

EGG DISPERSION IN CODLING MOTH: INFLUENCE OF EGG EXTRACT AND OF ITS FATTY ACID CONSTITUENTS

DENIS THIÉRY,^{1,*} BRUNO GABEL,^{1,4}
PAVEL FARKAS,² and MARC JARRY³

¹*Laboratoire de Neurobiologie Comparée des Invertébrés,
INRA-CNRS (URA 1190),
BP 23, 91440 Bures sur Yvette, France.*

²*Food Research Institute
Priemyselna 4,
82 006 Bratislava, Slovakia*

³*Laboratoire de Mathématiques Appliquées,
Université de Pau et des Pays de l'Adour,
64 000 Pau, France*

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Abstract—Codling moth females (*Cydia pomonella*, Lepidoptera: Tortricidae) (CM) usually lay single eggs and have a tendency to disperse. In a first experiment we observed that single females exposed to 20 apples distribute their eggs regularly among apples, suggesting a dispersive oviposition behavior. In a dual-choice situation, isolated females avoided oviposition on areas of cardboard treated with a methylene dichloride egg extract at the dose of ca. 1.0 egg equivalent/cm². A strong avoidance was obtained in response to a 20-fold dose, which was accompanied by a significant reduction of total oviposition. Seven major compounds found by GC analyses in the methylene dichloride extract of 2 to 3-day-old eggs were saturated or unsaturated C₁₄–C₁₈ straight-chain fatty acids: myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid. This was confirmed by GC-MS analysis of an extract made by ethyl ether. Smaller amounts of three methyl esters were also identified as methyl myristate, methyl palmitate, and methyl stearate. A blend of the seven fatty acids (FA) mimicked rather well the avoidance provoked by the extract, and this avoidance was confirmed by choice between treated and untreated fruits by single females. The treat-

*To whom correspondence should be addressed.

⁴Present address: Research Institute for Viticulture and Enology, Matuskova 25, 83311 Bratislava, Slovakia.

ment of apples with the 7FA mixture induced an aggregative distribution of the eggs among apples. We also found that the amounts of fatty acids harvested in the extract depend on the egg age. Amounts of fatty acids increased until eggs were 4 days old and then decreased before hatching. In this paper we discuss the possible role of simple molecules, such as fatty acids and their esters, as semiochemicals indicative of juvenile tissues.

Key Words—Behavior, *Cydia pomonella*, oviposition, pheromone, synomone, insect, offspring dispersion, fatty acids, egg age, Lepidoptera, Tortricidae.

INTRODUCTION

Investigations on oviposition deterrents relative to the dispersion of progeny yield fascinating information on host-plant selection behaviors by phytophagous insects. Reproductive strategies involve factors that influence how progeny are distributed over space and time. In many cases perception by the female of information produced by eggs or larvae is of primary importance to adjust population level to the size of available resources. Assessment of oviposition sites and egg load by ovipositing females and avoidance of plants occupied by conspecifics have been reported in Lepidoptera [Urquhart, 1960 quoted in Chew and Robbins, 1984; MacLellan, 1962, Rothschild and Schoonhoven, 1977], and more generally in a wide variety of phytophagous insects (Prokopy, 1981; Messina and Renwick, 1985; Anderson, 1986; Roitberg and Prokopy, 1987). This behavior involves intraspecific chemical cues (Hurter et al., 1987; Imai et al., 1990) or both intra- and interspecific ones (Schoonhoven, 1990; Thiéry and Gabel, 1993; Blaakmer et al., 1994). In the latter category, simple chemicals (fatty acids and derivatives) deter oviposition in several lepidopteran species (Thiéry and Le Quéré, 1991; Thiéry et al., 1992; Gabel and Thiéry, 1994), which led us to propose that fatty acids and/or derivative esters exist on the eggs of several lepidopteran species and may allow interspecific recognition (Thiéry and Gabel, 1993).

