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Infraspecific variability in the essential oil composition of *Cistus monspeliensis* leaves

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Abstract

The leaf essential oils of *Cistus monspeliensis* plants growing wild on calcareous and siliceous soils in Provence (South of France) were analysed by GC and GC–MS. Qualitative and quantitative differences are noted in the essential oil composition between the *Cistus* populations from the two soil types. For chemotaxonomic purposes, a characterisation of the two types of oil is proposed.

Keywords: *Cistus monspeliensis*; Cistaceae; Rockrose; Essential oil; Diterpenes; Sesquiterpenes; Infraspecific variability; Substrate

1. Introduction

The genus *Cistus* (Cistaceae) comprises ten species in France (Fournier, 1961). These shrubs are indigenous to the Mediterranean area (Juhren, 1966). Their germination is stimulated by fire, and they are opportunist species (Trabaud, 1995) with an increased distribution as a result of human disturbance (Luis-Calabuig, Tárrega & Alonso, 1996). Numerous chemical studies have been conducted on *Cistus* species (Vogt & Gülz, 1994; Danne, Petereit & Nahrsted, 1994; Urones et al., 1994), particularly on the essential oils (Gülz, Kobold, Michaelis & Vostrowsky, 1984; Simon-Fuentes, Sendra & Cuñat, 1987; Demetzos, Homatidou, Loukis & Philianos, 1989; Picci & Usai, 1994; Mariotti, Tomi, Bernardini, Costa & Casanova, 1993; Demetzos, Loukis, Siliotis, Zoakis, Stratigakis, Katerinopoulos, 1995), which contains a number of compounds used in the fragrance industry (Lawrence, 1984; Anonis, 1995). Moreover, the essential oils of some species show cytotoxic and antibacterial properties (Demetzos et al., 1995; Demetzos, Chinou, Harvala & Homatidou, 1990; Chinou, Demetzos, Harvala, Roussakis & Verbist, 1994).

Cistus monspeliensis is one of the most common *Cistus* species in the Mediterranean area, and can be found around the Mediterranean coast and in the Canary and Balearic Islands (Med-Checklist 1, 1984). Very common in Provence (South of France), it is a semi-deciduous shrub up to 1 m in height growing in both calcareous and siliceous soils, with resinous leaves (Cabezudo, Pérez Latorre, Navarro & Nieto Caldera, 1993) containing glandular trichomes (Gülz, Herrman & Hangst, 1996).

The essential oil of *C. monspeliensis* leaves has been studied by Gülz et al. (1984) from cultivated plants grown under artificial conditions. During a study of *C. monspeliensis* secondary metabolism, we discovered that the essential oil of individuals growing on calcareous soils was very different from plants growing on siliceous soils. The differences are reported here.

2. Results and discussion

Essential oil yields of *C. monspeliensis* were found to vary according to the study site and the sampling month. Whatever the population, oil yields were lower

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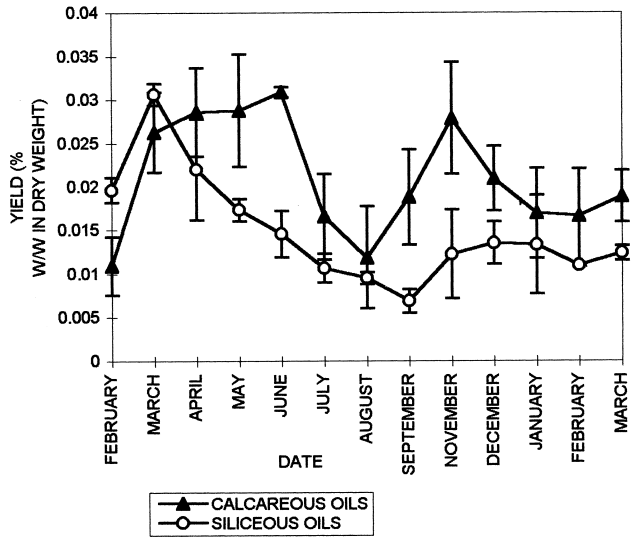

Fig. 1. Seasonal dynamics of *Cistus monspeliensis* essential oil yield (w/w) according to the substrate.

than those of *C. albidus* (Robles & Garzino, 1998) obtained using the same hydrodistillation conditions, corresponding to the lowest value obtained by Gülz et al. (1984): 0.02% (in fresh weight) from different *Cistus* sp. The annual mean yield (% w/w in dry weight) of essential oil from the population growing on calcareous soils (0.021 ± 0.007) was higher than that of plants growing on siliceous substrate (0.0149 ± 0.007). A Wilcoxon–Mann Whitney's U test showed that these differences are statistically significant ($p = 0.0014$), and are in contrast to results reported for *C. albidus*, which showed highest yields for populations on siliceous soils (Robles & Garzino, 1998). Therefore, the substrate influence depends on the species, and perhaps on its nutritional requirements.

