

## Differences in the Fragrances of Pollen and Different Floral Parts of Male and Female Flowers of *Laurus nobilis*

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The headspace analyses of pollen, whole living female and male flowers, and staminoids have been performed on *Laurus nobilis* L. (Lauraceae) from Italy to determine whether there are differences in the volatiles emitted in order to give a contribution to the roles of the different flower parts in the pollination ecology of dioecious plants. Also, the essential oils obtained from male and female plants have been studied to evaluate a possible correlation between the spontaneously emitted volatiles and the constituents stored in the glandular tissues. Furthermore, the headspace sampling technique has been improved, with respect to previously employed methods, by means of solid-phase microextraction (SPME).

**KEYWORDS:** *Laurus nobilis*; essential oil; pollen and flower volatiles; male and female plants

### INTRODUCTION

It is well documented that pollens of many species have distinctive fragrances as evaluated by the human nose (1–3). This situation has been confirmed by instrumental analyses (GC and GC–MS) of the volatiles sampled from air surrounding pollen (4–7). Besides visual clues, floral scents are very important in attracting pollinators: they can act both at long distances as attraction cues and at short distances as orientation cues among different parts of the flower or among different flowers (5, 8–10). Pollen odors probably evolved as defense compounds against pollen-feeding animals, but when plants became dependent on animals for pollination, some attractive compounds were included among pollen volatiles (10). Actually, plants must face two simultaneous contrasting pressures: the need to protect their pollen from nonpollinating insects, and the need to advertise it as a reward to pollinators.

The setup of the headspace technique to sample the air surrounding a whole plant or a plant organ permitted the discovery that pollen and different flower parts have distinct fragrance profiles. Furthermore, it has been shown that in angiosperms pollen has species-specific odors (5–7), giving new stimulus to pollination chemistry.

In the present paper we have analyzed the profiles of the volatiles obtained from male and female whole flowers, pollen, and staminoids of *Laurus nobilis* L. (bay, sweet bay), the sole species of the Lauraceae family growing in Italy (11). This is a dioecious plant, with scented flowers: the male flowers having 8–12 stamens, and the female flowers having four staminoids. The dried leaves are used as spices and its essential oil is employed in the flavoring industry. The fruits are also used pulverized in veterinary medicine in cows and mares to facilitate

removal of afterbirth, whereas the essential oil of the leaves has a depressive effect on the heart and causes hypotension (12). The pollination is entomophilous, with honey bees as main pollinators; because of the early blooming, bees employ its pollen and nectar mainly as food (13). The aim of this investigation is to ascertain if there are differences in the volatiles emitted from the whole living male and female flowers or from different parts and pollen in order to give a contribution to the roles of the different flower parts in the pollination ecology of dioecious plants. This is the first study about volatiles from a dioecious plant. We have also studied the essential oils obtained separately from male and female plants to evaluate a possible correlation between the spontaneously emitted volatiles and the constituents stored in the glandular tissues. Furthermore, because the sampling techniques employed in previous studies about pollen volatiles had the drawbacks of requiring considerable pollen amounts (50–200 mg), very long sampling times (24–48 h) with possible risks of sample contamination, and losses of volatiles during the following water-bath concentration of the solvents (4, 5, 7), we have also improved the sampling techniques.

### MATERIALS AND METHODS

**Samples.** The flowering aerial parts of *Laurus nobilis* L. were collected, during the morning, from cultivated plants in locality Alberaccio (San Giuliano Terme municipality, Pisa, Italy) at the end of March 2001. The samples also contained flower buds and were maintained in water. Six different samples were prepared: (1) whole female flowers (including sepals, petals, staminoids and gynecium); (2) whole male flowers (including sepals, petals, stamens and pollen); (3) only staminoids; (4) only pollen; (5) essential oil from flowering tops of female plants; and (6) essential oil from flowering tops of male plants.