The codling moth (CM), *Cydia pomonella* (Lepidoptera: Tortricidae), is an important pest of orchards, where it causes severe damage on apples and other fruits. Female CM normally deposit single eggs close to fruits (on bark or leaf) or directly on fruit. Eggs are always well distributed and more than one egg per single fruit or leaf occurs only occasionally (Geier, 1963; Wood, 1965; Jackson, 1979). As a possible result of that, more than one larva per apple is seldom found (Ferro and Harwood, 1973). Although Roitberg and Prokopy (1982) were unsuccessful in characterizing the occurrence of an oviposition deterring pheromone in CM, these reports motivated studies on egg dispersion in CM, with a dispersive oviposition behavior being hypothesized as the consequence of the moth perceiving fresh eggs (MacLellan, 1962). We

recently observed that CM females avoid ovipositing on apples treated with fatty acids and methyl esters isolated from *Lobesia botrana* at a dose of 72 egg equivalents per apple (Gabel and Thiéry, 1994).

The present study was intended to determine if simple chemicals acting as general oviposition deterrents occurred in CM eggs. The study had three objectives: (1) to quantify the distribution of eggs on apples under conditions of limited resource, (2) to verify the behavioral response of CM females to an extract of conspecific eggs, and (3) to determine the chemical constitution of this extract and the effect of egg age on its chemical constitution.

METHODS AND MATERIALS

Insects originated from a laboratory colony (strain: INRA Zoologie, Montfavet, France) reared for more than 20 generations on artificial diet (Guennelon et al., 1981) in climate chambers regulated with the following conditions: 16L:8D photocycle regime ($22 \pm 0.5^\circ\text{C}$, $85 \pm 5\%$ relative humidity during photophase and $20 \pm 0.5^\circ\text{C}$, $75 \pm 5\%$ relative humidity during scotophase). Behavioral observations were made with 2-day-old mated females. Mating was obtained by grouping 10 females and 10 males for two days in arenas with access to honey and water. Apples (cv. Golden Delicious) were harvested just before trials from an orchard free of pesticides, and those free of CM attacks and with horizontal diameter between 5 and 5.5 cm were used.

Extraction and Analysis.

Two- to Three-Day-Old Egg Extracts. Females were forced to oviposit on clean glass slides for two consecutive nights. Eggs were then counted and glass slides with eggs were washed either in methylene dichloride (12,000 eggs) or in ethyl ether (4200 eggs). Moth scales were removed by passing the extracts through a Millipore filter (GW, $0.22 \mu\text{m}$). The extract was then evaporated under nitrogen flow. An aliquot was devoted to GC analysis, and another aliquot devoted to bioassays was evaporated to dryness and then diluted according to the required concentration for application.

Effect of Egg Age. Age of eggs was studied by harvesting eggs during one night and extracting them with methylene dichloride as described above. We had four batches of 2000–2500 eggs each. One batch was immediately extracted (age < 12 hr), and the three others were kept in an incubator (climate conditions described above) for two, four, or six days before extraction.

Chemical Analysis. Analyses of the extracts were performed by gas chromatography (GC) with no prior treatment except filtration to remove scales as described above. GC, either coupled or not to a mass spectrometer (MS), was equipped with an HP Ultra 1 column ($0.20 \text{ mm ID} \times 25 \text{ m}$) and a flame

ionization detector. The temperature program started at 60°C with 2 min isothermal and then linearly increased by 5°C/min to 240°C with 10 min isothermal at the end. Mass spectra were obtained with an HP 5971 A mass selective detector with positive 70 eV ionization. Spectra were compared to an MS data bank and spectra obtained from pure standards.

Bioassays

All bioassays were conducted in climatic chambers under conditions described above.

Bioassay 1: Distribution of Eggs on Untreated Apples. To check the distribution of eggs on apples, one female and two males were offered during a night 20 equidistant apples positioned in four rows of five apples on the floor of an arena designed to prevent oviposition anywhere else than on apples (60 × 40 cm, 30 cm high). Twenty replicates were performed.

