HS-SPME-GC/MS analysis of volatiles from *Eucalyptus* with different susceptibility to the Eucalyptus Weevil *Gonipterus platensis* attack.

Eduardo P. Mateus[1] Marco Diogo R. Gomes da Silva [2] & Maria Rosa Paiva[1]

[1]*CENSE, Departamento de Ciências e Engenharia do Ambiente, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus Caparica, Portugal*

[2] *REQUIMTE, Dept. Chemistry, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus Caparica, Portugal*

INTRODUCTION

Eucalyptus plantations and its products (e.g. production of pulp and paper) are an important economic resource in Portugal. The *Gonipterus* is a genus of weevils in the Curculionidae family. Among them, the Eucalyptus Weevil (*Gonipterus platensis*), native from Australia, has become a major alien pest in most Eucalyptus plantations around the world where it became the most severe defoliator with a strong economic impact.

In order to study potential chemical factors that may mediate host tree selection, the leaf volatiles of different Eucalyptus, 3 hybrids of *E. Globulus* plus one *E.nitens*, with different, susceptibility to the attack of *Gonipterus*, were studied. The volatiles from each sample were extracted by solid phase microextraction (SPME), using a 100 μm Polydimethylsiloxane (PDMS) fiber and analysed by Gas chromatography (GC-FID) and Mass Spectrometry (GC/MS). The compounds separation was performed on non polar 5 % Phenyl 95 % Dimethylpolysiloxane capillary columns with 25 m x 0.25 mm i.d (ZB-5ms and ZB-5 from Phenomenex). The film thickness was 0.23 μm for GC/MS and 1.0 μm for GC-FID. The volatiles identification was performed by GC/MS and linear retention indices (LRI) calculated according to van den Dool and Kratz. 71 compounds were detected in the volatile fractions emitted by the Eucalyptus leaves, being 49 identified. The results show that samples belonging to more susceptible trees emitted qualitatively and quantitatively more compounds. By means of a principal component analysis it was possible to visualize a separation between the sample provenance driven by α-pinene, limonene, 1,8 cineol and viridiflorene.

MATERIALS AND METHODS

1) Materials

Needles from 3 hybrids of *E. Globulus* (YG15, VR1277 & MB43) plus one *E.nitens*

2) Sample Preparation

Eucalyptus samples collected at Quinta do Furadouro, Leaves extracted by SPME for 45 min, using a 100 μm Polydimethylsiloxane (PDMS) fiber .

3) GC – FID

Gas Chromatograph (GC-FID): Agilent 5890A

Analytical Column:
DB-5, 30 m x 0.32 mm i.d., 1.00 μm film

Oven Temperatures:
40ºC (2 min), ramped to 150 ºC at 4 ºC/min then to 250 ºC at 6ºC/min and to 290ºC at 10 ºC/min (held for 30 min)

Injection: splitless (1.0 min); 250 ºC

Carrier:
Hydrogen at 1.2 mL/min, constant flow

4) GC/qMS

Mass Selective Detector GC/MS System:
Agilent 5890 seriell with HP 5972 MSD

Analytical column:

ZB-5ms , 25 m x 0.25 mm i.d.; 0.25 μm film

Oven Temperatures:
40ºC (2 min), ramped to 150 ºC at 4 ºC/min then to 250 ºC at 6ºC/min and to 290ºC at 10 ºC/min (held for 30 min)

Injection : splitless (1.0 min); 250 ºC .

Carrier: Helium at 1.0 mL/min, constant flow

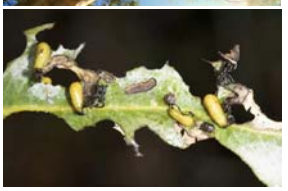

Table 1 - Volatile compounds emitted by the *Eucalyptus* leaves detected and identified by GC/MS, with retention times, calculated linear retention times (RLI) and reference linear retention times (RLI-Lib). X – compound detected.