Samples 1 and 2 were prepared using five flowers collected just after flower opening. The samples were cut a few mm below the calix,

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**Table 1.** Composition of the Volatiles of Whole Female and Male Flowers, Pollen and Staminoids (SPME), and of the Essential Oils from Flowering Tops of *Laurus nobilis*

compound	LRI <sup>a</sup>	sample					
		1 <sup>b,c</sup>	2 <sup>b,c</sup>	3 <sup>b,c</sup>	4 <sup>b,c</sup>	5 <sup>b</sup>	6 <sup>b</sup>
Terpenes							
α-thujene	933	0.1	0.1	tr <sup>d</sup>	0.3	0.4	0.3
α-pinene	940	1.9	2.5	0.9	3.8	3.2	3.1
camphene	955	0.4	0.4	0.3	0.5	0.1	0.6
sabinene	978	2.1	2.8	0.5	1.7	6.0	6.1
β-pinene	981	1.3	1.9	0.4	1.1	2.7	2.9
myrcene	992	0.3	0.7	0.4	1.7	0.8	0.8
2,3-dehydro-1,8-cineole	993					0.1	0.1
α-phellandrene	1007		0.1			0.1	0.1
3-carene	1013			0.2	2.1	0.6	
α-terpinene	1020			0.4	1.3	0.3	0.2
o-cymene	1023					0.2	
p-cymene	1028	0.1	0.1	1.1	5.4	0.3	0.1
limonene	1033	0.2	0.4	1.9	11.6	1.0	1.2
1,8-cineole	1035	20.5	26.3	7.9	9.1	42.8	38.8
(E)-ocimene	1051	65.3	45.7	17.8	4.2	0.1	0.1
γ-terpinene	1064	0.1	0.1	0.8	4.5	0.5	0.5
cis-sabinene hydrate	1070	0.1				0.6	0.5
cis-linalool oxide (furanoid)	1075		5.3	0.6	0.4		
terpinolene	1089			1.0	4.7	0.2	0.2
p-cymenene	1090			tr	0.5		
linalool	1101		0.8	0.6	1.8	14.4	12.1
trans-sabinene hydrate	1102	0.1					0.5
cis-p-menth-2-en-1-ol <sup>f</sup>	1123					0.1	0.1
allo-ocimene	1131	0.3	0.1	0.3			
(E)-2,6-dimethyl-1,3,5,7-octatetraene	1134	0.3	0.4				
neo-allo-ocimene	1144	0.1					
trans-p-menth-2-en-1-ol <sup>f</sup>	1145					0.2	0.1
camphor	1145	0.1		0.8	2.5		
umbellulone (= 3-thujen-2-one)	1173			1.3	4.6		
cis-linalool oxide (pyranoid)	1174		0.9				
borneol	1175		0.1				0.3
trans-linalool oxide (pyranoid)	1178		0.1				
isopinocamphe <sup>f</sup>	1180	0.1					
menthol	1181				0.3		
4-terpineol	1182	0.2	0.1	0.6	2.5	2.9	2.3
α-terpineol	1192	0.1		0.3	0.7	7.3	1.8
trans-piperitol <sup>f</sup>	1207					0.1	
nerol	1229					0.2	0.2
thymol methyl ether	1237				0.4		
carvacrol methyl ether	1246			tr	tr		
linalyl acetate	1258		0.3				0.2
piperitone	1258			tr	0.3		
isobornyl acetate	1286	0.1	0.2	0.6	0.5	0.1	0.6
thymol	1292				tr		
carvacrol	1300				tr		
δ-elemene <sup>f</sup>	1340		0.1	0.4	0.4		
α-terpinyl acetate	1351	0.3	0.5	1.1	1.1	5.1	12.0
α-ylangene <sup>f</sup>	1373	0.2	0.2	0.6	0.4	-	
α-copaene	1377	0.1		0.2	tr		
β-patchoulene	1382	0.1					
β-cubebene	1391	0.2		2.8			
β-elemene	1393	0.1	0.4	5.3	1.2	0.1	0.1
β-caryophyllene	1420	0.4	0.6	15.4	3.4	0.1	0.1
α-guaiene	1440		0.1	0.5	tr		
α-humulene	1456		0.1	1.6	0.3		
cis-murola-4(14),5-diene	1462				1.5		
γ-murolene	1478			0.2			
germacrene D	1482	0.1	0.5	5.1	1.5		
(Z,E)-α-farnesene <sup>f</sup>	1491	0.5	0.2	10.3	0.6		
β-selinene	1493					0.1	
bicyclogermacrene	1496	0.1	0.6	2.0	2.0	0.2	0.2
germacrene A <sup>f</sup>	1505					0.1	
(E,E)-α-farnesene	1509	0.1					
cis-γ-cadinene <sup>f</sup>	1511	0.1	0.1	1.2			
trans-γ-cadinene	1514	0.1		0.2		0.1	
δ-cadinene	1525			0.3		0.1	
germacrene D-4-ol <sup>f</sup>	1576			0.4			
spathulenol	1578					0.3	
caryophyllene oxide	1583			0.2		0.1	