Bioassay 2: Oviposition Responses to Extract and Synthetic Blend on Cardboard. Gravid females were isolated (no males) in an altuglass tube (6.5 cm ID, 13 cm long) and could only oviposit on a smooth cardboard providing treated areas (32 spots of 1.1 cm² each); the remaining area (109 cm²) was untreated (Gabel and Thiéry, 1992). The extract was diluted for application with 6 parts acetone and 4 parts purified water (API). Treatments were made by applying on the cardboard 32 equidistant wells (ca. 1.1 cm diam.) and filling each with 5 µl of the solution of test (Gabel and Thiéry, 1992). After complete evaporation of the solvents, the cardboards were exposed to females. We determined the response either to API or to the following quantities of extract expressed in egg equivalents per spot: 1 egg eq. (A) and 20 egg eq. (B). Females were individually exposed the first night to a blank cardboard, then on the second night to cardboard impregnated with treatment spots (API, extract, or synthetic blend), and on the third night to blank cardboard. The 3% of females that did not oviposit during the three consecutive nights were considered as unmated and then disregarded. The number of eggs deposited on treated areas (T) and untreated areas (UT) were counted, eggs in contact with boundaries being considered as T. We calculated for each female an avoidance index corrected to equivalent surface area (UT = 3T): $Ai\% = (UT - 3T)/(UT + 3T) \times 100$ (Gabel and Thiéry, 1992), which may vary from 100% (maximum avoidance) to -100% (maximum preference). An identical procedure was performed to verify the activity of a synthetic blend corresponding to seven identified fatty acids (7FA). Pure chemicals (GC standards, Sigma Ltd.) were dissolved in pure acetone (see Table 3 below for ratios) and the dose was adjusted in API to the higher dose of extract used (20 egg eq./spot).

Bioassay 3: Test of Synthetic Blend on Apples This bioassay was conducted in the same arenas as bioassay 1, with one female and two males per arena.

These experiments could not have been done with extracts of eggs because of the enormous number of eggs required. To prevent damaging effects of acetone on apple cuticles, we used a second solvent or application phase, AP2, consisting of 1 part acetone, 8.9 parts purified water, and 0.1 part wetting agents (Triton X-100, Prolabo). Apples were rapidly dipped into the treatment solution of 7FA (same proportions as in bioassay 2) dissolved in AP2. Oviposition assays were conducted either in dual-choice or no-choice situations. In the choice situation, females were exposed to two rows of five treated apples (7FA) alternating with two rows of apples treated with solvent only (AP2) (15 replicates). In the no-choice situation, all apples were dipped in the 7FA solution (18 replicates). The numbers of eggs on treated and control apples (C) were counted. We calculated in each experimental arena a simple avoidance index: $Ai\% = (C - T)/(C + T) \times 100$ (where T is the number of eggs on treated sites and C on control sites with C and T representing equal areas). This index is equivalent to that described above.

Statistics

Bioassays 1 and 3 (No-Choice Situation). Egg distribution on apples was analyzed using a nonparametric index of dispersion as described in Chessel (1978). The variable

$$D = \sum_{i=1}^n y_i^2,$$

where y_i is the number of eggs on apple i , describes the variability in the distribution of p eggs ($p = \sum_{i=1}^n y_i$) among n apples. D tends towards a maximum when eggs are concentrated on a single apple and towards a minimum when eggs are regularly dispersed. If the eggs are distributed randomly (null hypothesis), then the chance that a given egg is laid on apple i equals $1/n$. This hypothesis assumes that eggs are laid independently of each other and that apples have an equal chance of receiving an egg. Under this hypothesis, the mean (E) and the variance (V) of D equal:

$$\begin{cases} E(D) = \frac{p}{n} (n + p - 1) \\ V(D) = \frac{2p}{n^2} (p - 1)(n - 1) \end{cases}$$

For N replicates the following index of dispersion (Id) can be obtained:

$$Id = \frac{1}{\sqrt{N}} \sum_{i=1}^N \frac{D_i - E(D_i)}{\sqrt{V(D_i)}}$$

and the value of $Idc_i = [D_i - E(D_i)/\sqrt{V(D_i)}]$ represents the contribution of replicate i to Id . In practice, fitting the distribution of Id to a standard normal distribution is valid when $N \geq 15$.

