Main Agronomic–Productive Characteristics of Two Ecotypes of *Rosmarinus officinalis* L. and Chemical Composition of Their Essential Oils

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The productive potential of two different ecotypes of *Rosmarinus officinalis* (Cevoli and Lunigiana) cultivated in the littoral area near Pisa (northern Tuscany, Italy) and the differences in the yield and composition of the essential oils of leaves, flowers, and stems obtained from different positions of the plants were used to characterize the two ecotypes. The Cevoli ecotype plant produced the highest yield of dry matter (221 g plant^{-1}) in comparison to the Lunigiana ecotype (72 g plant^{-1}). There were significant differences in dry matter production of different organs of both ecotypes. The essential oil contents of Cevoli and Lunigiana ecotypes were similar. In contrast, the oil contents of the different plant parts showed marked differences. The apical part of the plant and the leaves gave the highest essential oil yields. The major difference between the oils of the two ecotypes consisted in the 1,8-cineole contents (6.6 and 37.9% in Cevoli and Lunigiana, respectively). The Cevoli ecotype was determined to be the most suitable for essential oil extraction because it was characterized by a preponderance of flowers and leaves in the apical portion. The Cevoli ecotype could be classifited as an α-pinene chemotype, whereas Lunigiana is a 1,8-cineole chemotype.

KEYWORDS: *Rosmarinus officinalis* (L.); ecotypes; yield; chemical composition; essential oil

INTRODUCTION

Rosmarinus officinalis L. (Lamiaceae) is a typical Mediterranean species. In Italy it can be found mainly in coastal "macchia", garigues of the whole peninsula, with the exception of the North and Middle Adriatic (*1*). It is an aromatic shrub with an intense pleasant smell. The flowering season is very long and gradual, from April to August, but often it flowers all year long.

Rosemary is cultivated mainly in Spain, Morocco, and Tunisia. Because of its rusticity it grows in every soil type, but it prefers a sandy, arid, calcareous, humus-poor soil. Usually the plant is clonally propagated because of the poor germinability of its seeds and the genetic diversity of the seedlings (*2*).

The main producers of rosemary oil are Spain, Morocco, and Tunisia; the United States, Japan, and the European Union countries are the principal importers.

Usually, the plant parts used for essential oil production are the flowering aerial tops, comprising leaves, twigs, and flowers, collected from spring to late autumn.

Rosemary oil is widely used by the cosmetic, food, and pharmaceutical industries (*3*, *4*) as a fragrance component of soaps, creams, lotions, and perfumes. The leaves are used in the preparation of alcoholic beverages (vermouth), herbal soft drinks, and cooked foods and sauces (*3*). Rosemary is used as a food preservative because of its antioxidative properties, due to the presence of phenolic diterpenes such as rosmarinic acid (*5*). In medicine it is used for its stimulatory activity on blood circulation, on the heart, and on the nervous system, probably because of its camphor content (*4*). Topically it is prescribed against articulation, muscular, rheumatic, and traumatic pains; it is also employed in lotions against baldness. Rosemary oil exhibits good microbicidal activity against mycetes and Gram-positive and Gram-negative bacteria; the main active components are 1,8-cineole, camphor, and pinenes (*6*–*10*).

Many studies have pointed out the variability, qualitative and quantitative, of the composition and yield of the essential oil, due to intrinsic (genetics and plant age) or extrinsic factors such as climate and cultivation conditions or isolation methods (*11*–*18*).

This paper deals with the evaluation of the productive potential of two different *R. officinalis* ecotypes cultivated in the littoral area near Pisa (northern Tuscany, Italy) and compares the chemical composition of the essential oils obtained from different parts and organs of the plants in order to classify and characterize the two ecotypes on the basis of oil yield and quality.

