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Semiochemicals from *Lobesia botrana* (Lepidoptera: Tortricidae) eggs deter oviposition by the codling moth *Cydia pomonella* (Lepidoptera: Tortricidae)

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***Cydia pomonella*, codling moth, behaviour, oviposition, egg associated molecules, fatty acids, esters of fatty acids**

Abstract. The intention of this study was to falsify a hypothesis of interspecific avoidance of semiochemicals from eggs by ovipositing tortricid females. The oviposition responses of *Cydia pomonella* (L.) females to apples baited with blends of semiochemicals identified in another tortricid species, *Lobesia botrana* (Den. et Schiff.) were investigated. Experiments were conducted in binary choice tests using natural oviposition substrates (apples). In each experimental arena, 4 mated females were offered 16 apples (8 treated and 8 untreated) and the number of eggs was compared. Females avoid ovipositing on apples treated with a blend of nine components characteristic of methanolic extracts of *L. botrana* eggs (fatty acids and esters) (complete blend), as well as a binary blend of palmitic acid and methyl palmitate (binary blend). Oviposition avoidance was already observed with these two blends at a dose of 72 eggs equivalent of *L. botrana* per apple and this effect increased with the dose. The blend of three major esters from complete blend (ternary blend) did not provoke significant avoidance. In the control, only 8.3% of apples bore no eggs, this percentage was increased with complete blend and binary blend (720 *Lobesia* eggs) respectively up to 37.5% and 26.4%, but only to 18.1% of apples with ternary blend. Complete blend and binary blend used at high dose strongly reduced the mean number of eggs/apple on treated fruits (1.6 ± 1.8; 9 compounds) (1.8 ± 2.2; 2 compounds) against 4.6 ± 4.0 in the control. This reduction was not observed with the blend of 3 esters. It is concluded that, from the present results, apples treated with compounds associated with *L. botrana* eggs are avoided by *C. pomonella* ovipositing females. Esters alone cannot explain such an avoidance, and palmitic acid may partly cause the avoidance response.

INTRODUCTION

The codling moth *Cydia pomonella* is an important economic pest of various deciduous fruits and causes severe damage in apple orchards. Typically, codling moths lay eggs on fruit or bark or on leaves in the vicinity of the fruit. Normally, females deposit a single egg, then re-orientate to other oviposition sites [Caesar, 1911; Hall, 1929 (quoted in Jackson, 1979)]. Densities greater than one larva per apple are seldom reported in natural conditions [Harris, 1880; Chapin, 1882; Howard, 1887 (quoted in Ferro & Harwood, 1973)]. This may be the result of egg distribution at apples, as reported by Wood (1965) and Geier (1963) who found averages of 0.48 to 0.09 eggs/apple respectively, in orchards they studied. Dispersed oviposition may limit competition and cannibalism which occurs in larvae (Putman, 1963; Ferro & Harwood, 1973) and regulate population density. MacLellan (1962) found that oviposition may be deterred by conspecific fresh eggs. Data

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of Roitberg & Prokopy (1982) contrasted with these previous observations. From laboratory observations on small experimental samples, they concluded that females could not discriminate between substrates harbouring 25 conspecific eggs and unoccupied sites. Recent data suggest that egg avoidance exists in tortricids (Poirier & Borden, 1991; Gabel & Thiéry, 1992; Thiéry et al., 1992). In dual choice bioassays, females of *Cydia pommonella* avoided oviposition on artificial substrates treated with a blend of 3 fatty acids and 6 esters of fatty acids identified from the eggs of another tortricid, the European grape vine moth, *Lobesia botrana*, and their behavioural response depended on the density of females per arena (Thiéry & Gabel, 1993).

Insect oviposition is influenced by various environmental factors, such as visual, mechanical and chemical. It is probable that both the nature and the abundance of potential substrates influence the behavioural response of females. This study was intended to design a dual choice bioassay suited to determine the codling moth oviposition behaviour in response to egg-associated semiochemicals applied to natural oviposition substrates.

MATERIAL AND METHODS

Insects and apples

Experiments were conducted on codling moths reared from a stock culture (INRA Montfavet, France) (Guennelon et al., 1981) on an artificial diet (Sender, 1970) and maintained at 26 ± 1°C under long day conditions (16L : 8D). Newly emerged females were isolated for 2 consecutive nights with males (1 female + 2 males) prior to the oviposition test.

Untreated apples free of pesticide (var. Golden delicious) were harvested just before the experiments from an abandoned orchard. Apple size was standardized with a mean horizontal diameter of ca 5 cm.