Table 1. Continued

compound	LRI <sup>a</sup>	sample					
		1 <sup>b,c</sup>	2 <sup>b,c</sup>	3 <sup>b,c</sup>	4 <sup>b,c</sup>	5 <sup>b</sup>	6 <sup>b</sup>
Fatty Acid Derivatives							
( <i>E</i> )-3-hexen-1-ol	852	0.2		0.9			
heptanal	901				0.4		
4-hydroxy-5-methyl-2-hexanone <sup>f</sup>	954				0.3		
6-methyl-5-hepten-2-one	986				0.6		
octanal	1003				0.4		
( <i>E</i> )-3-hexen-1-ol acetate	1006	0.3					
nonanal	1104	0.1		0.3	2.0		
dimethyl glutarate	1140			0.6	2.2		
decanal	1205		0.1		1.5		
dimethyl adipate	1246			tr	0.4		
1-tridecene	1292		0.2				
2-tridecene <sup>f</sup>	1296		0.1				
undecanal	1308				tr		
<i>n</i> -tetradecane	1400				tr		
oleic acid	2141	0.7					
stearic acid	2164	0.3					
Aromatics							
mesitylene	996			0.4	0.7		
pseudocumene	1027			tr	0.4		
phenylethyl alcohol	1113	0.1		1.4	0.7		
tetramethylbenzene 1 <sup>e</sup>	1120			0.5	1.8		
tetramethylbenzene 2 <sup>e</sup>	1124			0.7	2.1		
benzyl acetate	1164	0.2	0.1	0.2			
naphthalene	1180			0.6	1.6		
methyl salicylate	1192	0.1					
eugenol	1357	0.7	0.1	tr	tr	1.6	3.6
methyleugenol	1403	0.2		0.5	tr	4.8	7.6
benzyl tiglate	1498	0.1	0.1				
elemicin <sup>f</sup>	1555					0.2	0.3
essential oil yield (% w/w)						0.6	1.8

<sup>a</sup> Linear retention indices (HP-5 column). <sup>b</sup> Percentages obtained by FID peak-area normalization, all relative response factors being taken as one (HP-5 column); mean of three analyses. <sup>c</sup> SPME analysis. <sup>d</sup> tr < 0.01%. <sup>e</sup> Correct isomer not identified. <sup>f</sup> Tentative identification (no reference compound available).

and the ends were wrapped in aluminum foil to minimize water loss. They were introduced in a 10-mL septum-cap vial and allowed to equilibrate for 20 min at 25 °C before sampling.

Sample 3 was prepared using 20 staminoids obtained from freshly opened flowers, avoiding contamination from other flower parts. They were introduced in a 4-mL septum-cap vial and allowed to equilibrate for 20 min at 25 °C before sampling.

Sample 4 consisted of 3–5 mg of pollen obtained by gentle tapping from flowers after anther dehiscence. It was allowed to equilibrate as described above.

Samples 5 and 6 were obtained by hydrodistillation of fresh flowering tops (100 g each) for 2 h in a Clevenger-like apparatus.

Samples 1–4 were sampled by means of the solid-phase microextraction (SPME) technique.

**Gas Chromatography.** The GC analyses were accomplished with a HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness), working with the following temperature program and conditions: 60 °C for 10 min, ramp of 5 °C/min up to 220 °C; injector and detector temperatures 250 °C; carrier gas nitrogen (2 mL/min); detector dual FID; split ratio 1:30; injection of 0.5 μL. Identification of the components was performed, for both the columns, by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of *n*-hydrocarbons. All the reference compounds were obtained from Aldrich Italia (either normal or flavor and fragrances catalogs), except for the following: sabinene, α-copaene, and δ-cadinene (Sigma Italia); linalool oxide (mixture of isomers), 4-terpineol, ocimene (mixture of isomers), piperitone (ChromaDex, Santa Ana, CA). Some compounds, thymol methyl ether, carvacrol methyl ether, and (*E*)-3-hexen-1-ol acetate, were prepared by synthesis; whereas *cis*- and *trans*-sabinene hydrate, *p*-cymenene, umbellulone, β-elemene, *cis*-muurola-4(14),5-diene, γ-murolene, germacrene D, β-selinene, bicyclogermacrene, (*E,E*)-α-

farnesene, trans-γ-cadinene, and spathulenol were confirmed by NMR analyses of other essential oils.

