Análisis e identificación de los volátiles procedentes del barrenillo del olivo *Phloetribus scarabaeoides.* Contribución a la determinación de los semioquímicos asociados

Analysis and identification of volatiles associated to Olive Bark Beetle Phloetribus scarabaeoides (Bern.) Contribution to the determination of the semiochemicals involved

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RESUMEN

Se ha llevado a cabo un estudio sistemático de los semioquímicos asociados al barrenillo del olivo *Phloeotribus scarabaeoides* (Bern.) Que implica el análisis por CG y CG-EM de los volátiles procedentes de diferentes materiales (leños de poda, ramas del árbol, serrines e insectos)a fin de determinar las posibles kairomonas, feromonas y repelentes.
PALABRAS CLAVE: Phloeotribus scarabaeoides, semioquímicos, analisis mediante CG-EM.

ABSTRACT

A systematic study of the semiochemicals associated to the olive bark beetle* Phloeotribus scarabaeoides *(Bern.) is described. This study describes the analysis by GC and GC-MS the air volatiles from different sources (pruned logs, branches, frasses and insects) with the aim of determining the posible kairomones, pheromones and repellents.
KEY WORDS: Phloeotribus scarabaeoides, semiochemicals, GC-MS analysis.

INTRODUCTION

The olive bark beetle *Phloeotribus scarabaeoides* Bern. (Coleoptera, Scolytidae) is a very common insect infesting olive plants throughout the Mediterranean basin. Different pests, amongst which *Dacus oleae* or *Batrocera oleae, Prays oleae, Euzophera pinguis* and *Leperesinus varius,* among others, and *P. scarabaeoides* indeed, cause major damage to the olive groves, and have an important economic impact (González and Campos, 1994).

In order to contribute to the integrated pest control of the olive trees, we have studied the semiochemicals associated to *P. scarabaeoides.* The morphology and biology of the beetle have been described elsewhere (Russo, 1938a and b; Ballachowsky, 1949 and González, 1990). The adult olive bark beetles reproduce in waste wood resulting from pruning in galleries dug under the log´s bark. The new generation flies back to the trees, infesting the young olive branches, where they dig feeding and overwintering galleries. This is the most damaging period to the tree (Arambourg, 1984 and Benazoun, 1992).

Little is known on the semiochemicals involved in the infestation, aggregation, and sexual attraction of *P. scarabaeoides*. Ethylene has been reported to be released in large amounts by olive trees (Campos, 1994 and Campos and Peña, 1995) playing an important role as a primary attraction kairomone for the beetle. Also 2-decanone has been identified in abdominal washes of virgin female olive bark beetles, showing attractancy to *P. scarabaeoides* individuals (Peña, 1992), and a preliminary study on the response to semiochemicals in laboratory bioassays have been reported (Szauman-Szumski, 1998).

A sistematic study of the semiochemicals associated to the olive bark beetle would imply the analysis of the air volatiles from host plant in several states (to determine possible kairomones or repellents), the analysis of the frass where pheromone compounds are usually present, and finally, those emmited by the insects. Our preliminary results have been communicated (Plaza, 1996; Rodríguez, 1997 and 1998).

MATERIALS AND METHODS

The following samples have been analyzed:

1.- Olive wood

1a) Logs (**L**): Medium size branches directly cut from the trees. Those are the branches that, a few days after pruning will be infested by the pioneering females.

With the aim of determining the variation of the volatiles with ageing, they have been analyzed several times along 10 weeks next to their pruning.

1b) Old Logs (**O.L.**): Logs pruned in the former season, and left over in the olive grove. These logs are never infested by the beetles. The comparison of their components with the ones of both infested (**I.L.**) and uninfested (**L**) logs, would allow to know the components that have been formed during the ageing process (and could therefore act as repellents) and the components present in freshly cut logs and/or infested, and absent in **O.L.** which could act as attractants.

1c) Infested Logs (**I.L.**): Freshly cut logs stored in the olive grove and massively attacked by beetles.

1d) Young branches of living trees (**BR**): Usually infested by the new generation of beetles. Collected after the infestation, the analysis aims to determine the volatiles acting as attractants to the emerging beetles.

2.- Frasses

2a) Initiation frasses (**I.F.**): Frasses accumulated at the entrance holes of the reproduction galleries, and collected in the first 7 days after infestation of the prune logs. The sexual pheromones are thought to be released by the females in those frasses.

