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(*Z,E*)-α-Farnesene—An Electroantennogram-Active Component of *Maladera matrida* Volatiles

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Abstract—It has previously been shown in field-trapping experiments and laboratory olfactometer bioassays that virgin females of *Maladera matrida* Argaman (Coleoptera, Scarabaeidae) and their volatiles, both in the presence of food (cut peanut leaves), are efficient attractants for *M. matrida* males and females. In this study GC–EAD experiments using male antennae and GC–MS experiments revealed that (*Z,E*)-α-farnesene is an active component of *M. matrida* female volatiles. The identification and quantitative electrophysiological responses (EAG) of synthetic (*Z,E*)-α-farnesene were obtained with male and female antennae. It was also shown that (*Z,E*)-α-farnesene is not a component of the plant volatiles that serve as synergistic components of the mixture of attractants or of the source of food for *M. matrida*. Copyright © 1996 Elsevier Science Ltd

Introduction

The *M. matrida* Argaman beetle (Coleoptera, Scarabaeidae, Melolonthinae) (Fig. 1) was first detected in Israel in 1983 and was classified as a new species.[1] *M. maladera* is considered a serious polyphagic pest, causing damage to many agricultural crops during the grub and the adult stages. Its major host plants include peanuts, sweet potatoes, potatoes, flowers, orchard and citrus crops. The species is also a nuisance to man.[2,3]

Field-trapping experiments and laboratory olfactometer bioassays showed that *M. matrida* beetles are highly attracted by live virgin females in the presence of food (cut peanuts leaves) and by volatiles produced by live virgin females plus food.[3]

During the past half decade great progress has been made in the identification of scarab pheromones by applying uniform consistent bioassays in the form of electroantennograms (EAG) and/or gas chromatography in combination with an electroantenno detector (GC–EAD).[4]

In this paper we describe our progress in characterizing, by EAG and GC–EAD followed by GC–MS, the volatiles emitted by live virgin beetles as a means of chemical communication. Our study revealed that (*Z,E*)-α-farnesene is an active component of the female volatiles of *M. matrida.*

Key words: *Maladera matrida*, Coleoptera, Scarabaeidae, pheromone, (*Z,E*)-α-farnesene [(*Z*3,*E*6)-3,7,11-trimethyldodeca-1,3,6,10-tetraene], electroantennogram (EAG), gas chromatography electroantenno detector (GC–EAD).

Figure 1. *M. matrida* Argaman beetle (Coleoptera, Scarabaeidae) with the excited lamellet antenna (×20).

Results and Discussion

The live virgin *M. matrida* females and their volatiles proved to be bioactive in field trapping experiments and laboratory olfactometer bioassays.[3] In the course of our ongoing study of chemical communication in *M. matrida*, the next required step was to isolate the bioactive components in the female volatiles solution that are responsible for the attraction. GC showed that the minute amounts of volatiles produced by *M. matrida* females contain ca. 20–30 components, some of which might be bioactive (sex or aggregation pheromone?). A comparison of GC profiles of volatiles obtained from virgin females versus volatiles from wild females, virgin

males and wild males revealed a component (with $M_r = 166$ and a base peak of 83 for the two homolitic fragments). This material was identified by mass spectrometery as bicyclohexyl, which was also isolated from the volatiles solution of rose petals. Rose petals served as a food source for *M. matrida* and apparently the bicyclohexyl was transferred to the beetles and to their volatiles.

The application of GC–EAD and unique techniques and instrumentation[4] to the tiny lamellet antenna of the *M. matrida* makes it possible to single out bioactive volatile components from live virgin *M. matrida* females. Practical problems that still exist are connected to the short life time of the antennae (30–60 min) and the low intensity of the signal, which can be maximized by setting two antennae in between the two electrodes.