The relative proportions of the essential oil constituents were percentages obtained by FID peak-area normalization, all relative response factors being taken as one.

**GC/EIMS Analyses.** GC/EIMS was performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (both 30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; injection of 0.2 μL (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS 95) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data (14–19). Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing gas.

**SPME Analyses.** Supelco SPME devices coated with poly(dimethylsiloxane) (PDMS, 100 μm) were used to sample the headspace of samples 1–4. After the equilibration time, the fiber was exposed to the headspace for 15 min at 25 °C. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC and GC/MS systems, operating in the same conditions as above both for quantification and identification of the constituents, except that the splitless injection mode was used and the injector temperature was 250 °C.

All the analyses were performed in triplicate. The results were expressed as mean percentages obtained by FID peak-area normalization, all relative response factors being taken as one (HP-5 column).

**Table 2.** Compound Classes Identified in Whole Female and Male Flowers, Pollen and Staminoids (SPME), and in the Essential Oils from Flowering Tops of *Laurus nobilis*

compound class	sample					
	1	2	3	4	5	6
monoterpene hydrocarbons	72.5	55.3	26.0	43.4	16.5	16.2
1,8-cineole	20.5	26.3	7.9	9.1	42.8	38.8
oxygenated monoterpenes	1.1	8.3	5.9	15.1	31.1	30.8
monoterpene total	94.1	89.9	39.8	67.6	90.4	85.8
sesquiterpene hydrocarbons	2.1	2.9	46.1	11.3	0.8	0.4
oxygenated sesquiterpenes			0.6		0.4	
sesquiterpene total	2.1	2.9	46.7	11.3	1.2	0.4
terpene total	96.2	92.8	86.5	78.9	91.6	86.2
fatty acid derivatives	1.6	0.4	1.8	7.8		
aromatics	1.4	0.3	4.3	7.3	6.6	11.5
total identified	99.2	93.5	92.6	94.0	98.2	97.7

## RESULTS AND DISCUSSION

Solid-phase microextraction (SPME) is a fast, solventless technique that permits the establishment of an equilibrium between the sample matrix, the headspace above the sample, and a stationary phase coated on a fused silica fiber. The adsorbed analytes are then thermally desorbed from the fiber in the injector port of a gas chromatograph. This technique permits sampling of the volatiles emitted by living plants in a fast and easy way. We have obtained noteworthy improvements with respect to procedures reported in previous papers (4, 5, 7): the high concentration capability of this method permits use of considerably lower amounts of pollen with respect to those for the methods used in previous papers (3 mg instead of 50–200 mg); furthermore, the sampling time is very reduced (15 min instead of 24–48 h), minimizing the possibility of sample contamination due to the forced flow of air required by the former method; the absence of solvents prevents the loss of volatiles during concentration of the extractive solutions; and, finally, the higher concentration capability of this technique permitted identification of a greater number of compounds (98 different volatiles).

In the volatiles of the whole female flowers (sample 1) 45 compounds have been identified, and in the male flowers (sample 2) 39 constituents have been characterized (**Table 1**). Both the samples contained mainly terpenes (96.2% and 92.8% in female and male flowers, respectively). The other classes of compounds identified were fatty acid derivatives and aromatics. Monoterpenes were the dominating class (94.1% and 89.9%, respectively), whereas sesquiterpenes were less represented (2.1% and 2.9%, respectively). However, the low amounts of the latter class could be due to the short equilibration time used during sampling. The volatile fraction of the whole flowers was dominated by (*E*)-ocimene, which constituted 65.3% of the female flowers fragrance and 45.7% of the male ones, and by 1,8-cineole (20.5% and 26.3%, respectively). Other important compounds, found only in the male flowers, were linalool and its oxides (total percentage 7.1%).

