.* Experiments were carried out in three different laboratories; in 1983 in Geneva, NY and in Riverside, CA, and in Lund, Sweden in 1987. In Geneva and Riverside moths were reared on their natural diet consisting of green thinning apples according to procedures described by Baker et al. (1981). Both populations had been maintained in culture since 1975 and originated in Michigan apple orchards. A colony of *G. molesta* was established on apples at Lund University, Sweden in January 1987, from the Riverside culture. In Geneva and Riverside certain generations were raised on a synthetic diet of which the main constituent was lima beans; exact details of the recipe followed are given by Yokoyama et al. (1987).

*G. molesta* pupae reared on a synthetic diet were sent from Riverside, California, to Lund in 1987. The lima bean diet pupae were, on arrival, treated in exactly the same way as the Lund apple-reared pupae.

Adult *G. molesta* were removed from the cages where they emerged just before the start of the dark cycle each day. Adults reared on different diets were kept separately, as were males and females. Each container had a ball of cotton wool soaked in 5% sugar water to provide moisture.

*Individual variation and age dependence of pheromone titre.* In Geneva, male moths were separated from females as pupae and held in a separate room. The excised abdominal tip with the hairpencils and genital claspers from a male of a certain age was soaked at room-temperature for 5–15 min in 7 µl redistilled Skelly B (hexane), with 2 ng tetradecane added as internal standard. Subsequently the solvent was recovered with a 10 µl syringe and the sample analysed at once by gas chromatography (see below).

*Starving of males.* Males were emerged and maintained as adults without access to sugar water. Hairpencil extracts were prepared as above from 3–4 d old males and analysed by GC.

*Effect of G. molesta male larval diet on mating success.* In Riverside calling females reared as larvae on green apples were transferred to a 15×15 cm metal platform, on which they were covered by a Petri dish and placed in the downwind end of a flight tunnel (Kuenen and Baker 1982). A platform with a calling female was placed on an inverted metal table (Baker et al. 1981) and the Petri dish was removed. Then a 2–3 d old male, reared as larva either on apples or synthetic diet, was introduced into the pheromone plume some 3 m downwind from the calling female. The ensuing courtship interaction of males successfully locating the platform with the calling female was videotaped from above by means of a Sony RSC 1050 rotary shutter camera and Sony SLO 340 video cassette recorder. The resulting tapes were later analysed frame-by-frame on a Sony SVM 1010 motion analyser (Baker 1982).

For each male the number of attempts required to attract and mate with a female was counted. "Attempt" is defined here as synonomous with "display" used by Baker and Cardé (1979). A single attempt (at attracting a female) consists of the male turning to face away from the female and extruding and retracting his hairpencils, at least once, usually 3 to 5 times. The distance over which a successful male finally attracted the female was calculated.

*Ethyl* trans-*cinnamate treatment of males.* In Lund a method was developed for providing adult moths with the opportunity to ingest and sequester ethyl *trans*-cinnamate (EC), following the evidence by Nishida et al.

(1985) that adults do take up this compound during breeding. Munktell 9 cm diameter circular filter papers (Stora Kopparberg Filter Products, Sweden) were placed in the lids of Petri dishes and soaked with 1 ml distilled hexane solution of 1 ng $\mu l^{-1}$ ethyl cinnamate. Control filter papers were soaked with 1 ml distilled hexane. The filter papers were left for at least 10 min to allow the hexane to evaporate. Two ml 5% sugar water were then pipetted onto each filter paper. This amount was enough to moisten the filter paper sufficiently to allow uptake of liquid by the adult moths without flooding the floor of the Petri dish. Equal numbers of adult OFM males were introduced onto the control and ethyl *trans*-cinnamate-doctored papers. The Petri dishes were turned so that the lid (with filter paper) was uppermost. Female controls were also performed. Female moths were introduced into ethyl cinnamate-impregnated dishes and into the observation arena in exactly the same manner as male treatments. No more than 10 moths were placed in any one Petri dish.