Preliminary GC–EAD experiments with male antennae showed that the antennae were sensitive to and the electroantennogram responded to methyl benzoate and eugenol, which are known attractants. The signal-to-noise ratio was 4–8:1, which is considered satisfactory. GC–EAD experiments using male antennae performed on a solution of volatiles from live virgin *M. matrida* females showed that the GC peak (numbered as no. 1 in Figure 2) with an $t_R = 30.316$ min on a nonpolar column (DB-wax) exhibited a strong EAD response (0.0038 ± 0.0008 mV; $n = 3$; $S/N = 4$) and was reproducible for two beetle equivalents (each beetle equivalent was calculated as the total volatiles emited by a single female over 150 min per day, during 18 days of collection) in each experiment. The EAD response was also repeated on a polar column (HP-1) with $t_R = 24.595$ min (0.00115 ± 0.00021 mV; $n = 3$ for two beetle equivalents in each experiment; $S/N = 4.5$).

GC–MS analysis of the electrophysiologically active component (Fig. 3) revealed that (*Z,E*)-α-farnesene ($M_r = 204$) was an active component in the solution of volatiles from live virgin female *M. matrida* beetles.

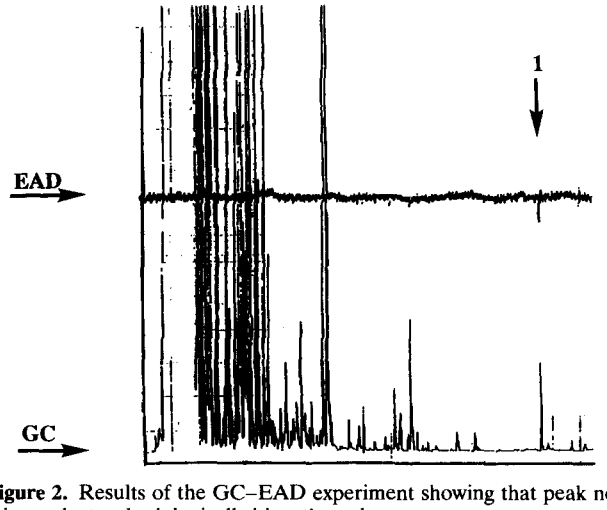


Figure 2. Results of the GC–EAD experiment showing that peak no. 1 is an electrophysiologically bioactive substance.

The mass spectrograph of the natural component (*Z,E*)-α-farnesene provided a good match with that in the NIH–MS data library.

Farnesene isomers, which have previously been reported to have pheromonal properties have been isolated from plant and insect sources;[5] e.g., (*Z,E*)-α-farnesene, i.e., (*Z*3,*E*6)-3,7,11-trimethyldodeca-1,3,6,10-tetraene has been isolated from the Dufour glands of three species of formicine ants and two species of *Myrmica* ants, *M. rubra* and *M. scabrinodis*.[5]

The identification and determination of any natural substance such as (*Z,E*)-α-farnesene as bioactive, and in this case as electrophysiologically active, is always based on reproducible inspection of the bioaction (or electrophysiological responses) of the corresponding synthetic material on the insect. We thus applied synthetic (*Z,E*)-α-farnesene (Scheme 1) to *M. matrida* male and female antennae. The electrophysiological responses were reproducible with strong intensity in the EAG instrument. On male antenna a pulse of 0.086 ± 0.017 mV ($n = 5$; $S/N = 8$) was detected for 0.08 μg (*Z,E*)-α-farnesene equivalent in each experiment and the female antenna responded 0.069 ± 0.021 mV ($n = 4$; $S/N = 7$). Further experiments with male and female antennae showed dependence of EAG intensity on the concentration of (*Z,E*)-α-farnesene. Dilution by 10 of the concentration of the inspected material (*Z,E*)-α-farnesene caused a decrease in EAG responses by a factor of 8 (Table 1).

We can therefore conclude that (*Z,E*)-α-farnesene, which is secreted by *M. matrida* females, is an a electrophysiologically active component for both *M. matrida* sexes. Field studies are needed to prove the real bioactivity of (*Z,E*)-α-farnesene.

To eliminate the possibility that the biologically active material, i.e., (*Z,E*)-α-farnesene, might also be present in sources other than *M. matrida* volatiles, we analyzed the composition of airborne volatiles of cut up peanut foliage, which was known to contain synergistic components of the attractant mixture for *M. matrida*,[3] and fresh rose petals, which are a food source for *M. matrida*.[3]

GC–MS analysis showed that (*Z,E*)-α-farnesene is not a component of peanut leaves volatiles, which act synergistically with the insect pheromones or of volatiles of rose flowers,[6] which serve as a food source for *M. matrida*.