Bioassay

Bioassays were conducted in a polyester-cloth wrapped cage (1 m × 0.4 m × 0.6 m) with 4 rows of 4 equidistantly spaced apples hung from the top of the cage. Eight treated and 8 control apples were arranged in a chess board-fashion, providing the females a dual choice (Fig. 1). Control apples were soaked in the solvent. Four mated females (2–3days old) were released into the cage and, after one night, the number of eggs oviposited per apple was counted. The number of ovipositing females was determined by isolating females in glass tubes the day following experimention. It was assumed that females which laid no eggs in the tubes did not start to oviposit during the experiment. All females were mated and this was confirmed by dissecting spermatophors from the bursa copulatrix after experimentation. We completed 9 trials per treatment using 36 females. Using numbers of eggs on treated apples (A) and control apples (B), we calculated a deterrence index (%) as follows: $DI = (A-B)/(A+B) \times 100$.

The different treatments consisted of a control (untreated control of 16 untreated apples per cage) and apples that were soaked in one of the following blends: a) the blend identified in the eggs of *L. botrana* with ratios according to those found in methanolic extract (Thiéry et al., 1992) (CB): myristic acid (0.26 parts), palmitic acid (1.37 p.), stearic acid (0.18 p.), methyl palmitate (0.59 p.), methyl palmitoleate (0.08 p.), methyl linoleate (0.23 p.), methyl linolenate (0.20 p.), methyl oleate (0.61 p.) and methyl stearate (0.29 p.); b) the 3 major esters (ternary blend – TB): methyl palmitate (0.59 p.), methyl linoleate (0.23 p.), methyl stearate (0.29 p.); and c) a binary blend of the two major components (BB) of the complete blend: palmitic acid (1.37 p.) and methyl palmitate (0.59 p.). Chemicals were diluted in a 10% methanolic solution in water with 1% of wetting substance (Triton X 100, Prolabo). Amounts of chemicals were adjusted to obtain two doses: 0.03 mg (low dose) and 0.3 mg (high dose) per apple which corresponded respectively to ca 72 and 720 *L. botrana* egg equivalents (LBE) per apple. This corresponeds to 0.9 and 9 LBE per cm^2 of apple surface.

Abbreviations: CB – complete blend, BB – binary blend, TB – ternary blend, LBE – *L. botrana* egg equivalent, CB1 – complete blend at 72 LBE, CB2 – complete blend at 720 LBE, BB1 – complete blend at 72 LBE, BB2 – complete blend at 720 LBE, TB2 – ternary blend at 720 LBE.

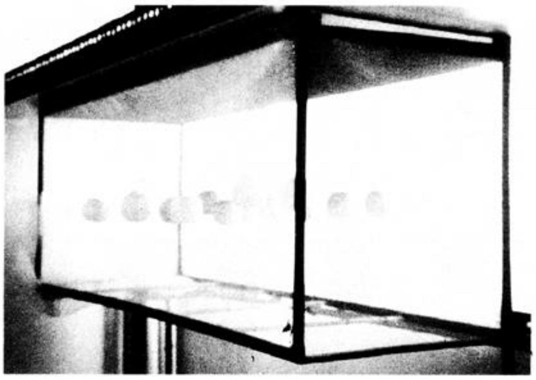

Fig. 1. Bioassay arenas used to study oviposition site selection in *C. pomonella.* Sixteen apples were hung from the top of the experimental arena and 4 mated females were released and allowed to oviposit freely during one night.

Statistics

The homogenity of egg distribution in the control replicates was assessed by a dispersion index calculated from each cage (I = variance / mean, I × (n – 1); values tested for fit to a Poisson distribution). Chi^2 analysis was used to compare: 1) frequency of oviposition between treated and untreated apples under treatment, 2) the mean number of eggs per female and the number of ovipositing females among treatments, and 3) the percentage of treated apples bearing no egg in each treatment [fourfold table (Sachs, 1984)]. The Mann Whitney U test (Sachs, 1984) was used to compare the deterrence index and number of eggs between treatments

RESULTS

Number of ovipositing females

In the control trials, 28 of the 36 mated females oviposited during one night on untreated apples. In the other treatments this number ranged from 20 (high dose CB) to 27 (low dose CB), and differences were not statistically significant (Chi^2 on the highest difference NS). Since all the females were mated, non oviposition could be attributed to behavioural factors.

Numbers of eggs per female

The mean number of eggs laid per female was not significantly different among dual choice trials. It ranged from 18.9 eggs per female (high dose CB) (no standard deviation because of grouped females) to 25.4 per female (low dose CB) with a value of 23.9 eggs in the control trials having all clean apples (Chi^2 on the highest difference, NS).

Oviposition site selection

In the control trials (when all apples were untreated), eggs were distributed homogeneously among the 16 apples. Analogous to a chess board type location of the eggs, totals of 334 eggs were deposited on the "white" and 337 on the "black" (Fig. 2). Dispersion indices calculated for each of the 9 control cages were not significantly different from a homogenous distribution. The mean number of eggs per apple in the control trials was 4.6 ± 4.0, with 8.3% of apples bearing no egg (Table 1).

