

Volatile Fractions from Three Cultivars of *Olea europaea* L. Collected in Two Different Seasons[§]

ELISABETTA CAMPEOL,* GUIDO FLAMINI, PIER LUIGI CIONI, AND IVANO MORELLI

Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, via Bonanno 33,
56126 Pisa, Italy

ROBERTO CREMONINI

Dipartimento di Scienze Botaniche, via L. Ghini 1, 56126 Pisa, Italy

LUCIA CECCARINI

Dipartimento di Agronomia e Gestione dell'Agroecosistema, via del Borghetto 21, 56126 Pisa, Italy

The chemical composition of the volatile fractions from leaves of three *Olea europaea* L. cultivars (Leccino, Frantoio, and Cipressino) harvested at two different times of the year were examined by GC and GC-MS. The results showed a high content of aliphatic aldehydes in the three cultivars during both harvesting periods and an increase of (*E*)-2-hexenal (an aldehyde with high antimicrobial properties) percentage from July to November.

KEYWORDS: *Olea europaea* L.; cultivars; Frantoio; Leccino; Cipressino; volatile compounds; aldehydes; terpenes

INTRODUCTION

Biodiversity is a precious heritage, a guarantee of progress for future generations, and a foundation of tenable agriculture. The drastic reduction in biodiversity over recent years has brought about a minor genetic variability leading to a concomitant reduced capability of plant populations to adapt to ever-changing environmental conditions and new diseases. The collection and retrieval of plant species must be increased with special attention to spontaneous species under the risk of extinction and those living in marginal lands, subjected to high levels of genetic erosion. The identification of sources of variability in natural populations, cultivars, and varieties must be taken into consideration for biological characterization, which will enable an accurate definition of conservation strategies.

Little information is available in the literature regarding phylogeny and biodiversity of *Olea europaea* (1–4). The study of the problem of the origin, identification, and certification of the varieties and the concomitant provision of more information for plant breeding are necessary to achieve more precise management and preservation of biodiversity.

Olea europaea L. is widely studied for its alimentary use (the fruits and the oil are important components in the daily diet of a large part of the world's population), whereas the leaves are important for their secondary metabolites such as secoiridoid

compounds oleacein and oleuropein, the first responsible for hypotensive activity (5, 6) and the latter also for hypoglycemic activity (7).

Papers concerning the analysis of the aroma components of the olive fruits and of the olive oil are present in the literature (8–10), but the investigation of the volatiles of the leaves is less considered.

In our previous work (11), the volatile fraction obtained from the leaves of three cultivars of *O. europaea* (Frantoio, Leccino, and Cipressino) collected in S. Piero a Grado (Pisa province, Italy), in the experimental fields of the University of Pisa, were analyzed by GC-MS, which was the first step of a wider investigation on phylogeny and biodiversity in *O. europaea*. The choice of these three cultivars was for their economic and agronomic importance in Tuscany; they are characteristic of this part of Italy, where they represent the greater part of the cultivated varieties.

We found that the three cultivars contained saturated and unsaturated nonterpenic aldehydes and many mono- and sesquiterpenes as main compounds.

It is known that α,β -unsaturated aldehydes, and in particular (*E*)-2-hexenal and (*E*)-2-heptenal, have antimicrobial properties (8); therefore, they could represent important defense phytochemicals against microbial invasion.

The aim of the present work was to analyze the biodiversity among the same three cultivars (Frantoio, Leccino, and Cipressino) grown in different habitats by comparison of the constituents of their volatile fractions and to evaluate possible differ-

* Author to whom correspondence should be addressed (e-mail camelis@farm.unipi.it; telephone +3905044074; fax +3905043321).

[§] In memory of Prof. Serena Catalano.

ences in the composition of the same cultivar in two different harvesting times, paying special attention to the defensive aldehyde compounds. Our results were compared with those previously published (11).

MATERIALS AND METHODS

Plant Material. Fresh leaves of three cultivars (Frantoio, Leccino, and Cipressino) of *O. europaea* (16-year-old clonal propagated trees) were collected at the agricultural farm Podere Contessa Beatrice in S. Vincenzo (Livorno province, Italy) in July and November 2001. These cultivars were never submitted to agronomical treatment, and Cipressino was used as windbreak only; cultivars Frantoio and Leccino were cultivated in "impianto a vaso" using organic fertilizer.