Leal,[7] in a recent article on the evolution of sex pheromones of the scarab beetle, refers to the pheromones of the subfamily *Melolonthinae*, and shows that terpenoid compounds, *inter alia*, are typical of this subfamily. (*Z,E*)-α-Farnesene falls into this category and thus lends support to his hypothesis that more primitive *Melolonthinae* (as compared with *Rutelinae*) use these chemicals because of their antifungal and

antibacterial properties and thus as defensive chemicals, which later developed into pheromones.

Experimental

Insects

M. matrida beetles were raised in the laboratory from grubs that were collected in the field. Adult insects were kept in separate jars and reared according to Yarden and Shani.[3] These insects were used for bioassays.

Collection of plant and insect volatiles

The airborne volatiles of male and female beetles were collected according to a previously reported method.[3] Volatiles of rose petals and peanut foliage were collected in a similar system. Fresh unsprayed rose petals from different species (Rosa chinesis: Banksiae, Lutea, Scarlett Knight, Samourai, Michelle Meilland, Nevada and Charleston) and peanut (Chanouch) foliage from a biological field served as sources of volatiles.

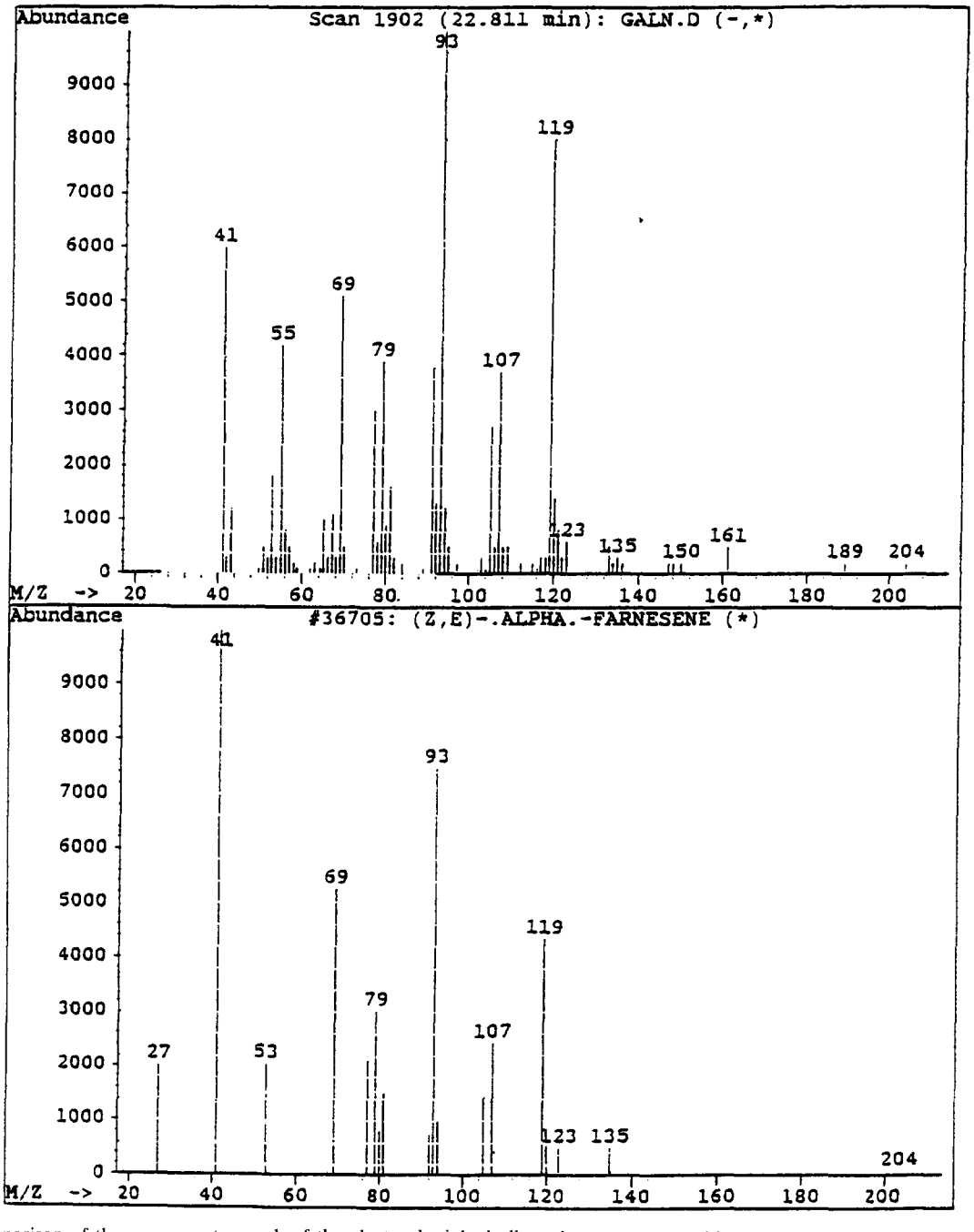
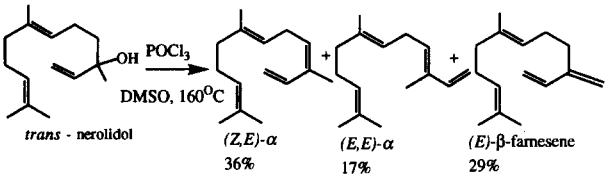