The complete blend (CB1) elicited a significant deterrence at the dose of 72 LBE per apple (Chi^2, 9.61 1df, $P < 0.01$) (DI = –31%) (Fig. 2). This avoidance was intensified in

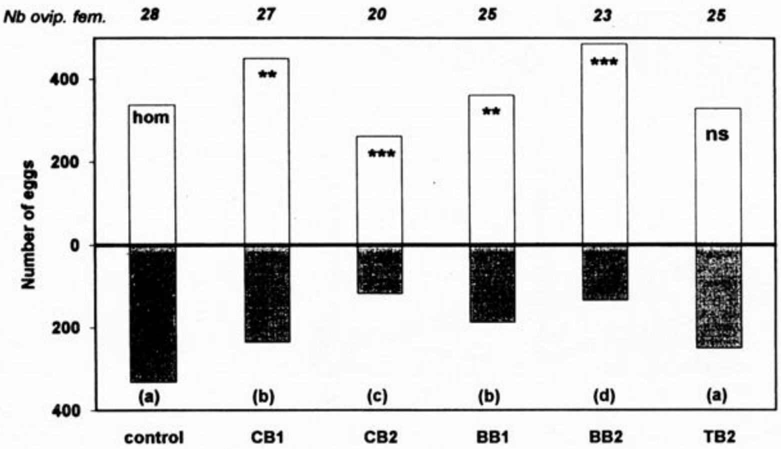

Fig. 2. Mean numbers of eggs laid by *C. pomonella* on treated (grey bars) and untreated apples (white bars) in dual choice experiments, using 36 females in all. For each treatment, 1 and 2 indicate doses of 72 and 720 *L. botrana* egg equivalents per apple respectivelly. Numbers above the frame are the number of females that oviposited during each experiment. Key to statistics: hom. indicates a distribution of eggs which is not different from a random distribution. Stars inside bars indicate statistical differences within experiment (** at $P < 0{,}01$, *** at $P < 0{,}001$, ns no difference; Chi^2 analysis). Different letters indicate statistical differences between treatments (see text for exact probabilities) (Mann Whitney U test).

response to the tenfold dose (CB2) in which a total of 118 eggs was laid on treated apples and 261 on the untreated ones (Chi^2: 14.24 1df, $P < 0.01$) (Fig. 2). The deterrence index at the 720 LBE dose of CB (Di = –38%) was greater than at the dose of 72 LBE (Di = –31%) ($P < 0.01$; Mann Whitney U test). Compared to control trials, trials involving CB1 reduced the mean number of eggs per apple (3.3 ± 2.9, $P < 0.0001$, Mann Whitney U test), although the percentage of apples bearing no eggs was not significantly different (Chi^2, NS) (Table 1). Trials with CB2 significantly reduced both the number of eggs/apple (1.6 ± 1.8) (Mann Whitney U test, $P < 0.0001$) and the percentage of attacked apples (37.5% of apples bore no eggs; (Chi^2: 24.15 1df $P < 0.001$) as compared with control trials (Table 1).

TABLE 1. Mean numbers of eggs of *C. pomonella* laid per apple and percentages of apples bearing no eggs as a consequence of the 3 different blends of components (CB: blend of 9 compounds, BB: blend of palmitic acid and methyl palmitate, TB: blend of 3 esters) at two doses (72 LBE per apple and 720 LBE per apple). TB was used only at the dose 2. Control consisted of 16 untreated applcs trials. Blends were presented in a binary choice with 8 treated vs 8 untreated apples per trial. Sd is standard deviation. Key to statistics: data with different letters are statistically different at the indicated probability.

	Control	CB1	CB2	BB1	BB2	TB2
Number of apples	144	72	72	72	72	72
Mean number of eggs/apple ± sd	4.6 ± 4.0	3.3 ± 2.9	1.6 ± 1.8	2.6 ± 2.8	1.8 ± 2.0	3.5 ± 4.4
statistics (at $P < 0.02$)*	(a)	(b)	(d)	(c)	(d)	(b)
% of apples without eggs	8.3	13.9	37.5	18.1	26.4	18.1
statistics (at $P < 0.05$)*	(a)	(a)	(c)	(b)	(b, c)	(b)

* lower values of P given in text

Apples treated with a binary mixture (palmitic acid and methyl palmitate) at the dose of 72 LBE per apple (BB1) were significantly avoided (361 eggs on untreated apples, 188 on treated ones) (Chi^2: 9.93 1df, $P < 0.01$) (Fig. 2). The deterrrence index (Di = –32%) was not statistically different from that observed with CB1 (Mann Whitney U test). However, BB2 provoked strong deterrency (487 eggs on untreated apples, 134 on treated) (Chi^2: 32.31 1df, $P < 0.001$) (Fig. 2). This deterrency was higher than that observed in any other treatments (Di = –57%) ($P < 0.005$; Mann Whitney U test). In trials involving BB1 and BB2, significantly fewer eggs than in control trials were found per apple: 2.6 ± 2.8 and 1.8 ± 2.0 respectively vs. 4.6 ± 4.0 in the control trials) (Mann Whitney U test, $P < 0.0001$) (Table 1). Percentages of apples with no eggs were also higher than those observed in control trials (18.1% and 26.4% respectively vs. 8.3% in the control) [Chi^2: 4.19 $P < 0.05$ (BB1); 11.42 $P < 0.001$ (BB2)].