Extraction and Identification. The fresh material (200 g) was hydrodistilled in a Clevenger-like apparatus for 2 h, and volatile compounds were collected in *n*-hexane (HPLC grade). The analyses were carried out twice on three distinct plants for each cultivar.

GC analyses were accomplished with an 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: 60 °C for 10 min, ramp of 5 °C/min to 220 °C; injector and detector temperatures, 250 °C; carrier gas, nitrogen (2 mL/min); detector, dual FID; split ratio, 1:30; injection, 0.5 μL. The identification of the components was performed, for both 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.

GC-EIMS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness = 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures, 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas, helium at 1 mL/min; injection, 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, by means of their LRI relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built from pure substances and components of known oils and MS literature data (12–16). Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

Chemical Suppliers. All of the reference compounds were obtained by Sigma-Aldrich (Sigma, Aldrich, and Fluka catalogs), with the exception of (*E*)-2-undecenal and (*E,E*)- α -farnesene (TCI America), (*Z*)-3-hexenyl butyrate (Austin Chemical Co.), (*Z*)-3-hexenyl benzoate (ABCR Product List), and (*E*)-2-decenal (Lancaster Synthesis Ltd.). The reference compounds missing were (*E,Z*)-2,4-hexadienal, (*E,E*)-2,4-hexadienal, (*Z*)-2-heptenal, 3-ethenylpyridine, (*E,Z*)-2,4-heptadienal, 2,3-dehydro-1,8-cineole, 3-hexen-1-ol-acetate, 1-undecene, *p*-cymen-8-ol, (*Z*)-2-decenal, dihydroedulan 1A, theaspiranes (Is I and Is II), (*E*)- β -damascenone, β -gurjunene, germacrene D, α -muurolene, β -oplophenone, humulene epoxide II, τ -muurolol, α -cadinol, humulane 1,6-dien-3-ol, selin-11-en-4- α -ol, 2-pentylfuran, and isocaryophyllene. (See **Figure 1** for structures of some of the more unusual compounds.)

Statistical Analysis. Percentage data were arcsin $\sqrt{\%}$ transformed using the ANOVA statistical package. In both cases, means were separated on the basis of the LSD test only when the *F* test of the ANOVA per treatment was significant at the 0.05 or 0.01 probability level (12).

RESULTS AND DISCUSSION

Results obtained from the three cultivars harvested in July and November are listed as means of three replications in **Tables 1** and **2**, respectively: in July we identified 37 compounds in cv. Frantoio (75.7%), 46 compounds in cv. Leccino (84.1%), and 38 compounds in cv. Cipressino (83.4%); in November we

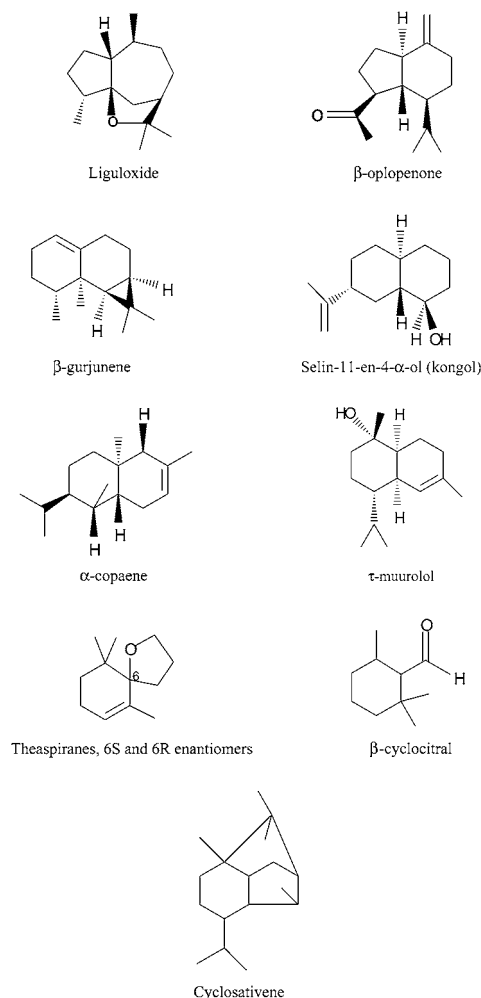


Figure 1. Structures of selected uncommon terpenes.

identified 32 compounds in cv. Frantoio (85.1%), 38 compounds in cv. Leccino (94.6%), and 36 compounds in cv. Cipressino (90.3%).