Figure 3. Comparison of the mass spectrograph of the electrophysiologically active component with the NIH–MS data library, showing a good match with (*Z,E*)-α-farnesene.



Scheme 1. Synthesis of (*Z,E*)-α-farnesene {(*Z*3,*E*6)-3,7,11-trimethyl-dodeca-1,3,6,10-tetraene}.

Gas chromatography combined with an electroantennographic detector (GC–EAD) and electroantennograms (EAG)

GC–EAD of the bioactive volatiles was performed according to the method of Struble and Arn[8] as modified by Leal et al.[4] The modification included an acrylic station for setting lamellet antennae of *M. matrida*. With the aid of binoculars the antennae were set such that the sensilla faced the flow from GC effluents. GC–EAD was carried out with a Hewlet Packard GC, model 5890 with a GC effluent splitter (1:1; FID:EAD) and a glass transfer system for the Tayo Corp, EAD amplifier model 11-T, both connected to a double-channel Yokogawa recorder model LR-4220. The volatiles were analyzed on two capillary columns (25 m long, 0.2 mm i.d. HP-1, and 30 m long, 0.25 mm i.d. DB-wax; J & W Scientific, Folson, California). The GC was programmed from 50 °C for 1 min, heating up to 180 °C at a rate of 4 °C/min, maintaining this temperature for 1 min, heating up to 240 °C at a rate of 10 °C/min and then maintaining this temperature for an additional 30 min.

The EAG was performed to test the synthetic (*Z,E*)-α-farnesene using an acrylic station (see the cross-section of Leal et al. diagram[4]) modified by size for setting tiny lamellet antennae (1 mm long when straightened out) of *M. matrida* (Fig. 4). The edges of the antennae were connected to the electrodes through Ringer's solution halls. The electrodes (silver wire, OD 0.5 mm, FSA Laboratory Supplies, Loughborough, U.K.) were connected to the stainless steel electrode holders, to the P-01 Universal probe and to an AM-05/b EAG/EAD amplifier and air delivery unit model Cs-05/b (all from Syntech, Hilversum, The Netherlands). The output from the amplifier was directed to a signal processing device (PC-computer to Barspec Chrom-A-set)

Table 1. Quantitative electrophysiological responses (EAG)[a] of *M. matrida* to synthetic (*Z,E*)-α-farnesene

Inspected antennae	Concentration of (*Z,E*)-α-farnesene[b] (μg)	Intensity (mV)
Males	0.08 μg	0.086 ± 0.017
Males	0.008 μg	0.0102 ± 0.0076
Females	0.08 μg	0.069 ± 0.021
Females	0.008 μg	0.0098 ± 0.0035

[a] The EAG was carried out using an acrylic station modified by size for setting the tiny lamellet antennae of *M. matrida* and to an Syntech AM-05/b EAG/EAD amplifier and air delivery unit model Cs-05/b. The output from the amplifier was directed to a signal processing device (PC computer to Barspece Chrom-A-set).
[b] (*Z,E*)-α-Farnesene (80% purity) was isolated from a mixture of farnesenes by preparative GC containing (*E,E*)-α-farnesene and (*E*)-β-farnesene (10% of each).

