3 Hölldobler, B., and Wilson, E. O., Behav. Ecol. Sociobiol. *3* (1978) 19.
4 Rettenmeyer, C. W., Topoff, H., and Mirenda, J., Annls ent. Soc. Am. *71* (1978) 519.
5 Markin, G. P., Annls ent. Soc. Am. *63* (1970) 1238.
6 Benois, A., Ins. Soc. *20* (1973) 267.
7 Aron, S., Deneubourg, J. L., Goss, S., and Pasteels, J. M., in: Lecture Notes in Biomathematics – Biological Motion, p. 533. Eds W. Alt and G. Hoffman. Springer Verlag, New York 1989.
8 Aron, S., Pasteels, J. M., and Deneubourg, J. L., Biol. Behav. *14* (1989) 207.
9 Siegel, S., and Castellan, N. J., Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York 1988.
10 Batschelet, E., Circular Statistics in Biology. Academic Press, New York 1981.
11 Aron, S., Ph. D. Thesis. Université Libre de Bruxelles, Belgium 1990.
12 Keller, L., Passera, L., and Suzzoni, J. P., Physiol. Ent. *14* (1989) 157.
13 Skaife, S. H., Trans. Roy. Soc. South Africa *34* (1955) 355.

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Identification of an oviposition-regulating pheromone in the European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae)

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Abstract. The oviposition of the European grapevine moth (EGVM) *Lobesia botrana* can be deterred by an extract of conspecific eggs corresponding to 20 egg equivalents. The reduction of the oviposition behavior is dose-dependent. Nine chemicals have been extracted from the eggs and identified as straight chain fatty acids and esters of fatty acids. A mixture of these rather simple molecules induces the same levels of deterrence as the total extract. It might be possible to use oviposition regulating pheromone in the future for the control of EGVM populations.
Key words. Oviposition-deterring pheromone; Lepidoptera; Tortricidae; *Lobesia botrana*; eggs; fatty acids; esters of fatty acids.

The European grapevine moth (EGVM) *Lobesia botrana* (Lepidotera: Tortricidae, Olethreutinae) is a major pest in vineyards in Europe. The yield reductions caused by this insect are due both to damage by the larvae and to further attack by fungi. The reduction varies according to the grape vine cultivars. It can attain more than 70% in Greek vineyards [1], while almost 100% of grapes have been reported to be attacked in Italian vineyards [2]. Like other Olethreutinae species, *L. botrana* females typically lay isolated eggs on the flower buds of the grapevines or on the grapes, depending on the adult generation involved, and the plant phenology [3]. Because of the low density of eggs naturally observed on the flowers or the grapes in vineyards, we hypothesized that the eggs carry an epideictic pheromone which influences the oviposition of conspecific females and regulates the egg spacing in *L. botrana*. Biological evidence has recently been presented for an oviposition-deterring pheromone (ODP) in this species, extracted from the egg surface [4]. We present here the chemical identification of nine constituents of this pheromone. The constituents are linear fatty acids and methyl esters of fatty acids.

Materials and methods

The insects used for egg extraction and bioassays originated from a stock culture, annually infused with insects collected in the field, reared on a semi-artificial diet [5]. The extraction method was adapted from that developed for the Lepidopteran Pyralidae *Ostrinia nubilalis* [6]. We collected about 21300 eggs from 386 two-day-old mated females individually placed for 24 h in glass tubes (diam. = 1 cm, L = 8 cm). After removing the females, the eggs were washed for 12 h with purified methanol (GC purity > 99.99%) (ca 2 ml/tube). Scales were removed by filtration on millipore filters (type GW, 0.22 µm), and the extract was concentrated under a stream of purified nitrogen. The oviposition assays were performed on artificial oviposition substrates (cardboard) providing a choice between treated areas (32 application circles of 1.1 cm diam., centers 2.1 cm apart) and non-treated areas (between the application circles). The treated area corresponded to 1/3 of the non-treated area (see Gabel and Thiéry [4] for a detailed description). Different dilutions of the extract (expressed in egg equivalents [e.e.]) were offered to groups of 10 mated females which had already

started to oviposit (same source as mentioned above). A deterrence index Di (in %) was calculated from the number of eggs laid on the treated (A) and the non-treated (B) areas as follows: $Di = (3A-B)/(3A+B) \times 100$, varying from −100% (maximum of deterrence) to +100% (maximum of preference). Five treatments were assayed: blank (carboard without any treatment), control (dots of pure methanol), 2 doses of extract (3 and 20 e.e.(dot [i.e. 0.6 and 4 e.e/µl]) and synthetic blend (equivalent to the dose of 20 eggs/dot). The numbers of eggs laid were compared by t-test analysis [7].