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MATERIALS AND METHODS

Plant Material. *R. officinalis* L. was cultivated in a field lot within the Experimental Center of Rottaia, owned by the Dipartimento di Agronomia e Gestione dell'Agroecosistema of Pisa University (43° 41′ N, 10° 23′ E). Soil chemical—physical properties were as follows: 29.5% sand; 53.6% silt; 16.9% clay; pH 7.7; 2.2% organic matter (Lotti method); 1.1% total nitrogen (Kjeldahl method); 35 ppm of assimilable P_2O_5 (Olsen method); 165 ppm of assimilable K_2O (Dirks—Sheffer method). This soil was particularly deep and fresh because of the presence of a shallow phreatic water-bearing stratum (120 cm at the most); the water capacity of the field (−0.033 MPa) and the withering point (−1.5 MPa) were 27.3 and 9.4% of dry weight, respectively. Tillage was carried out in the autumn of 1996 and consisted of medium-depth ploughing (30 cm). Seedbed preparation was conducted by a pass with a double-disking harrow and a pass with a field cultivator. Preplant fertilizer was distributed at a rate of 50 kg ha^{-1} of N (urea), 100 kg ha^{-1} of P_2O_5 (triple superphosphate), and 100 kg ha^{-1} of K_2O (potassium sulfate).

A further 50 kg ha^{-1} of nitrogen, in the form of urea, was applied as a top dressing.

After the transplanting operation, irrigation was performed to help the plant take root. Further irrigation was used to maintain optimal water conditions in the soil. The perforated hose was adopted as system irrigation to avoid wetting of the useful organs of the plant. Weed control was obtained with a field cultivator (hoeing, harrowing), mainly during the early developmental stages of the plants (from transplant to closing of the space between the rows).

Two different ecotypes of *R. officinalis* were cultivated: one originating from a 10-year-old cultivation from the Farm "Villa Vestri", situated in Cevoli di Lari, Pisa province (Cevoli ecotype), which was trasplanted in October 1997; and the other obtained from wild plants growing in Cinque Terre, La Spezia province (Lunigiana ecotype) planted in October 1996. From each ecotype about 200 scions were cut and placed in a phytocell in a cool greenhouse to root. The inter-row and interplant spacings were 1.0 and 0.7 m, respectively.

About two months later the Lunigiana ecotype showed a 93% root take, whereas for the Cevoli ecotype it was 88%. During April 1999, when all of the plants were in the full production phase, representative samples of the plants coming from each ecotype were sacrificed to quantify their productive potential in our environment and to quantify the dry material produced by the leaves, flowers, and stems coming from the apical, intermediate, and lower parts of the plants. Each sample plant was subdivided into three parts: the lower one, composed of the vegetative material present at the bottom of the plant (∼30 cm); the apical one, formed by the apical twigs, ∼20 cm long; and the intermediate one, composed of the remaining material in the middle of the plant. Leaves, flowers, and stems coming from each part were separately weighed. To individually characterize the oil composition of each sample, the different organs taken from different plant positions were dried in the shade until constant weight.

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 (*19*).

Essential Oil Analyses. The essential oils were obtained by hydrodistillation for 2 h in a Clevenger-like apparatus of the dried crushed material (100 g).

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 up 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 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.

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

Table 1. Analysis of Variance of Different Treatments on Dry Matter and Essential Oil Yield in *R. officinalis*

treatment	dry matter[a] (g plant^{-1})	essential oil yield[a] (%)
ecotype (E)[b]	*	NS
part (P)[c]	*	**
organ (O)[d]	**	**
E × P	NS	NS
E × O	**	**
P × O	**	**
E × P × O	**	**

[a] NS, *, **, nonsignificant or significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. [b] Cevoli, Lunigiana. [c] Apical, intermediate, lower. [d] Leaves, flowers, stems.

Table 2. Effects of Different Treatments on Dry Matter Yield in *R. officinalis* Ecotypes

		Cevoli[a]	Lunigiana[a]
apical	leaves	376 B	105 EH
	flowers	199 CG	30 GH
	stems	50.9 FH	23 H
intermediate	leaves	307 BD	105 EH
	flowers	18 H	3.6 H
	stems	319 BC	108 EH
lower	leaves	152 DH	17 H
	flowers	1.38 H	1.0 H
	stems	563 A	259 BE

[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.