Comparing the three cultivars collected in the same period, we can observe that there were some differences in the constituents of the volatile fraction: in July cv. Cipressino is distinguishable from the others for its higher content of (*E*)-2-hexenal (23.8% with respect to 6.4% in cv. Frantoio and 3.4% in cv. Leccino); the theaspiranes were absent in cv. Frantoio, whereas they were present in Leccino and Cipressino, but in different amounts (7.8 and 6.6% in Leccino and 1.9 and 1.4% in Cipressino, respectively); only in cv. Frantoio were 2,3-dehydro-1,8-cineole, phenylacetaldehyde, α -muurolene, and humulan-1,6-dien-3-ol present; these compounds were absent in the other two cultivars. *p*-Cymen-8-ol, (*Z*)-2-decenal, and *n*-hexadecane were present only in Leccino, whereas (*E,E*)-2,4-hexadienal, β -gurjunene, *trans*-nerolidol, β -oplophenone, humulene epoxide II, τ -muurolol, and δ -cadinol were detected only in Cipressino.

In November the percentage of (*E*)-2-hexenal in cv. Cipressino was still higher than those in cv. Leccino and Frantoio (34.2% against 22.0 and 21.2%); in cv. Frantoio the theaspiranes, absent in July, appear in November, and in cv. Cipressino they were still in lower percentage with respect to cv. Leccino; cv. Frantoio was distinguishable for its higher percentage of selin-11-en-4- α -ol (15.8%). Compounds present exclusively in one cultivar were α -muurolene for cv. Frantoio; 2-pentylfuran,

Table 1. Composition^a of the Volatile Fraction of the Leaves of *O. europaea* Harvested in S. Vincenzo in July