2b) Old frasses (**O.F.**): Frasses from the reproduction galleries, collected more than 7 days after infestation.

2c) Feeding galleries frasses (**F.F.**): Frasses from nutritional galleries dug by the emerging generation of beetles in young branches of the tree, and where they feed and overwinter.

3.-Beetles

The volatiles emmited by male (**M.B.**), female (**F.B.**) and mixed (**B**) live laboratory reared beetles were collected inmediately after the emergence of the individuals. To avoid a rapid death of the insects, due to the extreme need of the olive bark beetles for humidity, the extraction line (described below) was slightly modified, introducing between the rotameter and the vessel containing the sample, a gass-washing bottle with a small amount of water.

The air volatiles were collected in a cryogenic trap, using the method described by Browne (Browne, 1974), modified as follows: the air flow was generated from a high purity synthetic air cylinder (SEO N50). A rotameter maintained a constant flow at 50 mL/min. The air passed to a vessel containing the sample, then to an anhydrous silicagel trap to retain humidity, then to a glass U-tube placed inside a Dewar flask containing liquid nitrogen, and finally to a silicon bubbler.

The vessels containing the samples were adequate to their type and size: frasses in 10 cm glass tubes (5 mm i.d.); live beetles in gas-washing bottles,

and logs in 20 L glass desiccators. The air flow was kept for 8 hours. Afterwards, the U-tube was disconnected and the Dewar and U-tube were transferred to a freezer at -20ºC, and left overnight. A short glass tube filled with active charcoal was placed at every end of the U-tube. After the liquid air was completely evaporated, the tube was washed with 2 x 0.5 mL of high purity pentane. A small amount of $MgSO_4$ was added, and the solutions were transferred to a vial and stored at -20ºC. Prior to their analysis, the samples were evaporated under argon current until a volumen of c.a. 100 µL. Aliquots of 3 µL were injected in GC (HP 6890; column HP-5, 30 m, 0.25 µm ID, 0.25 mm film thickness) and GC-MS (Fisons VG, Mod. PLATFORM II, same column and temperature conditions). Two different temperature programmes were used for the sample analyses: Programme A: 80ºC, 3 min.; 80-120ºC at 10EC/min; 120ºC, 0.5 min.; 120-150ºC at 10EC/min; 150ºC, 3.5 min; flow (He): 1.1 mL/min.

Programme B: 45ºC, 3 min; 45-150ºC, 10ºC/min; 150ºC, 3.5 min; 150-250ºC, 5EC/min; 250ºC, 8 min; flow (He): 1.4 mL/min.

The chromatographic peaks were identified by comparison of their mass spectra with those in the libraries NIST/NBS and Wiley (6^{th}ed.), and, when possible, by comparison of their Rt with those of authentic samples (Aldrich, Sigma and Fluka). In Table I are indicated the Rt of standards used.

Tables II-IV show the analytical results for 18 products selected on the base of their significance in the semiochemistry of scolytids or their special abundance in most samples, and are expressed as the percentage of peak area in relation to the naphtalene peak. Naphtalene has been choosen as I-S because it is one of the major component in all samples. This method allowed to estimate the relative variations of every component in the different samples

TABLE I.

Standards	Rt A	Rt B	Standards	Rt A	Rt B
4-Methyl-2-pentanone	2.72	4.19	**Exobrevicomin**	6.71	10.44
Toluene	2.96	4.75	**1,6-Dioxaspiro[4.5] decane**	6.88	10.5
Hexanal	3.21	5.63	**1,7-Dioxaspiro[5.5] undecane**	8.56	11.1
Butyl acetate	-	5.66	**Nonanal**	7.95	11.53
o-Xylene	-	6.68	**Multistriatin**	8.66	12.22
p-Xylene	-	6.84	**2-Nonanone**	7.63	12.31
Dibutyl ether	4.10	7.16	**Benzoic acid**	9.11	12.76
2-Heptanone	4.17	7.30	**Endoborneol**	9.30	12.78
Heptanal	4.41	7.54	**2-decanone**	9.55	13.06
a-Pinene	5.00	8.33	**Naphtalene**	9.67	13.14
Benzaldehyde	5.41	8.86	**Decanal**	9.84	13.27
Sabinene	5.64	9.12	**Isobornyl acetate**	11.501	14.7
b-Pinene	5.74	9.21	**2-Undecanone**	11.401	14.8
6-Methyl-5-hepten-2-one	5.71	9.30	**Undecanal**	11.731	15.05
Octanal	6.07	9.63	**Dodecanal**	-	17.30
2-Ethyl-1-hexanol	6.43	10.13	**Transcaryophillene**	-	18.00
Limonene	6.61	10.22	**Tetradecanal**	-	22.55
Benzyl alcohol	6.65	10.29	**Methyl palmitate**	-	29.91