The volatile profile of pollen (45 compounds identified) was clearly different with respect to both female and male whole flowers (**Tables 1 and 2**; trivial names and CAS nomenclature of the volatile compounds are given in **Table 3**). We observed a decrease in monoterpene hydrocarbons; in particular (*E*)-ocimene dropped to 4.2% and 1,8-cineole dropped to 9.1. In contrast, oxygenated monoterpenes raised to 15.1%. A considerable increase was observed also in the percentage of sesquiterpenes, which reached 11.3%. Fatty acid derivatives and aromatics became important classes of compounds with percentages of 7.8% and 7.3%, respectively. Limonene was the

principal constituent of the pollen volatiles (11.6%), but it was present in very low amounts in the fragrance of the whole female and male flowers (0.2% and 0.4%, respectively). Other important compounds of the pollen were 1,8-cineole (9.1%), terpinolene (4.7%), and  $\gamma$ -terpinene (4.5%). The  $\alpha$ -methyl-ketones, considered defense compounds against both insects and pathogens (10), 4-hydroxy-5-methyl-2-hexanone and 6-methyl-5-hepten-2-one, even if in small amounts (0.3% and 0.6%, respectively) were exclusive of pollen volatiles. Also, many aldehydes and phenols were present exclusively, or in greater percentages, only among pollen chemicals.

In the volatiles from staminoids the total monoterpenes (86.5%) were only about 10% less than those found in whole female flowers (**Table 2**). From a closer inspection, however, the monoterpenes reached less than half the amount with respect to whole female flowers (94.1%), but sesquiterpenes experienced a considerable increase (46.7% vs 2.1%). Also, the aromatics percentage in staminoids raised from 1.4% to 4.3%. The main differences in chemicals between staminoids and whole female flowers were observed for (*E*)-ocimene (17.8% vs 65.3%, respectively),  $\beta$ -caryophyllene (15.4% vs 0.4%), (*Z,E*)- $\alpha$ -farnesene (10.3% vs 0.5%), 1,8-cineole (7.9% vs 20.5%),  $\beta$ -elemene (5.3% vs 0.1%), and germacrene D (5.1% vs 0.1%). These differences could represent, within the female flower, an olfactive gradient that could guide pollinators to the food rewards, thus acting analogously to the visive “nectar guides” on the petals of many plants (5, 6, 10).

The essential oils obtained separately from fresh flowering tops of female and male plants showed very similar compositions (**Table 1**). Both the essential oils showed as main constituents 1,8-cineole (42.8% and 38.8% in female and male plants, respectively) and linalool (14.4% and 12.1%, respectively). The principal differences were referred to the monoterpenes  $\alpha$ -terpinene and  $\alpha$ -terpinyl acetate and to the aromatics eugenol and methyleugenol (**Table 1**). Another difference was in the yield of the two oils: male plants showed a three-times greater production (0.6% vs 1.8% w/w). Qualitatively, pollen volatiles and essential oil constituents showed a good correspondence, with the exception of the fatty acid derivatives found only in pollen (10 compounds, 7.4% total). From the semiquantitative point of view, the main differences were observed for limonene, (*E*)-ocimene, and linalool. However, it must be pointed out that during hydrodistillation some artifacts could be produced.

Summarizing, our study demonstrated that different flower parts emitted chemically distinct fragrances, and these volatiles are not necessarily correlated, at least semiquantitatively, with the compounds stored in the secretory tissues. In the odorous

Table 3. Trivial Name and Corresponding CAS Nomenclature (9CI) of Volatiles Identified in *Laurus nobilis*