After emergence the moths were left for one day in treated/control Petri dishes, after which they were used for mating behaviour analysis and extraction of the hairpencils. Thus, all moths used were between 2 and 3 d old. At the time of peak responsiveness to sex pheromone by males and pheromone gland extrusion by females, beginning about 2 h before lights off (Baker and Cardé 1979), a calling female was transferred from one of the plastic cups to a glass vial and then onto the base of a 100×30×20 cm plexiglass box. A vacuum hose was attached at one end so that, with a circular hole and air filter at the other end, a slight air current was drawn through the chamber. A male was then quickly introduced ca. 10 cm downwind of the female, close enough to be able to detect and walk upwind in response to the female's sex pheromone.

The ensuing courtship interaction was videotaped and analysed as described above. Due to power supply problems with the video on some occasions a simple tape recorder and hand-held microphone were utilised (on these occasions two observers were always present to record an accurate commentary). The commentary was transcribed and analysed later in detail.

Each male was allowed at least 5 attempts to attract and/or mate with the female. Females that appeared distracted or disturbed on introduction into the mating arena were replaced by another female from the 'pool'. Likewise, females that had been waiting too long (>ca. 30 s) between introduction into the arena and courting by a prospective mate were replaced. Unsuccessful males were collected in separate small glass vials. Successful males were, within seconds after coupling teased apart from the female; the first few seconds of linkage being insecure (Baker 1982). They were then stored in glass vials identical to those used for unsuccessful males.

For each male, the proportion of attempts in which a male caused a female to 1) approach him; 2) approach him and touch his abdominal tip; 3) move away; and 4) remain unattracted; was calculated as a function of total attempts made by that male. Means of each proportion were then calculated for each set of males. Proportions were utilised to avoid giving unfair weighting in the final mean to males that had displayed a large number of times. Thus, each male only contributed one sample to the final mean. It was appreciated that this method of analysis does ignore the variance within each male's data subset.

From the video recordings, the speed with which an attracted female moved to touch the abdomen of a courting male was measured, using the distance of an attempt divided by the time from the moment the female started to move until the touch was registered by the male by a copulatory attempt (Baker and Cardé 1979).

Almost exclusively, the adults used in this main study had been reared as larvae on a lima bean diet (Yokoyama et al. 1987) and sent directly as pupae from U.C., Riverside, U.S.A.

After the mating experiments each individual male's hairpencils were chemically analysed whether the male was successful or not in coupling. About 2 h after lights-out, on the same day as the behavioural observations, each male's hairpencils and claspers were excised and extracted in 10 µl of distilled hexane in a microtube for between 10 and 15 min. The hexane solution contained 10 ng of a tridecyl acetate as an internal standard. The solvent was then recovered and transferred to a clean microtube. Particular caution was exercised when dissecting males whose diet had been ethyl cinnamate to avoid cross-contamination and also to ensure that only the hairpencils were snipped.

Due to the large number of males used it was necessary to store many of the microtubes containing extract within glass vials sealed with teflon in screw-top caps. The glass vials contained small amounts of distilled hexane. This saturated the atmosphere with hexane in the vial and, thus, prevented evaporation of the sample.

*Gas chromatographic analysis of hairpencil components.* Samples were analysed on a Hewlett-Packard 5840A (Geneva) or a 5830 (Lund) gas chromatograph equipped with flame ionisation detectors. The columns used were a 30 m × 0.31 mm id crosslinked methyl silicone column from Hewlett Packard, (in Geneva) or a 30 m × 0.25 mm id DB1 fused silica capillary column from J&W Scientific, Folsom, CA 95630 (in Lund). Conditions of chromatography were: In Geneva: injector temperature 150°C, column temperature maintained at 60°C for 1 min and then programmed at 20°C per min to 240°C. In Lund: injector temperature 250°C; column temperature maintained at 80°C for 3 min after injection and then raised at 15°C per min to 230°C. In both laboratories hydrogen was supplied as carrier gas at approximately 45 cm $s^{-1}$, splitless injections were made and the splitvalve was opened 1 min after injection.