The blend of 3 esters (methyl palmitate, methyl linoleate and methyl stearate) (TB2) provoked a slight deterrency (328 eggs on untreated apples, 253 on treated) (Chi^2, NS). The deterrence index (DI = –13%) was not significantly different from the control treatment (Mann Whitney U test). Trials with this blend also reduced significantly the number of eggs per apple compared to control trials (3.5 ± 4.4) (Mann Whitney U test, $P < 0.003$) and they were significantly more apples with no egg than in the control trials (Chi^2 4.19, $P < 0.05$) (Table 1). Trials with TB2 affected the number of eggs per apple equivalent to the effect observed in trials with CB1 (Mann Whitney U test, NS).

DISCUSSION

After having laid one egg, females of *C. pomonella* often take off and re-initiate sequences of oviposition site selection. Therefore, several eggs per apple is often considered as the result of different ovipositing females or at least as the consequence of consecutive complete ovipositional bouts by a single female (Caesar, 1911; Hall, 1929 both quoted in Jackson, 1979).

A slight but not significant decrease in the number of eggs laid per females was observed in the two cases where high dose of esters was applied (CB2 and TB2). This effect should be mentioned since it was observed in response to the 9 component blend applied on cardboard (Thiéry & Gabel, 1993). The effect of esters on such a reduction must be checked using isolated females. A reduction in the number of females that oviposited during exposure to the 9 component blend at the highest dose was noticed. Despite the lack of statistical significance, it is striking that this phenomenon occurred in response to the blend of components that also reduced the number of eggs laid per female.

In the control trials, only 8% of apples bore no eggs. It is probable that this was due to the high density of females per arena (4 females/16 apples). The blend of 9 and 2 components used at high dose increased the protection of apples, with respectively 38% and 26% of apples bearing no eggs. However, at a dose of 72 LBE per apple, increased protection was observed only with the binary blend. The blend of 3 esters at high dose also increased protection slightly (18% of apples with no eggs).

Apples treated with the blend of 9 components identified from *L. botrana* eggs were significantly avoided by *C. pomonella* ovipositing females. The deterrence index obtained in the present experiments is similar to that observed with 5 females per arena ovipositing on artificial substrates (Thiéry & Gabel, 1993). It is lower than that observed for females isolated with cardboard providing a treated/untreated dual choice (DI = –59%). On apples, the higher indices of deterrence always occurred in cages where it was suspected that a single female oviposited. However, the set of data was to accurately correlate female density with the selective behaviour. This is in agreement with observations made on isolated females laying on cardboard (Thiéry & Gabel, 1993).

Harvested apples produce α farnesene which stimulates *C. pomonella* oviposition behaviour (Wearing & Hutchins, 1973). Although it might be expected that it would compromise the effect of deterrents, the present results indicate that the ovipositional avoidance behaviour has similar expression on harvested apples in comparison with previous data obtained from artificial substrate (Thiéry & Gabel, 1993).

The binary mixture of palmitic acid and methyl palmitate produced identical or higher deterrencies when compared to corresponding doses of the 9 component blend. The ternary blend of methyl esters used at identical concentration had little effect on ovipositional site selection. Therefore, the deterrency observed in our experiments may be attributed in part to palmitic acid. We cannot explain why the binary blend produced higher deterrency in comparison with the complete blend, except that components which were not present in the binary mixture could have interfered with the behavioural response.

Recent chemical analysis of *C. pomonella* egg surface extracts (D. Thiéry, B. Gabel & P. Farkaš, unpublished data), revealed that palmitic acid is its major constituent. Therefore it is hypothesized that palmitic acid may be involved in egg spacing behaviour.

The occurrence of an oviposition deterring pheromone in *C. pomonella* has yet to be clarified, even if Roitberg & Prokopy (1982) concluded that females could not discriminate between occupied and non occupied resource. In the present experiments, it is evident that significant deterrency was provoked with compounds associated with eggs of another tortricid species, but this required higher doses of egg equivalents than those used by Roitberg & Prokopy (0.9 per cm^2 in our study against less than 0.02 per cm^2 in theirs). Our current work aims at further identification of *C. pomonella* egg extracts and assays of various blends of egg-associated components.

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