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 were as follows: 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 mL (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 LRI relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data (*20—25*). Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

RESULTS AND DISCUSSION

The productive potential of the species, expressed as amount of dry matter per plant, was found to be variable, depending on the examined ecotype, part, and organ. The data about dry matter production, submitted to ANOVA test, showed significant statistical differences relative to the different treatments (**Table 1**). The Lunigiana ecotype plants produced on the average a lower biomass amount than the Cevoli ecotype (∼33%). The highest yield of dry matter was obtained from the lower part of the plant (165 g/plant), followed by the intermediate (143 g/plant) and apical (131 g/plant) ones (**Table 2**).

The stems from the lower part of both ecotypes Cevoli and Lunigiana showed the highest yields of dry matter, 563 and 259 g/plant, respectively (**Table 2**). For the same plant part, leaves and flowers showed lower values, not significantly different from a statistically point of view. In the intermediate part of Cevoli there were no significant differences in the dry matter production of leaves (307 g/plant) and stems (319 g/plant); the same was true for Lunigiana, with 105 and 108

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Table 3. Effects of Different Treatments on the Essential Oil Yield in *R. officinalis* Ecotypes

		Cevoli[a]	Lunigiana[a]
apical	leaves	1.44 A	1.41 A
	flowers	0.79 BC	0.60 C
	stems	0.04 D	0.01 D
mean		**0.76**	**0.67**
intermediate	leaves	0.91 B	1.19 A
	flowers	0.68 BC	0.01 D
	stems	0.01 D	0.01 D
mean		**0.53**	**0.4**
lower	leaves	1.18 A	0.69 BC
	flowers	0.01 D	0.01 D
	stems	0.01 D	0.01 D
mean		**0.4**	**0.23**

[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.

g/plant, respectively (**Table 2**). The apical parts contained fundamentally leaves and flowers, which produced 92 and 85% of the dry matter of Cevoli and Lunigiana ecotypes, respectively. The dry matter obtained from the leaves of Cevoli population was significantly higher than that produced by the flowers and stems. In contrast, no statistically significant differences were observed for the three organs in Lunigiana plants. The Lunigiana population showed a higher degree of stem lignification also in the apical part than the Cevoli one, indicative of its greater wildness.

The dry matter production levels of the three examined organs were the same in the two ecotypes: the lowest value was found in the flowers (mean value = 126 g/plant), whereas the highest was in the branches (mean value = 661 g/plant).

The results of the ANOVA test on the essential oil yields of the two populations are reported in **Table 1**. There were no significant differences between the ecotype and its interaction with the plant part on the amount of oil obtained. On the contrary, there were significant differences in the oil contents of different plant parts and organs and in their interactions (**Table 1**). The apical part of the plant gave the highest oil yield (0.71%), whereas intermediate and lower parts gave 0.46 and 0.31%, respectively (**Table 3**). Among the various organs, the leaf oil content was ∼3 times higher than that of flowers, whereas it was practically absent in the stems.

Analysis of the mean values relative to the interaction of all the treatments (**Table 3**) showed that the highest percentages of essential oil were obtained from the leaves of the apical parts of both ecotypes, followed by the leaves of the intermediate part of the Lunigiana ecotype, and, finally, by the leaves of the basal part of the Cevoli ecotype. Therefore, the apical part of the plant was the richest in oil, mainly because of the lower amount of stems. Consequently, even if the essential oil yields of the intermediate (0.91 and 1.19% for Cevoli and Lunigiana ecotypes, respectively) and lower parts (1.18 and 0.69% for Cevoli and Lunigiana ecotypes, respectively) were satisfactory values, it could be not economically favorable to use these parts in the distillation process.

In light of these results about the oil yields of the different organs of the apical part, we can state that the best ideotype comprises erect plants, with many flowers and leaves in the apical portion, which should be not excessively woody, such as is found in the Cevoli ecotype. Therefore, from an agronomic point of view, it could be useful to obtain a genetic improvement of the "wilder" population, such as the Lunigiana ecotype, to increase the qualitative and quantitative yields for cultivation.