trivial name	CAS nomenclature (9CI)
	Terpenes
$\alpha$ -thujene	2-methyl-5-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene
(1R)-(+)- $\alpha$ -pinene	2,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene
(+)-camphene	2,2-dimethyl-3-methylene-bicyclo[2.2.1]heptane
(+)-sabinene	4-methylene-1-(1-methylethyl)-bicyclo[3.1.0]hexane
(1S)-(-)- $\beta$ -pinene	6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane
myrcene	7-methyl-3-methylene-1,6-octadiene
$\alpha$ -phellandrene	2-methyl-5-(1-methylethyl)-1,3-cyclohexadiene
(1S)-(+)-3-carene	3,7,7-trimethyl-bicyclo[4.1.0]hept-3-ene
$\alpha$ -terpinene	1-methyl-4-(1-methylethyl)-1,3-cyclohexadiene
<i>o</i> -cymene	1-methyl-2-(1-methylethyl)-benzene
<i>p</i> -cymene	1-methyl-4-(1-methylethyl)-benzene
(R)-(+)-limonene	1-methyl-4-(1-methylethenyl)-cyclohexene
1,8-cineole	1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane
( <i>E</i> )-ocimene	( <i>E</i> )-3,7-dimethyl-octatriene
$\gamma$ -terpinene	1-methyl-4-(1-methylethenyl)-1,4-cyclohexadiene
<i>cis</i> -sabinene hydrate	(1 $\alpha$ ,2 $\alpha$ ,5 $\alpha$ )-2-methyl-5-(1-methylethyl)-bicyclo[3.1.0]hexan-2-ol
<i>cis</i> -linalool oxide (furanoid)	5-ethenyltetrahydro- $\alpha$ , $\alpha$ -5-trimethyl-2-furanmethanol
terpinolene	1-methyl-4-(1-methylethylidene)-cyclohexene
<i>p</i> -cymenene	1-methyl-4-(1-methylethenyl)-benzene
linalool	3,7-dimethyl-1,6-octadien-3-ol
<i>trans</i> -sabinene hydrate	(1 $\alpha$ ,2 $\beta$ ,5 $\alpha$ )-2-methyl-5-(1-methylethyl)-bicyclo[3.1.0]hexan-2-ol
<i>cis</i> - <i>p</i> -menth-2-en-1-ol	<i>cis</i> -1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol
<i>allo</i> -ocimene	( <i>E,Z</i> )-2,6-dimethyl-2,4,6-octatriene
<i>neo</i> - <i>allo</i> -ocimene	( <i>E,E</i> )-2,6-dimethyl-2,4,6-octatriene
<i>trans</i> - <i>p</i> -menth-2-en-1-ol	<i>trans</i> -1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol
camphor	1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one
umbellulone (= 3-thujen-2-one)	4-methyl-1-(1-methylethyl)-bicyclo[3.1.0]hex-3-en-2-one
<i>cis</i> -linalool oxide (pyranoid)	<i>cis</i> -6-ethenyltetrahydro-2,2,6-trimethyl-2H-pyran-3-ol
borneol	<i>endo</i> -1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-ol
<i>trans</i> -linalool oxide (pyranoid)	<i>trans</i> -6-ethenyltetrahydro-2,2,6-trimethyl-2H-pyran-3-ol
isopinocamphe	(1 $\alpha$ ,2 $\beta$ ,5 $\alpha$ )-2,6,6-trimethyl-bicyclo[3.1.1]heptan-3-one
menthol	5-methyl-2-(1-methylethyl)-cyclohexanol
4-terpineol	4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol
$\alpha$ -terpineol	$\alpha$ , $\alpha$ -4-trimethyl-3-cyclohexene-methanol
<i>trans</i> -piperitol	<i>trans</i> -3-methyl-6-(1-methylethyl)-2-cyclohexen-1-ol
nerol	( <i>Z</i> )-3,7-dimethyl-2,6-octadien-1-ol
thymol methyl ether	2-methoxy-4-methyl-1-(1-methylethyl)-benzene
carvacrol methyl ether	2-methoxy-1-methyl-4-(1-methylethyl)-benzene
linalyl acetate	3,7-dimethyl-1,6-octadien-3-ol acetate
piperitone	3-methyl-6-(1-methylethyl)-2-cyclohexen-1-one
isobornyl acetate	<i>exo</i> -1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-ol acetate
thymol	5-methyl-2-(1-methylethyl)-phenol
carvacrol	2-methyl-5-(1-methylethyl)-phenol
$\delta$ -elemene	(3R- <i>trans</i> )-4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-cyclohexene
$\alpha$ -terpinyl acetate	$\alpha$ , $\alpha$ -4-trimethyl-3-cyclohexene—methanol acetate
$\alpha$ -ylangene	(1 $\alpha$ H,6 $\alpha$ H,7 $\alpha$ H)-1,3-dimethyl-8-(1-methylethyl)-tricyclo[4.4.0.0 <sup>2,7</sup> ]dec-3-ene
$\alpha$ -copaene	(1 $\beta$ H,6 $\beta$ H,7 $\alpha$ H)-1,3-dimethyl-8-(1-methylethyl)-tricyclo[4.4.0.0 <sup>2,7</sup> ]dec-3-ene
$\beta$ -patchoulene	[1S-(1 $\alpha$ ,4 $\alpha$ ,7 $\alpha$ )]-1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-4,7-methanoazulene