Peak (#)	RT (min)	RLI	Compound	RLI - Lib	YG15	VR1277	MB43	Nitens	GC-FID
1	8.73	933	α-pineno	939	x	x	x	x	1
2	9.40	952	Canfeno	954	vest	X	x		
3	10.36	978	β-pineno	979	x	x	x	x	2
4	10.78	990	Mirceno	991	x	x	x	x	3
5	11.42	1007	α-felandreno	1003	x	x	x	vest	4
6	11.85	1019	α-terpineno	1017	x	x	x		5
7	12.15	1027	ρ-cimene	1025	x	x	x	x	
8	12.34	1031	Limonene	1029	x	x	x	x	6
9	12.42	1034	1,8-cineol	1031	x	x	x	x	7
10	12.95	1047	*trans*- ocimene	1050	x	x	x	x	8
11	13.42	1060	γ-terpinene	1060	x	x	x	x	9
12	14.47	1087	terpinolene	1089	x	x	x	x	10
13	14.69	1093	ρ-cimenene	1091	x	x	x	x	11
14	15.54	1115	1,3,8-p-mentatriene	1110	x				
15	15.84	1123	Mentatriene isomero?		x	x	x		
16	16.17	1132	Mentatriene isomero?		x	x	x		
17	16.64	1144	Mentatriene isomero?		x				
18	17.75	1173			x	x	x	x	
19	18.06	1181	*cis* -linalil oxide	1177	x	x	x	x	
20	18.12	1183	4-terpineol	1177	x	x	x	x	
21	18.23	1186			x				
22	18.40	1190	*m* -8-cimenol/acetofenona	1180/1183	x	x	x	x	
23	18.69	1198	α-terpineol	1189	x	x	x	x	12
24	19.42	1218			x				
25	19.88	1231	Benzotiazol		x	x	x	x	
26	20.90	1258	Piperitona	1253	x				13
27	23.22	1326	Biciclocelemeno	1338*	x	x	x	x	
28	23.55	1336	δ-elemeno	1340*	x	x	x	x	14
29	24.02	1351	α-terpenil acetato	1349	x	x	x	x	15
30	24.26	1359			x	x	x	x	
31	24.79	1375	Isoledeno	1376	x	x	x	x	
32	24.95	1380	α-Copaeno	1377	x	x	x		16
33	25.32	1392	β-elemeno	1391	x				17
34	25.48	1397			x	x	x	x	18
35	25.80	1409	longifoleno +	1408	x	x	x	x	19
36	25.90	1413	α-Gurjuneno	1410	x	x	x	x	20
37	26.25	1426	β-cariofileno	1419	x	x	x	x	21
38	26.36	1430			x	x	x	x	
39	26.46	1434	β-copaeno	1432	x	x	x	x	22
40	26.56	1438	β-Gurjuneno (calareno)	1434	x	x	x	x	23
41	26.66	1441	α-maalieno	1440*	x	x	x	x	24
42	26.79	1446	Aromadendreno	1446	x	x	x	x	25
43	26.97	1453	Neocloveno +	1454	x	x	x	x	
44	27.02	1455	E-β-farneseno	1457	x				
45	27.10	1458	alloaromandandreno	1460	x	x	x		26
46	27.23	1462	α-humeleno	1455	x	x	x	x	
47	27.29	1465	coeluição		x	x			
48	27.36	1468	9-*chi*-β-cariofileno	1466	x	x	x	x	27
49	27.68	1480	γ-gurjuneno	1477	x	x	x	x	28
50	27.89	1488	Germacreno D	1485	x	x	x	x	29
51	28.03	1493	δ-Selineno	1493	x	x	x	x	30
52	28.10	1495	*cis* -β-guaieno	1493	x	x	x	x	31
53	28.15	1497	Viridifloreno	1497	x	x	x	x	32
54	28.28	1502	Biciclogermacreno	1500	x	x	x	x	33
55	28.36	1506	E,E-α-Farneseno	1506	x				34
56	28.44	1509			x		x		
57	28.48	1511			x	x			35
58	28.69	1520			x	x	x	x	
59	28.79	1524	δ-Cadineno	1523	x	x	x	x	36
60	29.91	1572			x	x	x	x	37
61	29.96	1574			x				
62	30.10	1580			x	x	x		
63	30.24	1587	Spathulenol	1578	x	x	x	x	39
64	30.46	1596	Veridiflorol	1593	x	x	x	x	40
65	30.67	1605			x	x	x	x	
66	30.75	1609			x	x	x	x	
67	30.90	1616			x	x	x		
68	31.31	1636			x	x	x	x	
69	31.51	1645			x	x	x		
70	33.61	1748			x				
71	36.28	1887			x				

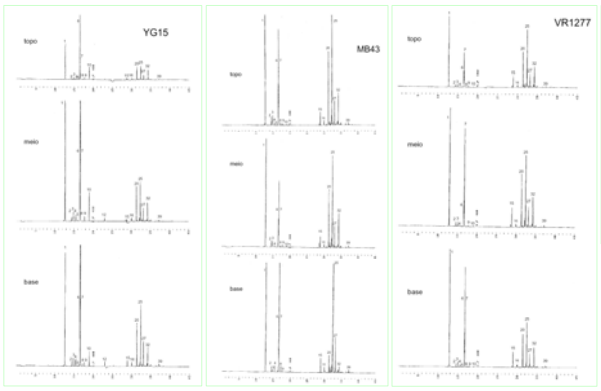

Figure 3: Volatile emission chromatograms from the sampled *Eucalyptus*. Top, middle and base leaves.

Peak numbers according to GC-FID (right column on table 1)

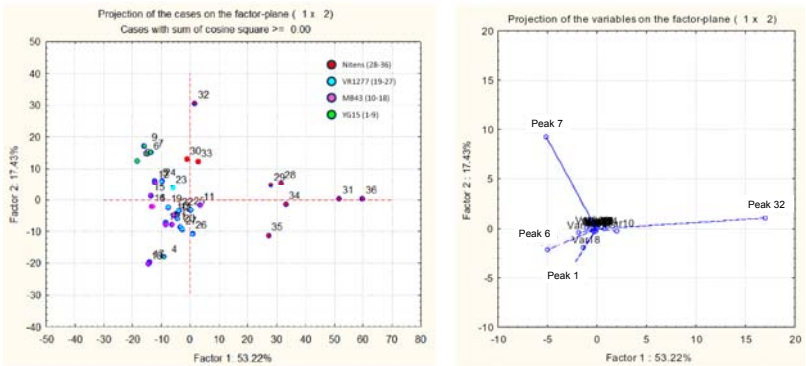

Figure 1. Principal component analysis (PCA) based on the volatiles emitted by leaves of the sampled Eucalyptus and loadings of the volatiles emitted (peak number according to GC-FID data)

RESULTS AND CONCLUSIONS

- 71 compounds were detected in the volatile fractions emitted by the Eucalyptus leaves, being 49 identified.
- Emitted volatile fractions mainly composed by: α-pinene, β-pinene, Mircene, α-felandrene, Limonene, 1,8 cineol, γ-terpinene, Terpinolene, Terpenil acetate, α-copaene, α-gurjunene, Aromadandrene, 9-*chi*-β-cariofilene and Viridiflorene.
- *E. nitens* leaves emitted qualitatively and quantitatively less volatiles than E. Globulus samples

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FCT FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

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