Gas chromatographic (GC) separations were performed on a HP 5890 II GC (split/splitless injector, HP Ultra 1 column [0.20 mm i.d., 25 m], flame ionization detector) using the following temperature program: 2 min isothermal at 60 °C linearly increased by 5 °C min^{-1} to 240 °C with a 10 min isothermal at the end. The chemicals were analyzed by the GC/MS method (GC described above coupled to a HP 5971 A mass selective detector with positive electron ionization, 70 eV). The identifications were confirmed by comparing mass spectra with data published in the literature, by comparison of spectra and GC retention times obtained with standards. The amount of each constituent was determined by comparison with an internal standard (standard = palmityl acetate).

Results

In the blank and in the controls, the eggs were evenly distributed on the oviposition substrate. This demonstrates that the methanol had evaporated or that oviposition behavior of *L. botrana* is not affected by the presence of methanol. Females avoided depositing eggs on the dots of extract corresponding to 20 conspecific eggs (Di = −50.3%). A slight deterrence of 15% was observed at the dose equivalent to 3 eggs/dot. The exposure to the egg extract did not significantly reduce the mean number of eggs per female. This indicates that the egg extract acts more like a choice-enhancing pheromone than an oviposition-reducing pheromone. Similar observations have already been reported in other species of Lepidotera [6, 8]. Interestingly, the number of eggs laid by *L. botrana* females 24 h after having been exposed to the total egg extract is reduced by about 25% [4]. This result suggests a lasting effect of the exposure to the semiochemicals extracted from the eggs. Aversive learning might be implicated in that phenomenon, but this has not yet been proven.

The nine constituents identified from the egg extract are C_{14} to C_{18} saturated straight chain fatty acids and methyl esters of C_{16} to C_{18} saturated and unsaturated straight chain fatty acids (fig. 2). The biological activity of a synthetic blend made from the nine identified chemicals was confirmed using the procedure described above. The chemicals (commercial source Sigma Chemical Co., USA; minimal indicated purity 95%) were dissolved in pure methanol. This blend was tested at the dose corresponding to the highest dose of the extract (4 e.e/µl). The mean deterrence index obtained with that synthetic blend was −54.8% with a maximum of −62.1% in one replicate which is slightly higher than the values observed with the natural extract. There was no statistically significant difference between the synthetic blend and the crude extract. The efficiency observed with the blend of nine chemicals confirms the structure elucidation; it sup-

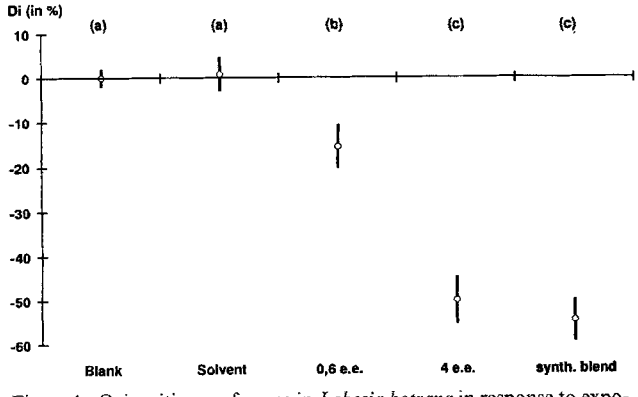

Figure 1. Oviposition preference in *Lobesia botrana* in response to exposure to blank treatment, to the extraction solvent and to different dilutions of egg extract (expressed in egg equivalents (e.e.) per µl). Open circles represent the index of deterrence calculated from at least 10 replicates, except with the synthetic blend (6 replicates). Vertical bars represent standard deviation; different letters indicate statistical differences (t-test, $p < 0.005$).