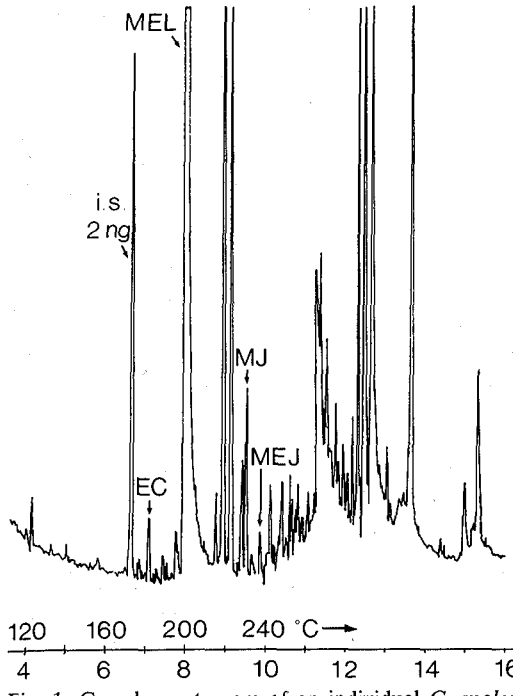


Fig. 1. Gas chromatogram of an individual *G. molesta* male reared on thinning apples as larva. EC = ethyl-*trans*-cinnamate, MEL = mellein, MEJ = methyl epi-jasmonate, MJ = methyl jasmonate.

## Results

*Individual variation and age dependence of pheromone titre.* The hairpencil constituents earlier reported by Nishida et al. (1982) were identified in the extracts on the correspondence of their rentention times with those of synthetic standards (Fig. 1). In newly emerged males mellein was usually the only component present in detectable amounts (Fig. 2). However, the titre of mellein in the hairpencil extracts increased dramatically on the 2nd-3rd day after emergence and so did the other compounds under study as well as a number of other unidentified peaks in the chromatograms. All four possible pheromone components peaked on day 2–4 and remained high on day 4–5 (Fig. 2). Whereas there was a substantial difference in pheromone titre between males of different age, there was only small variation in titres among males of the same age.

*Effect of larval diet on adult male pheromone titre.* Males reared as larvae on synthetic diet contained significantly lower amounts of ethyl *trans*-cinnamate than apple-reared ones did (Tab. 1). Mellein and methyl jasmonate titres were unaffected by larval diet.

*Effect of adult starvation.* Starvation of adult males lowered their ethyl *trans*-cinnamate titre, but starved males still contained on average 0.05 ng ethyl *trans*-cinnamate per male (Tab. 1). The titre of other compounds seemed not to be affected by starvation.

*Effect of larval diet on adult male courtship success.* There was no significant difference in mating success between males reared on apple vs males reared on synthetic diet. The number of hairpencil displays required for successful attraction of a female was equal in the two groups and the tendency for apple-reared males to be able to attract females over longer distances was not statistically significant (Tab. 2).

*Effect of G. molesta adult male diet on mating success.* The foremost result achieved was that of the measure of absolute mating success. The ethyl *trans*-cinnamate-treated males (EC males) experienced a success rate of 92% (34/37) whereas the control sugar water-treated males (SW males) achieved coupling 68% (26/38) of the time (Fig. 3A).

The proportion of attempts that cause attraction is also significantly greater for an EC male than for a SW male (Fig. 3B). It appears that once a male has succeeded in attracting the female to move towards him, a touch on the abdominal tip is likely to follow, because success at eliciting a touch from a female, given that she is already moving, does not differ significantly in EC compared with SW males (Fig. 3C).

On their successful attempts, EC males attracted females from significantly further away ($\bar{x}$ = 1.05 cm ± 0.15 SE) than did SW males ($\bar{x}$ = 0.73 cm ± 0.08 SE)

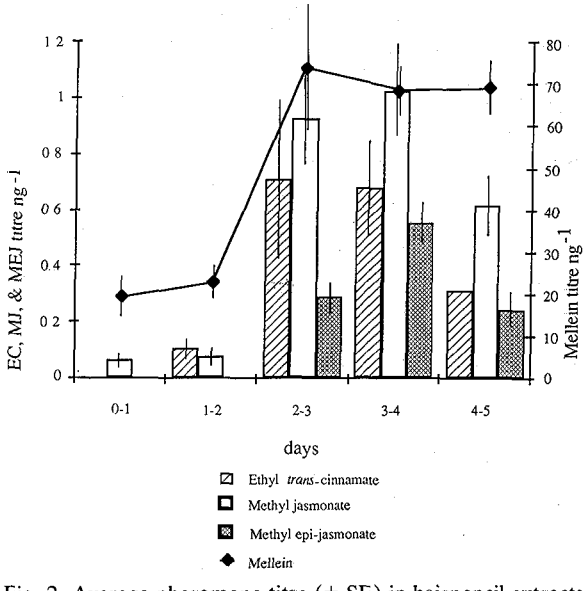


Fig. 2 Average pheromone titre (± SE) in hairpencil extracts from individual *G. molesta* males of different age.