Figure 4. Cross-sectional binocular photograph of the tiny antenna of *M. matrida* (1 mm long when straightened out) set in an acrylic station for GC–EAD so as to allow the sensilla to face the flow from GC effluents.

Synthesis of (*Z,E*)-α-farnesene

A mixture of farnesenes was produced by dehydration of *trans*-nerolidol with phosphoryl chloride (Frutarom Co., Israel) in dimethyl sulfoxide (Aldrich Co., Milwaukee, Wisconsin) at 160 °C for 5 min, followed by separation by elution chromatography and preparative GC on a Varian aerograph model 920 equipped with 2 m long, 2.5 mm i.d. 10% OV-17 on Chromsorb-W at 130 °C isothermal.[9] The structure was confirmed by ^{1}H NMR and GC–MS as well as with the NIH MS Database.

Gas chromatography–mass spectrometry

The volatiles were analyzed on two different GC–MS systems: System 1 comprised a Finnigan GC–MS spectrometer (model 4500) system (Finnigan Corporation, Sunnyvale, California), equipped with a 30 m long, 0.25 mm i.d. SE-30 capillary column, programmed at 50 °C for 3 min and heating up to 300 °C at a rate of 10 °C/min and then maintaining this temperature for an additional 30–60 min. The carrier gas was helium at a pressure of 20 psi and the ionization intensity of the mass spectrometer electron impact (EI) mode was 70 eV. Additional runs were carried out with a 30 m long, 0.25 mm i.d., RTX and a 30 m long, 0.25 mm i.d., DB-5 capillary columns. System 2 comprised a Hewlet Packard GC, model 5890, and a GC–MS, model 5872, mass selective system (Hewlet Packard,) equipped with a capillary column and programed as mentioned for the GC–EAD.

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References

1. (a) Argaman, Q. *Shapirit* **1986**, *4*, 40 (in Hebrew), 47 (summary in English); (b) Argaman, Q. *Isr. J. Entomol.* **1990**, *24*, 21.

2. Harari, A.; Ben-Yakir, D.; Rosen, D. *J. Chem. Ecol.* **1994**, *20*, 361.

3. Yarden, G.; Shani, A. *J. Chem. Ecol.*, **1994**, *20*, 2673.

4. (a) Leal, W. S.; Mochizuki, F.; Wakamura, S.; Yasuda, T. *Appl. Entomol. Zool.* **1992**, *27*, 289; (b) Leal, W. S., Sawada, M., Hasegawa, M. *J. Chem. Ecol.* **1993**, *19*, 1303.

5. Morgan, E. D.; Thompson, L. D. *J. Chem. Soc. Perkin Trans I* **1985**, 399.

6. (a) Masda, Y. In *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry*, Halsted, John Wiley and Sons: New York, 1976; pp 188–192; (b) Flament, I.; Debonneville, C.; Furrer, A. In *Bioactive Volatile Compounds from Plants*; Teranishi, R.; Buttery, R. G.; Sugisawa, H., Eds.; American Chemical Society Symposium Series 525: Washington, DC, 1993; pp 269–281.

7. Leal, W. S. In *Pheromone Research: New Directions* Carde', R. T.; Minks, A. K. Eds.; Chapman and Hall: New York, 1996, in press.

8. Struble, D. L., Arn, H. In *Techniques in Pheromone Research*; Hummel, H.; Miller, T. A., Eds.; Springer-Verlag: New York, 1984; pp 161–178.

9. (a) Anet, E. F. L. J. *Aust. J. Chem.* **1970**, *23*, 2101; (b) Vander Meer, R. K.; Williams F. D.; Lofgren, C. S. *Tetrahedron Lett.* **1981**, *22*, 1651.

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