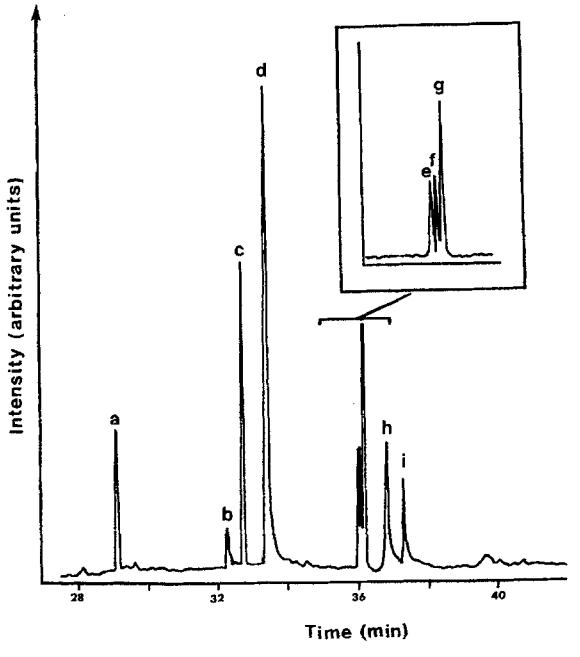

Figure 2. Part of the gas chromatographic analysis of *Lobesia botrana* egg extract. Amount of chemicals per egg equivalent shown in brackets. **a** = myristic acid (0.26 ng), **b** = methyl palmitoleate (0.08 ng), **c** = methyl palmitate (0.59 ng), **d** = palmitic acid (1.37 ng), **e** = methyl linoleate (0.23 ng), **f** = methyl linolenate (0.20 ng), **g** = methyl oleate (0.61 ng), **h** = methyl stearate (0.29 ng), **i** = stearic acid (0.18 ng). GC characteristics: HP Ultra 1 column (0.20 mm i.d., 25 m) split/splitless injector; heating program 2 min. isothermal at 60 °C, 5 °C × min − 1 to 240 °C ended by a 10-min isothermal.

ports the hypothesis that the C_{14} to C_{18} saturated fatty acids and the methyl esters of C_{16} to C_{18} saturated and unsaturated fatty acids regulate the oviposition behavior of *L. botrana*.

Five constituents (methyl palmitate, methyl palmitoleate, methyl linoleate, methyl oleate, methyl stearate) have already been found in the eggs of another Lepidopteran, *O. nubilalis*[6], methyl palmitate being one of the major constituents in both species. Esters of fatty acids may well be found in the eggs of other moth species. Myristic acid, palmitic acid, stearic acid and methyl linolenate analyzed in *L. botrana*, were not found in *O. nubilalis*. The total amount of methyl palmitate, methyl palmitoleate, methyl linoleate, methyl oleate and methyl stearate is about ten times higher in *O. nubilalis*[6] than in *L. botrana*. The difference in the amount of esters present on the eggs is probably due to the difference in egg size, but may also be related to differences in the biology of the two species and to differences in sensitivity of the females. Three of the compounds we have identified in *L. botrana* eggs were also identified in extracts of larval frass of the noctuid moth *Spodoptera litoralis* (Lepidoptera)[9], which act as oviposition deterrents[10].

Our present results confirm the role of such chemicals in insect communication and especially as oviposition deterrents. Esters of linear fatty acids have already been proposed as oviposition choice-enhancers in *O. nubilalis*[6]. The oviposition deterrence triggered by various C_{14} to C_{18} straight chain saturated and unsaturated fatty acids in the mosquito *Culex quinquefasciatus* (Diptera)[11] also reinforces the role of such molecules as semiochemicals. The simplicity of the molecules we have identified suggests that there is little specificity in the regulation of the oviposition behavior. Recent data indicate that such molecules can affect the oviposition behavior in other Lepidopteran species as well[6, 12] (Gabel and Thiéry, unpublished data).

Tortricid moths that lay isolated eggs probably represent good models for investigating the role of epideictic pheromones as oviposition regulators. In *L. botrana*, such pheromones may explain the dispersion of the eggs on the grapevine fruiting organs. Because of the relatively simple structure of the molecules involved, and the efficiency of synthetic formulations, the use of this oviposition-deterring pheromone as a method of controlling this pest may be expected in the future. Field experiments are now in progress to evaluate the efficiency of this pheromone under natural conditions.

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1 Savopoulo-Soultani, M., Angelakis, E., Hatzissiliadis, A., Tzanakakis, M. E., and Stavridis, D. G., in: Proc. CEC/IOBC int. Symp. on Plant protection problems and prospects of integrated control in viticulture, p. 47. Lisboa-Villa Real 1988.
2 Dalla Monta, L., in: Proc. CEC/IOBC Int. Symp. on plant protection problems and prospects of integrated control in viticulture, p. 75. Lisboa-Villa Real 1988.
3 Roehrich, R., and Boller, E., in: Tortricid pests: their biology, natural enemies and control, p. 507. Elsevier Sci. Publ. (1991) 507.
4 Gabel, B., and Thiéry, D., J. chem. Ecol. *18* (1992) 353.
5 Stockel, J., Roehrich, R., Carles, J. P., and Nadaud, A., Phytoma *412* (1989) 45.
6 Thiéry, D., and Le Quéré, J. L., Naturwissenschaften *78* (1991) 132.
7 Statgraphics statistics, Stat. graph. Corp. 2.6 (1987).
8 Schoonhoven, L. M., J. chem. Ecol. *16* (1990) 3043.
9 Klein, B., Schildknecht, H., Hilker, M., and Bombosch, S., Z. Naturforsch. *45c* (1990) 895.
10 Hilker, M., and Klein, B., J. chem. Ecol. *15* (1989) 929.
11 Hwang, Y. S., Schultz, G. W., and Mulla, M. S., J. chem. Ecol. *10* (1984) 145.
12 Thiéry, D., Gabel, B., and Pouvreau, A., in: Proc. 8th int. Symp. Insect-plant Relationships, Wageningen (1992) in press.

0014-4754/92/070697-03$1.50 + 0.20/0