Tab. 1. Influence of diet on the titre of hairpencil pheromone components in extraccts of individual 3–4 d old male *G. molesta*.

| Insects | EC | Pheromone component titre (ng)[a] (x̄±SD) | | |
|---|---|---|---|---|
| | | Mellein | MJ | MEJ |
| Apple-reared (n=5) | 0.68± .39[a] | 69±14 | 1.0±.4 | 0.6±.1 |
| Diet-reared (n=5) | 0.02± .01[b] | 61±15 | 0.9±.2 | 0.4±.2 |
| Starved apple-reared (n=5) | 0.06± .02[b] | 78±43 | 1.3±.4 | 0.6±.1 |

a) Diet had a significant influence only on ethyl *trans*-cinnamate (One-way ANOVA, P<0.001, followed Fisher's protected LSD method for multiple comparisons. Means followed by the same letter are not significantly different at the P<0.05 level).

(t-test, 0.025<P<0.05). The distance of an attempt was defined as the distance from the courting male's abdominal tip to the female's head. Females also moved faster toward EC males to touch their abdomens ($\bar{x}$ = 1.48 cm $s^{-1}$ ± 0.18, n = 12) than to SW males ($\bar{x}$ = 0.99 cm $s^{-1}$ ± 0.07, n = 15) (t-test; 0.0005<P<0.005).

When either an EC or SW male is not successful at attracting a female on one of his first displays then he tends to move in closer to the female to display on subsequent attempts. Since SW males that were successful at coupling on average took more attempts to do so ($\bar{x}$ = 2.54 ± 0.35 SE, n = 26) than EC males ($\bar{x}$ = 2.09 ± 0.29 SE, n = 34), their successful attempt tended to be later and closer than for EC males (t-test, P>0.05).

*Pheromone titre of individual males from courtship assay.* A typical gas chromatogram of the extract obtained from a SW male is shown in Fig. 4. No ethyl cinnamate peak was seen in any SW male (n = 28). The methyl jasmonate compounds were also absent. The mellein peak usually measured about 50 ng.

On average, the hairpencils of EC males contained 6.7 ng ± 0.47 SE (n = 26) of ethyl *trans*–cinnamate. Fig. 4 includes a typical gas-chromatogram from an EC treated male. Analyses of control females on ethyl cinnamate that had their abdomens snipped and extracted in a similar way to the males' also contained no trace of ethyl cinnamate (n = 9). The two methyl jasmonate components of the courtship pheromone could also not be detected in any EC male hairpencil extraction.

Mellein, a fourth constituent of the extrusible organs, was present in large quantities in all hairpencils analysed. The average level found in EC males ($\bar{x}$ = 48.1 ng ± 3.6 SE, n = 26) was not significantly different (t-test, p>0.05) from that found in SW males (x = 51.2 ng ± 3.8 SE, n = 29).

## Discussion

The hairpencils of *G. molesta* males play a crucial role in mating success. This study demonstrates rigorously that the chemical message contained within the hairpencils is a critical factor as to whether a female accepts or rejects a particular male, how quickly she does so, and that OFM females have a preference for increased development of this secondary sexual character. Adult males fed on the ethyl *trans*–cinnamate diet had a significantly greater success rate in coupling. In a natural situation, several males may arrive at a calling female simultaneously or within a few seconds of each other (Baker and Cardé 1979). The ability to attract a female quickly and to get the female to move speedily are critical in order to couple successfully before the arrival of another male. Baker (1982) has shown that competing males may 'sneak' copulations or disrupt the first-arriving male's courtship display. This fact makes the speed of attraction even more important.

The pheromone titre results show that the adult males offered ethyl cinnamate are able to take-up and sequester the chemical into the hairpencils (also shown by Nishida et al. 1985). If the high levels of ethyl cinnamate present had simply been due to contamination via gut-contents extracted with the hairpencils, then ethyl cinnamate should also have shown up in the control EC

Tab. 2. Mating behaviour of male *G. molesta* reared on different diets as larvae.

| Insects | % matings[a] | Mating data no. of displays for successful attraction | Distance of successful attraction (m m.)[b] |
|---|---|---|---|
| Apple-reared (n=27) | 69% | 2.6 | 9.4 |
| Diet-reared (n=25) | 80% | 2.6 | 4.4 |

a) difference in % mating was not significant according to $\chi^2$ analysis ($\chi^2$=0.315, P=0.57)
b) Apple-reared males were not able to attract females over significantly longer distance (Mann-Whitney U-test; one-sided P=0.18.