At the level $\alpha = 0.05$, we may reject the null hypothesis when $Id > 1.96$ (aggregative distribution) or when $Id < -1.96$ (regular distribution).

Bioassays 2 and 3 (Choice Situation). Data are presented as mean numbers of eggs and mean $Ai(s)$ per female. Nonparametric statistics were used: Mann-Whitney U test (M-W) for comparisons between experimental groups and Wilcoxon matched-pair signed-ranks test (WX) for comparisons within groups (oviposition during consecutive nights). Numbers of eggs laid on treated and untreated apples (bioassay 3) were compared by χ^2 analysis.

RESULTS AND DISCUSSION

Distribution of Eggs on Untreated Apples. Females laid a mean of 10.7 ± 4.7 eggs per arena. Among the 400 apples, 50% had no egg, 39% contained a single egg, and only 6% had more than one egg, with one apple carrying seven eggs. The global index of dispersion calculated from the 20 replicates ($Id = -1.54$) did not permit excluding a random distribution of eggs. However, 16 experimental arenas had a negative value of Id_{ci} , which indicates a tendency for egg dispersion. One female laid more eggs than the number of available apples and distributed the eggs in a completely different pattern than the 19 other females. This female constituted the most important contribution to $Id(5.83)$. After excluding this arena, $Id = -2.94$ allowed us to reject the hypothesis of a random distribution with $P < 0.003$. This regular distribution suggests a dispersive oviposition behavior which is consistent with previous analysis of egg dispersion in orchards, in which egg distribution among spurs, within spurs, and on leaves deviated from randomness (estimated as a Poisson distribution) (Jackson, 1979). In our experiment, the large number of apples bearing no or one egg suggest a dispersive oviposition behavior.

Oviposition on Cardboard Before and After Exposure to Treatment. The mean numbers of eggs laid per female are presented for the three consecutive nights of the experiment (Table 1). During the night before any exposure, the numbers of eggs were remarkably constant among experimental groups and varied from 36.2 ± 15.9 to 30.3 ± 15.1 , which was very close to that observed in previous experiments (32.5 ± 15.1 ; Thiéry and Gabel, 1993). In all groups, this number regularly decreased by one third each night (difference between two consecutive nights: WX, $P < 0.001$). On night 3, values were similar between groups (from 9.4 ± 8.5 to 11.9 ± 4.7 eggs) no matter what the exposure on the second night was. As in other tortricids, mating stimulates egg production by CM: here females were exposed to mates prior to the observations and no

TABLE 1. EGGS (MEAN \pm SD) LAID ON ARTIFICIAL SUBSTRATE BY *Cydia pomonella* FEMALES^a

Treatments	Females (N)	Night 1, blank cardboard	Night 2, treated and untreated areas	Night 3, blank cardboard
Application solvent	22	33.2 \pm 15.9a	21.7 \pm 11.2b	10.1 \pm 9.5c
Extract A	23	31.2 \pm 12.5a	19.1 \pm 8.8b	9.4 \pm 8.5c
Extract B	15	36.2 \pm 15.9a	15.6 \pm 5.4c	10.2 \pm 7.6c
7FA B	15	30.3 \pm 15.1a	23.2 \pm 9.9b	11.9 \pm 4.7c

^aDuring night 2, females had a choice between treated areas and an untreated area. Dose A = 1 egg eq./spot, dose B = 20 egg eq./spot. Seven fatty acids (FA) were tested at dose B. Different letters indicate statistical differences based upon Wilcoxon matched-pairs at $P < 0.001$ within lines and Mann-Whitney U test, $P < 0.04$ within columns.

additional mating occurred. The reduction in egg laying was thus considered as normal.