Tables 4 and **5** report the essential oil composition of the oils obtained from the different parts and organs of the Lunigiana and Cevoli ecotypes, respectively. Some of the constituents were present or absent depending not only on the ecotype but also on the organ or part of the plant.

Table 6 reports the mean values originating from the interaction of the treatments performed to reveal the effects of the ecotype, plant part, and organ on some of the oil constituents.

Table 4. Chemical Composition of the Essential Oil from Different Parts and Organs of the Plants in *R. officinalis,* Ecotype Cevoli

compound[a]	leaves (%)			flowers (%)			stems (%)			retention index
	apical	intermediate	lower	apical	intermediate	lower	apical	intermediate	lower	
α-pinene	28.6 ± 0.82	26.05 ± 0.7	30.3 ± 0.36	21.8 ± 0.86	15.1 ± 0.41	6.59 ± 0.14	20.0 ± 0.52	20.7 ± 0.71	16.2 ± 0.34	940
camphene	7.44 ± 0.08	5.6 ± 0.04	6.65 ± 0.05	10.6 ± 0.29	6.58 ± 0.10	2.98 ± 0.02	11.26 ± 0.18	11.0 ± 0.26	12.0 ± 0.09	953
sabinene	0.9 ± 0.01	0.96 ± 0.01	0.97 ± 0.01							978
β-pinene	1.37 ± 0.44	0.38 ± 0.03	0.53 ± 0.01	2.79 ± 0.07	1.57 ± 0.03	1.01 ± 0.01	2.44 ± 0.01	1.85 ± 0.04	1.63 ± 0.03	981
myrcene	3.24 ± 0.09	3.13 ± 0.06	3.57 ± 0.03	2.25 ± 0.07	1.82 ± 0.05	1.32 ± 0.02	1.27 ± 0.17	-	0.69 ± 0.01	993
α-terpinene	0.47 ± 0.05	0.36 ± 0.05	0.38 ± 0.01	0.56 ± 0.01						1020
p-cymene	0.74 ± 0.01	1.23 ± 0.02	1.23 ± 0.01	0.55 ± 0.02					0.56 ± 0.04	1027
limonene	3.8 ± 0.02	4.58 ± 0.02	4.21 ± 0.03	3.54 ± 0.03	2.74 ± 0.01	2.27 ± 0.01	2.55 ± 0.03	3.36 ± 0.01	1.82 ± 0.04	1032
β-phellandrene										1033
1,8-cineole	8.50 ± 0.15	7.28 ± 0.08	8.66 ± 0.02	7.72 ± 0.23	5.95 ± 0.08	5.87 ± 0.07	7.0 ± 0.05	5.09 ± 0.09	3.35 ± 0.16	1035
γ-terpinene	0.83 ± 0.20			0.68 ± 0.15	0.64 ± 0.01	0.66 ± 0.01				1064
linalool	1.76 ± 0.01	2.84 ± 0.02	1.64 ± 0.06	0.68 ± 0.02	1.08 ± 0.04	0.60 ± 0.03	2.48 ± 0.08	2.28 ± 0.01	0.62 ± 0.01	1100
camphor	9.26 ± 0.32	10.06 ± 0.21	9.47 ± 0.25	10.1 ± 0.34	10.6 ± 0.17	11.5 ± 0.13	8.21 ± 0.19	8.18 ± 0.05	4.88 ± 0.29	1145
borneol	5.97 ± 0.34	8.21 ± 0.53	6.66 ± 0.46	14.7 ± 0.41	17.0 ± 0.4	18.8 ± 0.69	29.9 ± 0.34	25.7 ± 0.1	12.4 ± 0.10	1168
terpinen-4-ol					1.17 ± 0.01	1.35 ± 0.01	1.31 ± 0.12			1179
α-terpineol		1.71 ± 0.33	1.55 ± 0.03		1.42 ± 0.01	1.85 ± 0.09	1.47 ± 0.18			1191
verbenone	5.97 ± 0.34	8.21 ± 0.53	6.66 ± 0.46	14.6 ± 0.41	17.0 ± 0.49	18.8 ± 0.69				1205
thymol	0.9 ± 0.04	0.39 ± 0.07	0.85 ± 0.14	0.86 ± 0.04						1236
geraniol	1.37 ± 0.11	0.99 ± 0.07	1.36 ± 0.04							1256
bornyl acetate	4.7 ± 0.10			6.82 ± 0.11	9.57 ± 0.2	9.0 ± 0.17			1.61 ± 0.05	1287
α-cedrene		0.88 ± 0.04	0.58 ± 0.09	1.09 ± 0.02			1.61 ± 0.20	1.74 ± 0.23	2.67 ± 0.16	1411
β-caryophyllene										1420
α-humulene										1457
germacrene D									2.15 ± 0.20	1482
germacrene B										1558
caryophyllene oxide								4.77 ± 0.27	4.06 ± 0.06	1583