$\beta$ -cubebene	[3aS-(3 $\alpha\alpha$ ,3 $\beta\beta$ ,7 $\alpha$ ,7 $\alpha$ S*)]-octahydro-7-methyl-3-methylene-4-(1-methylethyl)-1H-cyclopenta[1,3]cyclopropa[1,2]benzene
$\beta$ -elemene	[1S-(1 $\alpha$ ,2 $\beta$ ,4 $\beta$ )]-1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane
$\beta$ -caryophyllene	[1R-(1R*,2E,9S*)]-4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-2-ene
$\alpha$ -guaiane	[1S-(1 $\alpha$ ,4 $\alpha$ ,7 $\alpha$ )]-1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-azulene
$\alpha$ -humulene	( <i>E,E,E</i> )-2,6,6,9-tetramethyl-1,4,8-cycloundecatriene
$\gamma$ -muurolene	(1 $\alpha$ ,4 $\alpha\alpha$ ,8 $\alpha\alpha$ )-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene
germacrene D	[1S-( <i>E,E</i> )]-1-methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene
( <i>Z,E</i> )- $\alpha$ -farnesene	( <i>E,Z</i> )-3,7,11-trimethyl-1,3,6,10-dodecatetraene
$\beta$ -selinene	[4aR-(4 $\alpha\alpha$ ,7 $\alpha$ ,8 $\alpha\beta$ )]-decahydro-4-a-methyl-1-methylene-7-(1-methylethenyl)-naphthalene
bicyclogermacrene	(1R*,2E,6E,10S*)-3,7,11,11-tetramethyl-bicyclo[8.1.0]undeca-2,6-diene
germacrene A	[1S-( <i>E,E</i> )]-1,5-dimethyl-8-(1-methylethenyl)-1,5-cyclodecadiene
( <i>E,E</i> )- $\alpha$ -farnesene	( <i>E,E</i> )-3,7,11-trimethyl-1,3,6,10-dodecatetraene
<i>cis</i> - $\gamma$ -cadinene	(1 $\alpha$ ,4 $\alpha\alpha$ ,8 $\alpha\alpha$ )-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(methylethyl)-naphthalene
<i>trans</i> - $\gamma$ -cadinene	(1 $\alpha$ ,4 $\alpha\beta$ ,8 $\alpha\alpha$ )-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(methylethyl)-naphthalene
$\delta$ -cadinene	(1S- <i>cis</i> )-1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene
germacrene D-4-ol	[1S-(1R*,2E,4R*,7E)]-1,7-dimethyl-4-(1-methylethyl)-2,7-cyclodecadien-1-ol
spathulenol	[1aR-(1 $\alpha\alpha$ ,4 $\alpha\alpha$ ,7 $\beta$ ,7 $\alpha\beta$ ,7 $\beta\alpha$ )]-decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulen-7-ol
caryophyllene oxide	[1R-(1R*,4R*,6R*,10S*)]-4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.0 <sup>4,6</sup> ]dodecane
	Fatty Acid Derivatives
dimethyl glutarate	pentanedioic acid dimethyl ester
dimethyl adipate	hexanedioic acid dimethyl ester
	Aromatics
mesitylene	1,3,5-trimethylbenzene
pseudocumene	1,2,4-trimethylbenzene
methyl salicylate	2-hydroxybenzoic acid methyl ester
eugenol	2-methoxy-4-(2-propenyl)-phenol
methyleugenol	1,2-dimethoxy-4-(2-propenyl)-benzene
benzyl tiglate	2-methyl-2-butenic acid phenylmethyl ester
elemicin	1,2,3-trimethoxy-5-(2-propenyl)-benzene



profile of whole female and male flowers (*E*)-ocimene and 1,8-cineole were the dominant volatiles. The former is a quite common constituent of flower fragrances, but it is the main one only in a few species of some families (7). Our analyses have evidenced two  $\alpha$ -methyl ketones only in the pollen volatiles: this kind of chemical is considered a defense compound against insects and pathogens, as confirmed also by their abundance in wind-pollinated plants (10); this finding could further strengthen this hypothesis. However, other highly attractive compounds in the pollen odor or in the flower fragrance could overshadow the deterrent effect of defense chemicals, as already observed for eugenol or tetradecyl acetate in *Rosa rugosa* (20). In *L. nobilis* eugenol and its derivative methyl eugenol were present in low percentages in whole flowers (Table 1) and in trace amounts in pollen volatiles; we could hypothesize that in this species other compounds are responsible for pollinator attraction.

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Received for review March 1, 2002. Accepted May 20, 2002.

JF020269X