	compound	Frantoio	Leccino	Cipressino	LRI ^b	LRI ^c
1	(<i>E,Z</i>)-2,4-hexadienal ^d	2.5 ± 0.6	0.9 ± 0.3	1.9 ± 1.1	841	
2	(<i>E</i>)-2-hexenal	6.4 ± 0.6	3.4 ± 1.0	23.8 ± 2.5	854	1220
3	heptanal	0.4 ± 0.0	0.6 ± 0.0		901	1174
4	(<i>E,E</i>)-2,4-hexadienal ^d			0.6 ± 0.0	911	
5	benzaldehyde	3.0 ± 0.2	1.4 ± 0.4	2.7 ± 0.2	963	1495
6	(<i>Z</i>)-2-heptenal ^d	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.0	964	
7	3-ethenyl pyridine ^d	6.1 ± 1.4	4.0 ± 0.4	5.5 ± 0.3	975	
8	(<i>E,Z</i>)-2,4-heptadienal ^d		1.1 ± 0.3	0.5 ± 0.1	988	
9	6-methyl-5-hepten-2-one	0.4 ± 0.1	0.5 ± 0.0		993	1336
10	2,3-dehydro-1,8-cineole ^d	0.4 ± 0.1			1000	
11	octanal		2.1 ± 0.2	0.7 ± 0.01	1002	1280
12	3-hexen-1-ol-acetate ^d	1.2 ± 0.2	1.2 ± 0.4		1004	
13	(<i>E,E</i>)-2,4-heptadienal	1.4 ± 0.3	0.8 ± 0.3	0.3 ± 0.0	1017	1373
14	phenylacetaldehyde	1.1 ± 0.2			1045	1625
15	(<i>E</i>)-2-octenal	0.5 ± 0.0	0.9 ± 0.2	0.5 ± 0.1	1064	1345
16	1-undecene ^d	2.6 ± 0.0	2.0 ± 1.0	3.4 ± 0.8	1075	
17	linalool	0.4 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	1099	1547
18	nonanal	18.2 ± 1.0	12.5 ± 1.5	8.0 ± 1.9	1103	1385
19	(<i>E</i>)-2-nonenal	0.5 ± 0.0	0.6 ± 0.1	0.6 ± 0.1	1165	1447
20	<i>p</i> -cymen-8-ol ^d		0.5 ± 0.08		1185	
21	(<i>Z</i>)-3-hexenyl butyrate	0.5 ± 0.6	1.2 ± 0.2		1187	1452
22	α-terpineol	0.3 ± 0.0	0.4 ± 0.1		1190	1698
23	methyl salicylate	0.6 ± 0.1	0.8 ± 0.1	0.5 ± 0.0	1192	1745
24	decanal	0.8 ± 0.0	0.8 ± 0.1	0.5 ± 0.0	1205	1484
25	β-cyclocitral	0.6 ± 0.1	0.6 ± 0.1	Tr ^e	1223	1205
26	(<i>Z</i>)-2-decenal ^d		0.4 ± 0.1		1252	
27	(<i>E</i>)-2-decenal	5.3 ± 0.2	6.3 ± 0.5	5.6 ± 1.2	1264	1590
28	dihydroedulan IA ^d		2.6 ± 0.9	3.5 ± 0.6	1292	
29	theaspirane Is I ^{d,f}		7.8 ± 2.5	1.9 ± 0.1	1298	
30	theaspirane Is II ^{d,f}		6.6 ± 1.7	1.4 ± 0.0	1315	
31	(<i>E,E</i>)-2,4-decadienal	0.6 ± 0.1	1.2 ± 0.0	0.8 ± 0.0	1317	1710
32	(<i>E</i>)-2-undecenal	0.3 ± 0.0	0.6 ± 0.0	0.8 ± 0.0	1368	1710
33	cyclosativene	1.4 ± 0.0	0.8 ± 0.2		1370	1545
34	α-copaene	2.8 ± 0.1	0.7 ± 0.1		1377	1521
35	(<i>E</i>)-β-damascenone ^d	6.3 ± 0.3	7.9 ± 0.5	4.7 ± 1.1	1381	
36	(<i>E</i>)-β-damascone ^d	2.1 ± 0.1	2.9 ± 0.2	4.1 ± 0.7	1411	
37	β-caryophyllene	1.0 ± 0.15	2.1 ± 0.27	0.6 ± 0.01	1419	1604
38	β-gurjunene ^d			0.3 ± 0.0	1432	
39	(<i>E</i>)-geranyl acetone ^d	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	1455	
40	germacrene D ^d	0.9 ± 0.2	0.8 ± 0.1		1481	
41	(<i>E</i>)-β-ionone		Tr	0.5 ± 0.0	1485	1952
42	α-murolene ^d	1.6 ± 0.2	Tr		1502	
43	(<i>E,E</i>)-α-farnesene	0.3 ± 0.0	0.5 ± 0.1		1510	1700
44	δ-cadinene	0.5 ± 0.2	0.4 ± 0.0		1523	1764
45	kessane ^d		0.4 ± 0.0	0.4 ± 0.0	1530	
46	liguloxide ^d		1.3 ± 0.2	0.5 ± 0.1	1532	
47	<i>trans</i> -nerolidol			0.9 ± 0.1	1564	1997
48	caryophyllene oxide		0.5 ± 0.0	2.8 ± 0.3	1571	2071
49	<i>n</i> -hexadecane		0.8 ± 0.1		1582	1600
50	β-oplophenone ^d			0.4 ± 0.0	1600	
51	humulene epoxide II ^d			0.6 ± 0.0	1606	
52	τ-murolol ^d			0.3 ± 0.0	1608	
53	α-cadinol ^d			0.6 ± 1.1	1619	
54	(<i>Z</i>)-3-hexenyl benzoate	0.5 ± 0.2	0.6 ± 0.0		1643	1555
55	humulan-1,6-dien-3-ol ^d	0.8 ± 0.3			1655	
56	selin-11-en-4-α-ol (kongol) ^d	8.7 ± 2.2	1.0 ± 0.3	1.7 ± 1.4	1660	
	total %	75.73	84.08	83.40		

^a Percentages obtained by FID peak-area normalization (HP-5 column). ^b Linear retention indices (HP-5 column). ^c Linear retention indices (HP-Wax column). ^d Tentatively identified (reference compound missing). ^e Tr = <0.1%. ^f Natural theaspirane is a mixture of the (6*R*) and (6*S*) isomer compounds. MS of Is I: *m/z* (relative intensity) 138 (100), 96 (39), 82 (22), 39 (18), 41 (13), 109 (12), 95 (11). MS of Is II: *m/z* (relative intensity) 138 (100), 96 (41), 82 (26), 39 (20), 41 (12), 109 (12), 139 (12), 95 (11), 83 (11).

cyclosativene, isocaryophyllene, and kessane for cv. Leccino; and germacrene D, δ-cadinene, and caryophyllene oxide for cv. Cipressino.