Response to Egg Extraction on Cardboard. The application phase (AP1) alone had no effect on the oviposition preference ($A_i = -1 \pm 24\%$) (Table 2), with a total of 120 eggs being laid on areas treated with AP1 and 362 eggs on the untreated area. This is in agreement with observations based on other solvents (pure methanol or methanol in water) (Thiéry and Gabel, 1993; Gabel and Thiéry, 1994). Females avoided ovipositing on the application spots of extracts, and this behavior was dose dependent (Table 2). Dose A (1 egg eq./spot) induced a slight avoidance: the 23 females laid a total of 66 eggs on treated

TABLE 2. OVIPOSITION PREFERENCES OF *Cydia pomonella* FEMALES BETWEEN AREAS OF CARDBOARD TREATED WITH DIFFERENT SUBSTANCES AND UNTREATED AREA^a

Type of exposure and doses	N	Eggs per female (Mean \pm SD)		Avoidance index (% \pm SD)
		Treated areas	Untreated area	
Control (AP1)	22	5.5 \pm 2.9	16.5 \pm 8.8	1 \pm 24a
Extract A	23	2.9 \pm 1.8	16.2 \pm 7.3	34 \pm 22b
Extract B	15	0.9 \pm 0.8	14.6 \pm 4.9	75 \pm 23c
7FA B	15	1.5 \pm 0.6	21.7 \pm 9.4	66 \pm 8c

^aAP1 was application solvent, egg extract dose A (1 egg eq./spot), egg extract dose B (20 egg eq./spot), and 7FA is a synthetic blend of seven fatty acids at dose B. Data are means \pm SD of N observed females, different letters indicate statistical differences among avoidance indexes at $P < 0.001$, Mann-Whitney U test).

areas and 372 on the untreated area. The mean Ai ($34 \pm 22\%$) differs statistically from that observed in response to AP1 (M-W U test, $P < 0.0002$). At dose B (16 egg eq./spot), the 15 females laid 13 eggs on the treated areas and 219 on the untreated area. The mean Ai ($75 \pm 23\%$) differed from the previous dose (M-W U test, $P < 0.0005$) (Table 2). Six of the 15 females accounted for 68 eggs, and they laid no egg on treated areas. Females exposed to dose B of extract laid fewer eggs than females exposed to AP1 (15.6 ± 5.4 vs. 21.7 ± 11.2 ; MW, $P < 0.04$) (Table 1).

The avoidance of CM females to its egg extract is higher than that observed in the tortricid (*L. botrana*) exposed to its own extract (Ai = 50%; Gabel and Thiéry, 1992). In the present work, avoidance by *C. pomonella* was already evident at the dose of 1 egg eq./spot (32 egg eq./arena), whereas a weaker response by *L. botrana* required three times more egg equivalents.

Identification of Active Compounds. Seven major compounds were found in the GC analysis of 2 to 3-day-old eggs (Table 3). These molecules are C_{14} to C_{18} saturated or unsaturated straight-chain fatty acids. We found in the methylene dichloride extract myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), palmitoleic acid (Z9)($C_{16:1}$), stearic acid ($C_{18:0}$), oleic acid (Z9)($C_{18:1}$), linoleic acid (Z9,Z12)($C_{18:2}$), and linolenic acid (Z9,Z12,Z15)($C_{18:3}$) in the amounts shown in table 3. Slightly lower amounts of each of these seven fatty acids were found in the ethyl ether extract (except for $C_{14:0}$) (Table 3). Three methyl esters were detected in the ethyl ether extract. These were methyl myristate (Me $C_{14:0}$), methyl palmitate (Me $C_{16:0}$), and methyl stearate (Me $C_{18:0}$) (Table 3). Only the Me $C_{16:0}$ and the Me $C_{18:0}$ were detected in the methylene dichloride extract.