Changes in yield during the year for populations on calcareous substrate was different than for plants on siliceous soils (Fig. 1), suggesting that the process of essential oil production is different for the two types of populations. For both populations, there was no significant correlation between essential oil yield and climatic conditions (mean temperatures and precipitation of the corresponding study sites given by an official national agency: Meteo France). Variations during the year are probably caused by a combination of environmental parameters (irradiance, climate, nutrients, water availabilities in soil, etc.). The seasonal dynamics of essential oil yield in both population types were very different from those of other semi-deciduous shrubs studied by Vokou and Margaris (1986) or by Arras and Grella (1992), which showed the lowest values during winter.

The major components of the essential oils from *C. monspeliensis* leaves are presented in Table 1, arranged in order of elution. Although some of them could not be identified, they are included in Table 1 because of their high concentration.

A comparison of the data in Table 1 demonstrates the qualitative difference between the composition of essential oils from *C. monspeliensis* leaves of plants grown on both substrates. The essential oil of plants growing on siliceous soils is characterised by the absence of α-cadinol (compound M) regardless of the study site and sampling month. This qualitative difference suggests that the plants growing on the two substrates may correspond to two chemical races (one from calcareous soils and the other from siliceous soils).

Moreover, comparison of values found for all of the calcareous populations (39 values) to those of the siliceous populations (26 values), using a Wilcoxon–Mann Whitney's U test, shows significant concentration differences for 11 compounds. The essential oil from *C. monspeliensis* on calcareous soils was significantly richer in components 1,5,5-trimethyl-9-oxabicyclo-[4,3,0]-non-2-en ($p < 0.0001$), compound N ($p < 0.0001$), α-bisabolol ($p < 0.0001$) than the oils from plants on siliceous substrate. They are significantly poorer in compound R ($p < 0.0001$), epimanoyl oxide ($p < 0.0001$), unknown diterpene: constituent T ($p < 0.0001$), compound U ($p < 0.0001$), heptacosane ($p = 0.0002$), myristic acid ($p < 0.0001$), nonacosane ($p = 0.0038$) and palmitic acid ($p = 0.0007$). However, comparisons between the values of CaA and SiA, CaA and SiB, CaB and SiA, CaB and SiB, CaC and SiA, CaC and SiB (Wilcoxon–Mann Whitney's U test) show significant concentration differences for all of the comparisons for only four compounds: B, R ($p < 0.05$); O, S ($p < 0.01$). Differences in environmental conditions may induce changes in plant secondary metabolite production (Rice, 1984); the influence of the soil type on the essential oil composition has been already described for *C. albidus* (Robles & Garzino, 1998). However, qualitative differences were not observed in that study and fewer compounds showed significant concentration differences between the two types of oil. The qualitative and quantitative variations in essential oil found in *C. monspeliensis* leaves were even greater than those found in chemotypes of several species of *Mentha* (Kokkini, Karousou & Lanaras, 1995) and *Thymus* (Granger & Passet, 1973). However, they were less pronounced than those observed for other Lamiaceae (Grayer, Kite, Goldstone, Bryan, Paton & Putievsky, 1996). In this study, the existence of chemotypes cannot be suggested, because leaves were harvested from more than one individual plant at each study site, and more importantly, because plants grew on different soil types. However, these results may indicate that the populations on siliceous soils and on calcareous substrate represent two separate ecotypes. The ratios of

Table 1
Constituents detected in the leaf essential oils of *Cistus monspeliensis* plants grown on different substrates