These results confirmed that each cultivar can be distinguishable from the other as demonstrated in our previous work (11); furthermore, interesting differences between the two times of harvest were observed.

The main constituents of the volatile fraction from the leaves of cv. Frantoio in July, calculated as average values of three

replications, were nonanal (18.2%), selin-11-en-4-α-ol (8.7%), (*E*)-2-hexenal (6.4%), (*E*)-β-damascenone (6.3%), 3-ethenylpyridine (6.1%), and (*E*)-2-decenal (5.3%). In November the principal compounds were (*E*)-2-hexenal (22.0%), selin-11-en-4-α-ol (15.8%), nonanal (7.9%), (*E*)-β-damascenone (6.4%), and α-copaene (5.5%).

Even if these two samples showed the same main constituents, there was a considerable quantitative difference: from July to November increasing (*E*)-2-hexenal (from 6.4 to 22.0%), selin-

Table 2. Composition^a of the Volatile Fraction of the Leaves of *O. europaea* Harvested in S. Vincenzo in November

	compound	Frantoio	Leccino	Cipressino	LRI ^b	LRI ^c
1	(<i>E,Z</i>)-2,4-hexadienal ^d	4.6 ± 0.1	3.6 ± 0.0	3.6 ± 0.3	841	
2	(<i>E</i>)-2-hexenal	22.0 ± 2.2	21.2 ± 1.5	34.2 ± 2.2	854	1220
3	heptanal	0.2 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	901	1174
4	(<i>E,E</i>)-2,4-hexadienal ^d	0.7 ± 0.2	0.3 ± 0.1	0.8 ± 0.3	911	
5	benzaldehyde	1.6 ± 0.3	1.4 ± 0.0	1.6 ± 0.2	963	1495
6	(<i>Z</i>)-2-heptenal ^d	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	964	
7	3-ethenylpyridine ^d	0.4 ± 0.1	0.4 ± 0.1	1.1 ± 0.2	975	
8	6-methyl-5-hepten-2-one	0.3 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	988	1336
9	2-pentylfuran ^d		0.8 ± 0.1		993	
10	2,3-dehydro-1,8-cineole ^d	0.6 ± 0.1		0.6 ± 0.1	994	
11	(<i>E,Z</i>)-2,4-heptadienal ^d	0.5 ± 0.1	2.1 ± 0.3	1.8 ± 0.5	1000	
12	octanal	0.7 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1002	1280
13	(<i>E,E</i>)-2,4-heptadienal		0.6 ± 0.1	0.5 ± 0.2	1017	1373
14	phenylacetaldehyde	0.2 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	1028	1625
15	(<i>E</i>)-2-octenal		0.4 ± 0.0	0.3 ± 0.0	1045	1345
16	1-undecene ^d	1.3 ± 0.1	1.2 ± 0.2	1.1 ± 0.2	1064	
17	<i>p</i> -cymene	0.3 ± 0.0		0.3 ± 0.0	1075	1274
18	linalool	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	1099	1547
19	nonanal	7.8 ± 0.3	11.5 ± 0.4	11.8 ± 1.4	1103	1385
20	(<i>E</i>)-2-nonenal		0.2 ± 0.0	0.3 ± 0.0	1165	1447
21	α -terpineol	0.3 ± 0.0	0.3 ± 0.0	0.8 ± 0.1	1190	1698
22	methyl salicylate	0.2 ± 0.1	0.4 ± 0.0		1192	1745
23	decanal	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	1205	1484
24	β -cyclocitral	0.3 ± 0.1	0.6 ± 0.1	0.5 ± 0.0	1223	1205
25	(<i>E</i>)-2-decenal	2.7 ± 0.1	2.8 ± 0.3	2.2 ± 0.1	1264	1590
26	dihydroedulan IA ^d	1.4 ± 0.13	1.7 ± 0.19	2.6 ± 0.03	1292	
27	theaspirane Is I ^{d,e}	1.9 ± 0.2	9.6 ± 0.4	2.3 ± 0.1	1298	
28	theaspirane Is II ^{d,e}	1.6 ± 0.3	7.6 ± 0.4	1.8 ± 0.0	1315	
29	cyclosativene		0.5 ± 0.1		1370	1549
30	α -copaene	5.5 ± 0.6	1.1 ± 0.1	0.5 ± 0.1	1377	1521
31	(<i>E</i>)- β -damascenone ^d	6.4 ± 0.2	10.7 ± 1.0	7.4 ± 0.1	1381	
32	isocaryophyllene ^d		0.6 ± 0.0		1405	
33	(<i>E</i>)- β -damascone ^d	1.7 ± 0.4	4.0 ± 0.7	4.7 ± 0.5	1411	
34	β -caryophyllene	0.4 ± 0.1	1.6 ± 0.1	1.9 ± 0.5	1419	1604
35	α -humulene		0.4 ± 0.0	0.4 ± 0.1	1456	1676
36	germacrene D ^d			0.9 ± 0.2	1481	
37	β -selinene ^d	1.5 ± 0.4	1.0 ± 0.3		1487	
38	α -muurolene ^d	2.1 ± 0.6			1502	
39	δ -cadinene			0.3 ± 0.0	1523	1764
41	liguloxide ^d		1.7 ± 0.1	0.4 ± 0.1	1532	
42	caryophyllene oxide ^d			0.5 ± 0.0	1582	
43	humulane-1,6-dien-3-ol ^d	1.0 ± 0.2	0.5 ± 0.1		1619	
44	selin-11-en-4- α -ol (kongol) ^d	15.8 ± 0.4	1.4 ± 0.4	1.2 ± 0.3	1660	
	total %	85.13	94.63	90.34		