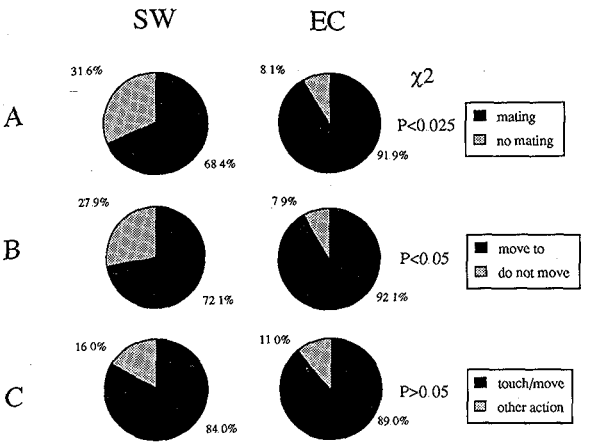


Fig. 3. Parameters of courtship success estimated for control males (SW, fed sugar-water only, n = 38) and ethyl *trans*-cinnamate treated males (EC, fed sugar-water with ethyl *trans*-cinnamate, n = 37). A) The proportion of males achieving coupling; B) The proportion of attempts, as a function of total attempts, resulting in female movement towards the male; C) The proportion of attempts, as a function of attempts eliciting movement, resulting in female touch of the abdominal tip of a displaying male. The statistical significance of differences between SW and EC males was tested by $\chi^2$ 2×2 analysis with Yates' correction.

females. However, the precise dissection technique and the fact that females offered ethyl cinnamate had no corresponding peak indicates that this is not the case. Clearly, in view of the observed behavioural traits, the ability to sequester ethyl cinnamate from the diet and into the hairpencils is a very important feature. Indeed, ethyl *trans*-cinnamate has been found to be one of the components from rotting Japanese pears that actually attracted OFM in the orchard (Nishida et al. 1985). Baker et al. (1981) showed ethyl *trans*-cinnamate to be the only component of the courtship pheromone that elicited a significant attraction response in female OFM when presented alone. The present study shows that the consequences of not having the opportunity to sequester ethyl cinnamate can have important implications, in terms of mating success.

The question naturally arises as to where *G. molesta* males obtain their ethyl cinnamate in the wild. Male Oriental fruit moths are known to possess all hairpencil components without ingesting fruit juice as adults (Baker et al. 1981, Nishida et al. 1982, 1985). The present study indicates that not even adult feeding per se is obligatory for the hairpencils to contain ethyl cinnamate, as starved males also contained some cinnamate. This indicates that the larvae may be able to sequester pheromone components for later use.

It was apparent from an early stage of our study, in concordance with Nishida et al. (1982, 1985), that males reared on artificial diet as larvae and kept on sugar water as adults had a very low ethyl cinnamate titre. Nishida et al. (1985) reported that both ethyl cinnamate and jasmonic esters and/or their derivatives were plant constituents, and that these compounds might be sequestered from the host-plant by the insect for use at a later stage. Methyl 2-epijasmonate can be isolated from lemon (Nishida, Acree, and Roelofs, unpubl.) and methyl jasmonate from jasmine, and could be present in fruits fed upon by *G. molesta* larvae (Nishida et al. 1982). Nishida et al. (1982) posited that "a reduction of these compounds (in the courtship pheromone) due to larval feeding on deficient hosts might affect male mating success".