[a] Listed in elution order from a DB-5 column.

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Table 5. Chemical Composition of the Essential Oil from Different Parts and Organs of the Plants in *R. officinalis*, Ecotype Lunigiana

compound[a]	leaves (%)			flowers (%)		stems (%)			retention index
	apical	intermediate	lower	apical	intermediate	apical	intermediate	lower	
α-pinene	18.6 ± 0.03	11.5 ± 0.15	23.1 ± 0.19	10.2 ± 0.2	12.9 ± 0.18	7.57 ± 0.21	10.9 ± 0.20	11.0 ± 0.05	940
camphene	2.65 ± 0.07	2.57 ± 0.07	3.47 ± 0.13	2.06 ± 0.05	2.25 ± 0.06	3.54 ± 0.04	6.63 ± 0.07	16.7 ± 0.15	953
sabinene								0.83 ± 0.01	978
β-pinene	6.79 ± 0.02	6.52 ± 0.04	4.16 ± 0.03	11.5 ± 0.08	10.6 ± 0.04	5.79 ± 0.11	6.96 ± 0.05	4.55 ± 0.1	981
myrcene	1.57 ± 0.05	1.67 ± 0.05	1.21 ± 0.09	1.29 ± 0.02	1.96 ± 0.05	0.78 ± 0.04	1.18 ± 0.02		993
α-terpinene	0.59 ± 0.01	0.63 ± 0.01	0.69 ± 0.02	0.56 ± 0.01					1020
p-cymene	0.57 ± 0.01	0.86 ± 0.02	1.47 ± 0.02	0.33 ± 0.01			1.01 ± 0.01		1027
limonene									1032
β-phellandrene	2.24 ± 0.01	2.16 ± 0.02	2.27 ± 0.03	1.57 ± 0.01	2.84 ± 0.23	1.12 ± 0.22	1.42 ± 0.35	0.98 ± 0.1	1033
1,8-cineole	43.3 ± 0.01	55.3 ± 0.02	42.5 ± 0.01	46.4 ± 0.01	31.5 ± 0.14	43.9 ± 1.5	46.8 ± 0.09	11.6 ± 0.31	1035
γ-terpinene	1.04 ± 0.02	0.98 ± 0.02	0.88 ± 0.02	1.09 ± 0.02			0.89 ± 0.02		1064
linalool	0.86 ± 0.1	0.38 ± 0.07	0.48 ± 0.07	0.45 ± 0.01		0.59 ± 0.18			1100
camphor	4.6 ± 0.03	8.14 ± 0.04	9.13 ± 0.02	2.45 ± 0.02	4.76 ± 0.03	5.52 ± 0.01	8.41 ± 0.09	5.2 ± 0.1	1145
borneol	8.96 ± 0.32	3.0 ± 0.35	4.22 ± 0.36	9.32 ± 0.34	10.3 ± 0.41	14.0 ± 1.04	4.95 ± 0.01	7.04 ± 0.09	1168
terpinen-4-ol									1179
α-terpineol	3.59 ± 0.01	3.34 ± 0.02	3.18 ± 0.01	3.27 ± 0.01	5.3 ± 0.02	3.17 ± 0.26	3.19 ± 0.03		1191
verbenone									1205
thymol									1236
geraniol									1256
bornyl acetate	1.22 ± 0.01				2.83 ± 0.02				1287
α-cedrene				-					1411
β-caryophyllene	0.92 ± 0.04	0.15 ± 0.15	0.56 ± 0.01	3.31 ± 0.01	6.85 ± 0.33	0.85 ± 0.06	0.57 ± 0.03	1.48 ± 0.03	1420
α-humulene	0.40 ± 0.4	-	0.34 ± 0.01	0.39 ± 0.01				1.76 ± 0.1	1457
germacrene D								2.51 ± 0.48	1482
germacrene B							1.61 ± 0.05		1558
caryophyllene oxide					3.18 ± 0.11	3.86 ± 0.01	0.49 ± 0.02	6.71 ± 0.01	1583