^a Percentages obtained by FID peak-area normalization (HP-5 column). ^b Linear retention indices (HP-5 column). ^c Linear retention indices (HP-Wax column). ^d Tentatively identified (reference compound missing). ^e Natural theaspirane is a mixture of the (6*R*) and (6*S*) isomer compounds. MS of Is I: *m/z* (relative intensity) 138 (100), 96 (39), 82 (22), 39 (18), 41 (13), 109 (12), 95 (11). MS of Is II: *m/z* (relative intensity) 138 (100), 96 (41), 82 (26), 39 (20), 41 (12), 109 (12), 139 (12), 95 (11), 83 (11).

11-en-4- α -ol (from 8.7 to 15.8%), α -copaene (from 2.8 to 5.5%), (*E,Z*)-2,4-hexadienal (from 2.5 to 4.6%), and α -muurolene (from 1.6 to 2.1%) were observed, whereas the percentage of other constituents, such as benzaldehyde (from 3.0 to 1.6%), nonanal (from 18.2 to 7.8%), (*E*)- β -damascone (from 2.1 to 1.7%), (*E*)-2-decenal (from 5.3 to 2.7%), 3-ethenylpyridine (from 6.1 to 0.4%), and 1-undecene (from 2.6 to 1.3%), decreased.

Some compounds were present only in one of the harvests: (*E*)-2-octenal, (*E*)-2-nonenal, (*Z*)-3-hexenyl butyrate, (*E,E*)-2,4-decadienal, cyclosativene, (*E*)-geranyl acetone, germacrene D, and δ -cadinene were present in July and absent in November, whereas in this latter period (*E,E*)-2,4-hexadienal, (*E,Z*)-2,4-heptadienal, octanal, *p*-cymene, dihydroedulan IA, β -selinene, and the theaspiranes (Is I and Is II) appeared.

The main constituents of the volatile fraction from leaves of cv. Leccino collected in July were nonanal (12.5%), (*E*)- β -damascenone (7.9%), theaspirane Is I (7.8%), theaspirane Is II (6.6%), (*E*)-2-decenal (6.3%), and (*E*)-2-hexenal (3.4%), whereas in November they were (*E*)-2-hexenal (21.2%), nonanal (11.5%),

(*E*)- β -damascenone (10.7%), theaspirane Is I (9.6%), and theaspirane Is II (7.6%).