TABLE 3. FATTY ACIDS AND RELATED ESTERS EXTRACTED FROM 2 TO 3-DAY-OLD *Cydia pomonella* EGGS AS IDENTIFIED BY GC-MS ANALYSIS^a

Compound		Methylene dichloride (ng/egg eq.)	Ethyl ether (ng/egg eq.)
Myristic acid	$C_{14:0}$	4.8	0.5
Palmitic acid	$C_{16:0}$	13.6	11.2
Palmitoleic acid	(Z9) $C_{16:1}$	2.7	1.8
Stearic acid	$C_{18:0}$	7.3	3.7
Oleic acid	(Z9) $C_{18:1}$	15.3	10
Linoleic acid	(Z9, Z12) $C_{18:2}$	16.6	11.7
Linolenic acid	(Z9, Z12, Z15) $C_{18:3}$	9.1	3.7
Methyl myristate	Me $C_{14:0}$	0	0.015
Methyl palmitate	Me $C_{16:0}$	0.024	0.67
Methyl stearate	Me $C_{18:0}$	0.01	0.02

^aAmounts are expressed in ng per egg equivalent; numbers in parentheses are double bond positions.

Remarkably, we found the same kind of fatty acids as in *L. botrana*, the two major compounds [$C_{16:0}$ and (Z9) $C_{18:0}$] being identical to both species (Thiéry et al, 1992; Farkas, Gabel, and Thiéry, unpublished data), although we found them in higher amounts in CM. Methyl palmitate and stearate have also been identified as oviposition deterrents in *Ostrinia nubilalis* (Thiéry and Le Quééré, 1991).

Biological Activity of Synthetic Blend of Fatty Acids. We have verified the biological activity of a synthetic blend made according to the amounts of the seven fatty acids found in the 2 to 3-day-old egg extract. The exposure of 15 females to a dose of the seven fatty acids equal to 20 egg eq./spot evoked a mean Ai of $66 \pm 8\%$, which was not statistically different from the extract at an identical dose (M-W U test) (Table 2). A total of 20 eggs were laid on treated areas and 326 on untreated ones. Females in total laid more eggs (23.2 ± 9.9) than when exposed to the extract applied at an identical egg equivalent dose (15.6 ± 5.4) (M-W U test, $P < 0.03$). The first value was similar to that observed in the control treatment (Table 1).

In previous work, CM females exposed to a synthetic blend of three acids [$C_{16:0}$, $C_{18:0}$ and (Z9) $C_{18:1}$] and six esters [Me $C_{16:0}$, Me (Z9) $C_{16:1}$, Me $C_{18:0}$, Me (Z9) $C_{18:1}$, Me (Z9,Z12) $C_{18:2}$, and Me (Z9,Z12,Z15) $C_{18:3}$] significantly reduced their oviposition (Thiéry and Gabel, 1993), suggesting that esters of fatty acids could be candidates to explain such a reduction.

Responses of Females to Apples Treated with a Blend of Fatty Acids. Females exposed to 10 apples treated with the blend of fatty acids (7FA) and to 10 untreated apples laid more eggs (20.1 ± 11.4 eggs) than those exposed to 20 untreated apples or those exposed to 20 treated apples. They strongly avoided the treated apples. A total of 38 eggs were found on the treated apples and 323 on the untreated ones ($\chi^2 = 225$, 1 df, $P < 0.0001$), producing a mean Ai of $82 \pm 12\%$. In eight of the 14 replicates, the Ai value was between 80% and 100%.

The 18 females exposed to uniform treatment aggregated their eggs among certain apples. Thirteen values of Id_{ci} were strongly positive, and after excluding one female that laid a single egg, the global index of dispersion ($Id = 15.56$) allowed us to reject the null hypothesis of a random distribution at $P < 0.0001$. The aggregation of eggs is probably due to a perturbation during oviposition bouts. It might also be the result of modification in locomotion patterns because of repulsion. Interpretation of the increased oviposition in the dual-choice situation is difficult. One possibility is that, because of restricted areas, the number of eggs laid is related to the time spend on apples. Short-range repulsion provoked by uniformly treated apples might have reduced the time invested in oviposition site selection, while in dual-choice assays females setting for longer periods on untreated apples would lay more eggs. In the present experiments, females laid almost 30% fewer eggs when exposed to apples than when exposed

to cardboard. One possibility is that preoviposition sequences are more complete and may last longer on apples than on artificial substrates. This needs, however, detailed quantification of flying, preoviposition and oviposition bouts.