[a] Listed in elution order from a DB-5 column.

Table 6. Effects of the Ecotype and of the Part and Organ of the Plant on Some Components of the Essential Oil in *R. officinalis*

		α-pinene	β-pinene	camphene	myrcene	1,8-cineole	camphor	linalool	borneol
					Cevoli				
apical	leaves	28.57 B	1.37 GH	7.44 E	3.24 B	8.50 GH	9.26 CE	1.76 C	5.97 A
	flowers	21.8 E	2.79 F	10.59 D	2.25 C	7.72 GH	10.12 BC	0.68 EF	14.66 E
	stems	19.98 F	2.44 F	11.26 C	1.27 E	6.99 HI	8.21 E	2.48 B	29.92 A
intermediate	leaves	25.97 C	0.38 L	5.6 G	3.13 B	7.28 GI	10.06 BC	2.84 A	8.21 HI
	flowers	15.13 I	1.57 G	6.58 F	1.82 D	5.95 IL	10.64 AB	1.08 D	17 D
	stems	20.66 F	1.85 G	10.98 CD	1.0 FG	5.09 L	8.18 EF	2.28 B	25.71 B
lower	leaves	30.3 A	0.5 IL	6.65 F	3.57 A	8.66 G	9.47 BD	1.64 C	6.66 L
	flowers	6.59 P	1.01 HI	2.98 I	1.32 E	5.87 IL	11.46 A	0.6 FG	18.82 C
	stems	16.18 H	1.63 G	12.04 B	0.69 H	3.35 M	4.88 G	0.62 FG	12.4 F
					Lunigiana				
apical	leaves	18.64 G	6.79 C	2.65 IL	1.57 D	43.3 C	4.60 G	0.86 E	8.96 GH
	flowers	10.19 N	11.47 A	2.06 L	1.29 EF	43.4 B	2.45 I	0.45 GH	9.32 GH
	stems	7.57 O	5.79 D	3.54 H	0.78 GH	43.9 C	5.52 G	0.59 FH	13.97 E
intermediate	leaves	11.52 M	6.52 C	2.57 LM	1.67 D	55.3 A	7.14 F	0.38 H	3.0 N
	flowers	12.9 L	10.58 B	2.25 M	1.96 C	31.5 D	4.76 G	0.1 I	10.29 G
	stems	10.86 MN	6.96 C	6.63 F	1.18 EF	46.7 B	8.41 DE	0.1 I	4.95 M
lower	leaves	23.07 D	4.16 E	3.47 H	1.21 EF	42.5 C	9.13 CE	0.48 FH	4.22 MN
	flowers	5 Q	1 HI	1 N	1.0 FG	20 E	4.0 H	0.1 I	9.0 GH
	stems	10.95 MN	4.55 E	16.67 A	1.0 FG	11.64 F	5.2 G	0.1 I	7.04 IL

[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.

Table 6 shows that the α-pinene content of the Cevoli ecotype was higher (20.6%) than that of the Lunigiana ecotype (12.3%) and, on average, it was present mainly in the apical part of the plant (17.8, 16.2, and 15.3% in the apical, intermediate, and lower part, respectively). Leaves were the plant organ richest in this constituent. The highest amount of α-pinene, as evidenced by the interaction of all the treatments, was found in the leaves of the lower part of the Cevoli population. The same trend was also found for the Lunigiana ecotype. Both plant populations showed the lowest values for α-pinene in the flowers of their lower parts.