As for cv. Frantoio, the main components in cv. Leccino were nearly the same for the two periods of harvest even if in different amounts: from July to November the percentage of some compounds such as α -copaene (from 0.7 to 1.1%), selin-11-en-4- α -ol (from 1.0 to 1.45%), (*E,Z*)-2,4-hexadienal (from 0.9 to 3.6%), (*E*)- β -damascenone (from 7.9 to 10.7%), theaspirane Is II (from 6.6 to 7.6%), and theaspirane Is I (from 7.8 to 9.6%) increased; the highest changes were experienced by (*E*)-2-hexenal (from 3.4 to 21.2%).

On the other hand, the percentage of some other constituents such as nonanal (from 12.5 to 11.5%), (*E*)-2-decenal (from 6.3 to 2.8%), 3-ethenylpyridine (from 4.0 to 0.4%), and 1-undecene (from 2.0 to 1.2%), decreased.

As for cv. Frantoio, also in this cultivar several compounds were present only in July: (*Z*)-3-hexenyl butyrate, (*E,E*)-2,4-decadienal, (*E*)-2-undecenal, (*E*)-geranyl acetone, germacrene D, (*E,E*)- α -farnesene, and caryophyllene oxide. Others were present only in November: (*E,E*)-2,4-hexadienal, 2-pentylfuran,

Table 3. Effects of Cultivar and Time of Harvesting on Some Components of the Volatile Fraction Obtained from Olive Leaves

compound	Frantoio		Leccino		Cipressino		mean
	July	Nov	July	Nov	July	Nov	
(<i>E,Z</i>)-2,4 hexadienal	2.5 LM	4.6 IM	0.9 M	3.6 KM	1.9 M	3.6 KM	2.9 E
(<i>E</i>)-2-hexenal	6.4 IL	22.0 BC	3.4 KM	21.2 BC	23.8 H	34.2 A	18.5 A
nonanal	18.2 CD	7.9 GI	12.5 EF	11.5 FG	8.0 GI	11.8 EG	11.6 B
(<i>E</i>)- β -damascenone	6.6 IL	6.4 IL	7.9 GI	10.8 FH	4.7 IM	7.4 HK	7.3 C
selin-11-en-4- α -ol (kongol)	8.7 FI	15.8 DE	1.0 M	1.5 M	1.7 M	1.2 M	5.0 D
mean cv.	8.5	11.3	5.1	9.7	8.0	11.6	
		9.9 A		7.4 B		9.8 A	

^a Each value is the mean of three replicates. Means followed by the same letters are not significantly different at the 0.01 probability level according to LSD test.

phenylacetaldehyde, isocaryophyllene, β -selinene, and humulan-1,6-dien-3-ol.

In the case of cv. Cipressino, the main components of the volatile fraction in July were (*E*)-2-hexenal (23.8%), nonanal (8.0%), (*E*)-2-decenal (5.6%), 3-ethenylpyridine (5.5%), (*E*)- β -damascenone (4.7%), and (*E*)- β -damascone (4.1%), and the same compounds, with the exception of 3-ethenylpyridine, were the principal ones also in November, even if in different amounts [34.2% for (*E*)-2-hexenal, 11.8% for nonanal, 7.4% for (*E*)- β -damascenone, 4.7% for (*E*)- β -damascone, and 2.2% for (*E*)-2-decenal].

The greatest variations between July and November were observed mainly for (*E*)-2-hexenal (from 23.8 to 34.2%), nonanal (from 8.0 to 11.8%), and (*E*)- β -damascenone (from 4.7 to 7.4%) and for 3-ethenylpyridine (from 5.5 to 1.1%), benzaldehyde (from 2.7 to 1.6%), 1-undecene (from 3.4 to 1.1%), and (*E*)-2-decenal (from 5.6 to 2.2%).

In July methyl salicylate, (*E,E*)-2,4-decadienal, (*E*)-2-undecenal, β -gurjunene, (*E*)-geranyl acetone, (*E*)- β -ionone, kessane, *trans*-nerolidol, β -oplophenone, humulene oxide II, τ -muurolol, and α -cadinol and in November heptanal, 6-methyl-5-hepten-2-one, 2,3-dehydro-1,8-cineole, phenylacetaldehyde, *p*-cymene, α -terpineol, α -copaene, α -humulene, germacrene D, and δ -cadinene characterized the different periods of harvest.