However, we failed to demonstrate a clear-cut effect of larval feeding on mating success. This could be because we did not use females of a well-defined age for these experiments. Not only aspects of male courtship behaviour but also the behavioural state of the female influences the mating success, and any uncontrolled variation in female performance might have added to the overall variance in mating success and obscured the possible significance of larval diet. Another explanation may be related to the surprising result that males reared as larvae on a reasonably natural diet of apples in Lund and Riverside 1987 had no ethyl cinnamate present in hairpencil extracts. This group included on several occasions males reared as larvae on the definitive thinning-apple diet (Baker and Cardé 1979, Nishida et al. 1982). Perhaps seasonality and the quality of the apples used as

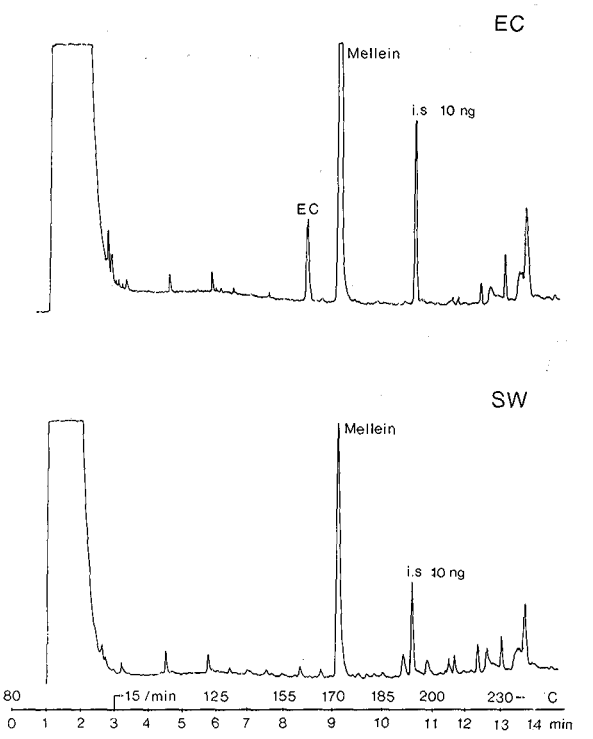


Fig. 4. Typical gas chromatograms of a hairpencil extract from a control sugar water male and an ethyl *trans*-cinnamate treated male. Note the complete absence of ethyl cinnamate but normal level of mellein in SW male.

food has a part to play, as the pheromone titre of the males in one of our cultures in 1983 decreased during the autumn after the apples had been stored for several months. The influence of larval diet on courtship pheromone titre appears to be a complex and perplexing issue that will not be easily resolved.

The OFM is reminiscent of *Asclepias*-feeding danaid butterflies. As larvae these lepidopterans feed on milkweeds from which they sequester poisonous cardiac glycosides. These compounds protect both the larval and adult stages. However, the adult males have another secondary compound requirement, not provided by the milkweed (Edgar and Culvenor 1974, Edgar et al. 1974). The males are able to obtain pyrrolizidine alkaloids from *Senecio* and some Boraginaceae. The male butterfly uses these compounds to manufacture pheromones, which are in turn used in its courtship display to attract the female (Dethier 1977). In the OFM the sequestered compounds do not provide protection, and thus, the initial advantage to females mating with pheromone displaying males is less obvious. However, it appears likely that in the OFM a process of sexual selection, perhaps of a runaway type, may have been triggered by the avoidance of interspecific mating mistakes by those females initially selecting males that emitted specific host-plant derived volatiles, as suggested by Phelan and Baker (1986). An alternative, not necessarily exclusive, explanation may be that as high EC titres are associated with adult male feeding, a female selecting for EC will mate with a well-fed male, able to provide a large spermatophore. This hypothesis remains to be tested.

It would be enlightening to find out why so many females in 1983 and 1987 responded to males lacking the behaviourally most important chemical ingredient in their hairpencils as determined in 1979 (Baker et al. 1981). Pheromone-deficient (SW) males had a success rate of 68%. Presumably, the wind generated by the fanning male, the mellein, the visual and maybe audio channels of communication provide enough sensory input for the female to respond by walking upwind. Baker et al. (1981) showed mellein on its own to be inactive in behavioural bioassays on females, and Nishida et al. (1982, 1985) indicated that the female electroantennogram response to mellein was not above background. However, all three papers realise that a pulsed wind of 45–90 cm $s^{-1}$ did not accompany the chemical treatments as it does during a display; the additional wind is known to significantly increase female locomotion (Baker and Cardé 1979) and may be the reason why some females respond to SW males. The possibility exists that by 1987, the quality of the male display and the female preference for it had declined over so many generations of courtship under confined, competitive conditions. These might favour males performing alternative behaviours instead of attraction of the female over several cm (Baker 1982).

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