RESULTS AND DICUSSION

1.- Ageing of logs (**L**). Logs with a total weight of 2.3 Kg, colleted from the trees in 11-18th-1997, were extracted four times (L1 to L4) and analyzed using programme B. The results are shown in Table II.

2.- Analysis of old logs (**O.L.**). Three experiments were carried out on logs from the 1996 prunning season:

2a.- Extracted in 2-24th-97. Weight 4.15 Kg. Temperature programme A.

2b.- Extracted in 4-25th-97. Weight 3.17 Kg. Temperature programme A.

2c.- Extracted in 6-17th-97. Weight 3.17 Kg. Temperature programme B.

Results in Table II show the mean values of the three experiments for every selected compound.

3.- Infested logs (**I.L.**). Four experiments were carried out on I.L.:

3a.- Logs (0.61 Kg) infested on June 1996, and extracted 1 month later. Temperature programme A.

3b.- Logs (0.46 Kg) cut and infested in February 1996, and extracted about 10 days after infestation. Programme B.

3c.- Logs (2.28 Kg) cut and infested in March, 5th, 1996 and extracted about 10 days after infestation. Programme B.

3d.- Logs (4.5 Kg) infested in June 1997, and extracted 3 weeks later. Programme B.

In Table II are shown the mean values of these analysis.

4.- Branches (**BR**): Young branches (1.240 Kg, with leaves) cut from the trees in June 3th 1996, and extracted the next day (programme B). Results in Table II. In an independent experiment, a sample of branches in full bloom, cut in May 1997 has been analized. The results are not included in Table II, but it is worth saying that exobrevicomin, absent in the free of flowers samples of 1996, has been found in the 1997 samples in 59 %

5.-Initiation frasses (**I.F.**): Eight samples of initiation frasses (0 to 6 days after infestation, 0.7 to 6 g), collected either in the field or in laboratory have been analized, using programme B. Five samples were collected in different days of April 1996, and three more in March and May 1997. The mean values are shown in Table III.

6.- Old frasses (**O.F.**): Six samples of old frasses (8 to 60 days after infestation, 0.2 to 22 g) have been analyzed using programme B. Five samples were collected in laboratory from March to May 1996, and one more in the field in April and May 1997 (frasses were accumulated in that sample up to two months after infestation). No significative evolution of the air volatile compounds has been observed in frasses from the same origin, collected in different weeks after infestation, except for exobrevicomin, absent in frasses originated during the first four weeks, and present in the 1997 sample (2 month old) in 43 %. The mean values for the rest of compounds are shown in Table III.

TABLE II.

COMPOUND	L-1	L-2	L-3	L-4	O.L.	I.L.	BR
Hexanal	45	60	11	11	62	11	36
4-Hydroxy-4-methyl-2-pentanone	23	64	77	20	1	2	15
2-Heptanone	10	18	33	7	8	4	9
Heptanal	-	19	18	31	7	2	25
α-Pinene	50	80	199	12	22	6	84
β-Pinene	7	9	4	13	5	7	48
6-Methyl-5-hepten-2-one	4	7	11	10	9	3	13
Octanal	21	9	55	73	14	4	38
2-Ethyl-1-hexanol	20	38	n.d	26	13	3	6
Exobrevicomin	6	8	32	20	87	7	-
2-Nonanone	33	28	62	42	9	2	6
Nonanal	114	75	248	122	30	7	67
2-Decanone	11	20	130	37	18	5	-
Naphtalene	100	100	100	100	100	100	100
Decanal	15	9	14	30	43	6	41
2-Undecanone	15	11	67	26	1	1	6
Undecanal	11	32	59	68	33	5	16
Junipene	60	60	89	140	60	64	117

n. d.: not determined.

7.- Feeding frasses (**F.F.**): Two samples of feeding galleries frasses (both weighing 0.7 g), collected in May and July 1997 have been analyzed, using programme B. The mean values are shown in Table III.