The greatest difference between the oils of the two populations was in their 1,8-cineole content. The Cevoli ecotype had a mean value of 6.6%, whereas in the Lunigiana ecotype it was 37.9%. From the interaction of the treatments, the highest value was found in the leaves of the intermediate part of the Lunigiana ecotype. The same ecotype also showed high values of 1,8-cineole in all of the organs of the apical and intermediate parts, whereas stems (11.6%) and flowers (20%) of the lower part contained the lowest amounts. In the Cevoli population the highest value was verified in the leaves of all the plant parts, whereas the lowest was in the stems of the lower part. On

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average, camphor was present in higher amounts in the oils of the Cevoli ecotype (9.1%) than in the oils of the Lunigiana ecotype (5.7%). The richest plant part was the intermediate one (8.2%), followed by the lower (7.4%) and the apical (6.7%) ones. The Cevoli population had the highest camphor content in the flowers of the lower and intermediate plant parts, whereas the lowest values were found in the stems of each portion. On the contrary, in the Lunigiana ecotype, the stems and leaves of the intermediate and lower parts were the camphor-richest plant organs; flowers from the apical part showed the lowest content (2.5%). The camphene amount of the Cevoli ecotype was, on average, higher than that of the Lunigiana one. It was localized principally in the lower part of the plant, particularly in the stems. The interaction of the treatments revealed that the highest values were found in the stems of the lower part of both Cevoli (12%) and Lunigiana (16.7%) ecotypes. The quantity of myrcene was greater in the Cevoli ecotype; there were no significant differences between the mean content of the apical and intermediate parts, whereas the lower one contained a lesser amount. Among the examined organs, leaves were richest in myrcene (2.4%), followed by flowers (1.6%) and stems (1%). This trend was the same for both populations. From the interaction of the treatments, it appears that in the Cevoli population the highest myrcene content was localized in the leaves of the lower part of the plant, whereas in the Lunigiana ecotype it was in the flowers of the intermediate part.

β-Pinene was present mainly in the oil of the Lunigiana ecotype (6.4%) rather than in the Cevoli one (1.5%). An increasing content passing from the basal (2.1%) to the intermediate (4.6%) to the apical (5.1%) part of the plant was found. Linalool was found in higher percentages in the Cevoli than in the Lunigiana ecotype. It was localized mainly in the apical and intermediate parts of the plant, particularly in the leaves (1.3%), followed by stems (1.0%) and flowers (0.5%). In the Cevoli ecotype the leaves of the intermediate part showed the highest values, whereas the flowers and stems of the lower part contained significantly lesser amounts. On the contrary, in the Lunigiana plants the leaves of the apical part were the organ richest in linalool, whereas the flowers and stems of the intermediate and lower parts were the organs with the poorest content. The borneol content of Cevoli plants was nearly 2-fold the amount (15.5%) of Lunigiana plants (7.9%). From the interaction data, high values of borneol were found in the stems of the apical (29.9%) and intermediate (25.7%) parts of Cevoli plants, whereas only the branches of the apical part of the Lunigiana ecotype showed high percentages of borneol. Both ecotypes showed the lowest amount of borneol in the leaves of the intermediate and lower parts of Lunigiana plants and in apical and lower portions of Cevoli plants.

The compositions of the essential oils obtained from the apical parts of each ecotype, the portion usually used for the production of the essential oil, were clearly different, thus characterizing the two populations. The Lunigiana plants could be defined as a 1,8-cineole-chemotype because of their 44.5% mean content, whereas the Cevoli plants, despite the absence of a high percentage of a single constituent, could be considered an α-pinene/borneol chemotype (23.4 and 17%, respectively).

However, the most significant datum was the higher essential oil yield of leaves and flowers than that of stems. This suggests that it should be advisable to discard the latter to obtain a better yield. This evidence, however, must be supported by an economical evaluation of the separation process, considering that in the industrial manufacture this operation should be mechanized, due to the high cost of the manual separation in industrialized countries. Therefore, it could be necessary to breed populations with little or, better, no woody structure in the apical part of the plant.

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