Furthermore, a statistical evaluation of the data related to the five main compounds identified in the three cultivars during each period of harvest has been carried out, and the results are listed in **Table 3**.

Cv. Leccino showed the lowest percentages (7.4%) of constituents identified in its volatile fraction with respect to cv. Frantoio (9.9%) and cv. Cipressino (9.8%) (**Table 3**).

The volatile fraction obtained from the leaves was composed mainly by (*E*)-2-hexenal (18.5%) followed by nonanal (11.6%), (*E*)- β -damascenone (7.2%), selin-11-en-4- α -ol (5.0%), and (*E,Z*)-2,4 hexadienal (2.8%).

In November the percentages were significantly higher (11.0%) in comparison to those observed in July (7.0%); in this period the leaves of the three cultivars had the highest (*E*)-2-hexenal content with respect to the leaves harvested in July. During the second harvest the amounts of (*E*)-2-hexenal increased by 84, 70, and 30%, respectively, for cv. Leccino, Frantoio, and Cipressino. The nonanal content showed significant differences in relation to the examined cultivar: in cv. Frantoio the highest percentage of nonanal was in July (18.2%), whereas for cv. Cipressino and Leccino there were no significant differences during the two harvests.

The amount of (*E*)- β -damascenone did not show changes in relation to time of harvest; cv. Cipressino had the lower (*E*)- β -damascenone content in July.

The percentage of selin-11-en-4- α -ol in the leaves of cv. Frantoio harvested in November was significantly higher (16%) than that in July (8.7%). On the contrary, no significant differences were observed for cv. Cipressino and Leccino.

No differences were evident in the treatments imposed with respect to the amount of (*E,Z*)-2,4-hexadienal in the volatile fraction of olive leaves.

In our investigation the percentages of total aldehydes identified in the two harvests were the same for cv. Frantoio (41.7% in July and 41.9% in November), whereas for cv. Leccino (34.7% in July and 47.4% in November) and cv. Cipressino (47.4% in July and 60.6% in November) the percentages increased. The number of aldehydes identified in the two periods was nearly the same for all of the cultivars (15 in July and 13 in November for cv. Frantoio, 17 in July and 16 in November for cv. Leccino, and 15 in July and 16 in November for cv. Cipressino), but some of these compounds increased and other decreased in the interval from July to November. We can observe an increase of C-6 aldehydes and in particular of (*E*)-2-hexenal that undergoes a particular increment (from 6.4 to 22.0% in Frantoio; from 3.4 to 19.2% in Leccino, and from 23.8 to 34.2% in Cipressino); other aldehydes, such as (*E*)-2-decenal, decrease in all three cultivars, whereas nonanal, one of the main compounds, experienced a decrement only for cv. Frantoio and Leccino.

In plant tissue linolenic and linoleic acids, the most abundant polyunsaturated fatty acids, are enzymatically oxidized to the 9- and 13-hydroperoxides; hydroperoxide lyases (HPLs) catalyze the cleavage of these hydroperoxides, yielding C-9 and C-6 aldehydes, respectively. The significant increase of (*E*)-2-hexenal observed in November could be attributed either to the increment of substrates or to enzyme activity; perhaps this behavior could be related to the particular requirements of the plants in November.

In 1995 Kubo et al. (3) evaluated the antimicrobial activity of the compounds identified in the distillate obtained from olive and olive oil extract and commercial fruits extracts: all of the aldehydes α,β -unsaturated exhibited a broad activity against all of the microorganisms tested (Gram-positive and Gram-negative bacteria and fungi). In particular, (*E*)-2-hexenal and (*E*)-2-heptenal showed the best effectiveness and were the only compounds active against *Pseudomonas aeruginosa*, a Gram-negative bacteria (few phytochemicals exhibit activity against Gram-negative bacteria, especially *Pseudomonas* species).

The high content of aliphatic aldehydes in the three cultivars during both of the harvesting periods could have a significant importance in the defensive mechanisms of the plant against microbial pathogens; they may be produced, or their production may be increased, when the plant needs to react against a microorganism attack. The increasing of percentage of (*E*)-2-hexenal observed in November could support this hypothesis because of the rising defensive needs of the plant during fruit ripening.

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Received for review October 11, 2002. Revised manuscript received January 29, 2003. Accepted February 1, 2003.

JF026025U