8.- Mixed male and female live insects (**B**): Aproximately 1000 individuals were extracted inmediately after their emergence, in July 24th, 1996. Programme B. Results in Table IV.

TABLE III.

COMPOUND	I.F.	O.F.	F.F.
Hexanal	29	22	37
4-Hydroxy-4-methyl-2-pentanone	20	2	18
2-Heptanone	3	6	8
Heptanal	8	5	7
α-Pinene	12	10	18
β-Pinene	8	3	8
6-Methyl-5-hepten-2-one	8	8	3
Octanal	16	5	7
2-Ethyl-1-hexanol	10	3	8
Exobrevicomin	-	*	3
2-Nonanone	9	5	3
Nonanal	34	15	16
2-Decanone	1	-	4
Naphtalene	100	100	100
Decanal	26	13	13
2-Undecanone	1	1	3
Undecanal	28	7	11
Junipene	66	41	67

*: See text.

9.- Live male insects (**M.B.**): Four experiments were carried out with live male beetles (120 to 310 individuals). The air volatiles were collected inmediately after their emergence which occurred between May 21th and June 28th, 1997. The insects kept alive at least for 2 days into the extraction vessel, and were therefore extracted for two consecutive days. Programme B. Mean results are shown in Table IV.

TABLE IV.

COMPOUND	B	MB	FB
Hexanal	11	45	40
4-Hydroxy-4-methyl-2-pentanone	8	-	4
2-Heptanone	8	8	7
Heptanal	16	12	8
α-Pinene	12	23	20
β-Pinene	28	11	12
6-Methyl-5-hepten-2-one	14	13	19
Octanal	23	16	23
2-Ethyl-1-hexanol	9	n.d.	n.d.
Exobrevicomin	2	49	48
2-Nonanone	5	15	8
Nonanal	55	47	36
2-Decanone	-	1	5
Naphtalene	100	100	100
Decanal	55	29	32
2-Undecanone	-	1	1
Undecanal	8	8	9
Junipene	n.d.	88	62

n. d.: not determined.

10.- Live female insects (F.B.): Four experiments parallel to the ones decribed in 9 were carried out with recently emerged female beetles (120 to 360 individuals). Mean results also shown in Table IV.

A systematic analysis of the data shown in Tables II, III and IV allowed to determine the following items: a) evolution with aging of the air volatiles emmited by the logs; b) comparison of the volatiles emmited by young branches and pruned logs; c) comparison of the volatiles emmited by uninfested and infested logs; d) comparison of the volatiles associated to different types of frasses; e) comparison of the volatiles emmited by both male and female beetles. It can be deduced from Table II that both 4-

hydroxy-4-methyl-2-pentanone and α-pinene, described elsewhere as primary attractant for several spcies of scolytids (Byers, 1989), are present in significant amount in young branches (samples BR) and recently infested logs (samples L-1 to L-3), and in lower amount in old logs (OL), so these compounds could be considered as primary attractants from both materials. β-Pinene is present in significant amount only in BR, and therefore it could be a selective attractant from young branches. The exobrevicomine has not been detected in **BR**, but it has been determined in important amount in old logs. As **OL** are never been infested by *Phloeotribus scarabaeoides*, exobrevicomine could be considered as a repellent for the beetles. Methylketones 2-nonanone, 2-decanone and 2-undecanone reach their highest level in **L-3**, but very low levels in **BR**, so they could be considered as selective attractants from logs, and not from young branches.

From Table III it can be deduced that *n*-aldehydes C-8 to C-11 show highest levels in infested frasses (samples IF) than in other samples of frasses, thus indicating its probably participation as sex pheromone. 4-Hydroxy-4-methyl-2-pentanone as well as junipene are present in significant amount in IF and feeding galleries frasses (FF) and they are practically absent or in lower level, respectively, in OF thus, they could be components of aggregation pheromone. As a result, we hereby propose the following conclusions: 4-hydroxy-4-methyl-2-pentanone and a-pinene could act as common kairomones for both logs and young branches, 2-nonanone, 2-decanone and 2-undecanone as specific kairomones for logs, β-pinene as specific kairomone for young branches, 4-hydroxy-4-methyl-2-pentanone and junipene as aggregation pheromones, octanal, nonanal, decanal and undecanal as sex pheromones, and finally exobrevicomine as repellent.

Several mixtures of these compounds are currently been bioassayed, with promising results.

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