Age-Related Composition in Fatty Acids. We focused on the variation in concentration of the seven fatty acids identified from the methylene dichloride egg extracts. Methylene dichloride was used to study this variation because it extracted a larger amount of the seven acids (Table 3). In each age class, palmitic, oleic, and linoleic acids were always the major compounds present and were present in almost equal proportions (Figure 1). The amount of all fatty acids increased until day 4 and then decreased just before hatching. The maximum increases was observed between 2-day-old and 4-day-old eggs, where it ranged from 80% ($C_{14:0}$) to 300% ($C_{18:0}$).

The mucus that coats the eggs contains free fatty acids and esters that contribute to retardation of dessication. Egg extraction certainly harvests large amounts of chemicals from the surface, but also extracts constituents from within the egg yolk. The exact proportion of both chemicals has not been determined. The permeability of the egg chorion might allow natural passage of chemicals to the surface and could to some extent allow relations between the external and internal constitutions. Anecdotally, the large increase in the amount of linoleic and linolenic acids is noteworthy since *de novo* synthesis of these fatty acids has been seldom reported in insects. We can not exclude these FA as being liberated from phospholipids or triglycerides stores. From an ecological point of view, the variation in amount of oviposition regulators released may represent dynamic information to CM because it is related to egg age. Probably because

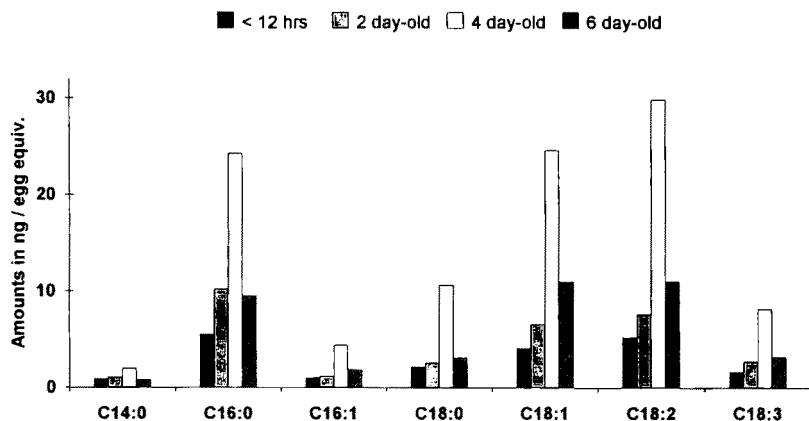


FIG. 1. Variation in the amount of fatty acids from methylene dichloride extracts of *Cydia pomonella* eggs of different ages.

of their simplicity and general occurrence, fatty acids and derivative esters have not drawn very much attention in insect communication. However, the literature provides interesting insights relative to their role as semiochemicals (Hwang et al., 1984; Rickli et al., 1992; Trouiller, 1993).

The present study is a first step towards demonstrating that chemical information produced by CM eggs regulates the oviposition site selection of conspecific females. This study has been conducted with CM reared for generations on artificial diet, and one might expect females from natural populations to be more egg dispersive than mass-reared ones. Such a comparison between natural and laboratory-reared populations has not yet been done.

We have identified free fatty acids in CM eggs whose amounts vary according to the egg developmental stage. This finding does not exclude that species-specific and more sophisticated chemicals may also influence egg dispersion. The results reported here reinforce the hypothesis that oviposition can be deterred by general chemical markers of juvenile tissue (eggs or larvae) (Thiéry and Gabel, 1993). Eggs of several moths (*O. nubilalis*, *L. botrana*, and CM) have characteristic odors very close to that of several fatty acids and esters of the fatty acids. The fact that C_{16} acids and esters released electroantennographic responses in *O. nubilalis* females (our unpublished data) suggests to us that long-chain fatty acids and related esters produce odors that might be involved in the perception of occupied oviposition substrates at a short range distance.

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