Peaks[a]	Retention time	Name	Concentrations (μg/μl)						
			CaA[b]	CaB[b]	CaC[b]	SiA[b]	SiB[b]	Mean Ca[c]	Mean Si[d]
A	21.1	Unknown	0.45 ± 0.25	1.00 ± 0.63	0.88 ± 0.65	0.40 ± 0.38	0.50 ± 0.47	0.77 ± 0.58	0.45 ± 0.42
B	28.4	1,5,5-Trimethyl-9-oxabicyclo[4,3,0]non-2-ene	0.66 ± 0.33	1.15 ± 0.66	0.87 ± 0.55	0.34 ± 0.23	0.28 ± 0.22	0.89 ± 0.56	0.36 ± 0.22
C	35.7	3 Nonen-2-one	2.15 ± 1.40	2.69 ± 1.54	2.27 ± 1.73	1.10 ± 0.63	1.92 ± 0.97	2.37 ± 1.54	1.51 ± 0.91
D	42.4	β-Caryophyllene	1.65 ± 1.01	2.07 ± 1.18	1.74 ± 1.35	0.97 ± 0.59	1.44 ± 0.69	1.82 ± 1.17	1.20 ± 0.67
E	49.2	Unknown	2.92 ± 2.07	2.57 ± 1.77	3.01 ± 2.50	2.17 ± 1.41	1.88 ± 0.87	2.83 ± 2.09	2.02 ± 1.16
F	62.8	Unknown	1.24 ± 0.76	2.22 ± 1.14	1.54 ± 0.67	1.48 ± 0.86	1.45 ± 1.01	1.66 ± 0.95	1.47 ± 0.92
G	68.9	Dihydro-β-ionone	2.02 ± 1.29	5.31 ± 1.97	4.38 ± 1.90	2.73 ± 1.26	3.13 ± 1.28	3.90 ± 2.21	2.93 ± 1.26
H	70.7	α-Ionone	Trace	Trace	Trace	Trace	Trace	Trace	Trace
I	78.8	Unknown	1.51 ± 0.89	3.12 ± 1.62	2.06 ± 0.87	2.07 ± 1.39	1.93 ± 0.94	2.23 ± 1.33	2.00 ± 1.17
J	79.6	β-Ionone	Trace	Trace	Trace	Trace	Trace	Trace	Trace
K	91.5	Ethyl myristate	Trace	Trace	Trace	Trace	Trace	Trace	Trace
L	96.8	Spathulenol	3.30 ± 1.09	3.97 ± 1.53	3.88 ± 1.63	4.32 ± 1.42	2.39 ± 0.86	3.72 ± 1.43	3.36 ± 1.52
M	99.7	α-Cadinol	4.09 ± 0.93	2.67 ± 1.54	2.76 ± 1.35	–	–	3.17 ± 1.43	–
N	108.0	Unknown	47.21 ± 15.72	28.91 ± 13.08	36.74 ± 16.42	22.92 ± 7.79	7.40 ± 2.78	37.62 ± 16.58	15.16 ± 9.77
O	110.1	α-Bisabolol	59.83 ± 41.35	21.91 ± 17.17	23.14 ± 18.23	1.58 ± 0.65	1.63 ± 0.72	34.96 ± 32.49	1.60 ± 0.67
P	116.1	Ethyl palmitate	Trace	Trace	Trace	Trace	Trace	Trace	Trace
Q	121.9	Tricosane	9.96 ± 4.23	13.87 ± 5.17	12.52 ± 6.51	13.96 ± 5.96	4.15 ± 2.93	12.11 ± 5.50	9.05 ± 6.80
R	127.6	Unknown	5.57 ± 3.78	15.99 ± 6.17	16.07 ± 3.94	19.95 ± 4.55	41.46 ± 11.95	12.54 ± 6.81	30.70 ± 14.10
S	129.4	Epimanoyl oxide	9.98 ± 2.96	9.38 ± 2.96	10.89 ± 3.28	20.54 ± 5.91	18.89 ± 7.50	10.09 ± 3.06	19.71 ± 6.67
T	136.1	Unknown diterpene	46.85 ± 16.22	41.75 ± 20.04	46.49 ± 22.18	93.71 ± 36.69	75.80 ± 40.84	45.03 ± 19.26	84.75 ± 40.50
U	144.9	Unknown	0.61 ± 0.28	2.19 ± 1.18	1.76 ± 1.32	2.28 ± 1.02	2.60 ± 1.04	1.52 ± 1.21	2.71 ± 1.02
V	145.5	Pentacosane	Trace	Trace	Trace	Trace	Trace	Trace	Trace
W	147.6	Ethyl Linoleate	Trace	Trace	Trace	Trace	Trace	Trace	Trace
X	157.3	Heptacosane	2.79 ± 1.56	3.80 ± 1.76	3.48 ± 1.82	5.03 ± 1.82	4.49 ± 0.92	3.35 ± 1.72	4.76 ± 1.44
Y	158.3	Myristic acid	2.93 ± 1.10	3.37 ± 1.40	3.09 ± 1.13	4.97 ± 1.48	5.71 ± 1.13	3.13 ± 1.20	5.34 ± 1.35
Z	166.8	Nonacosane	2.96 ± 1.19	3.49 ± 1.30	3.45 ± 1.25	4.18 ± 1.48	4.26 ± 0.96	3.30 ± 1.24	4.22 ± 1.22
AA	168.6	Palmitic acid	2.03 ± 0.85	3.53 ± 1.37	3.25 ± 0.86	3.77 ± 0.80	3.73 ± 0.73	2.94 ± 1.22	3.75 ± 0.75
AB	170.0	Triacontane	Trace	Trace	Trace	Trace	Trace	Trace	Trace

[a] In bold letters, compound checked by injection of commercial standard.
[b] Mean of the 13 samplings (±S.E.).
[c] Mean of the calcareous samplings (±S.E.).
[d] Mean of the siliceous samplings (±S.E.).
CaA, CaB, CaC are the three calcareous sites; SiA and SiB are the two siliceous sites.

Major mass fragments and their intensities:
compound N: 136 (100%), 121 (85%), 81 (69%), 55 (45%), 272 (16%).
compound R: 107 (100%), 93 (94%), 257 (60%), 147 (59%), 286 (56%).
compound T: 189 (100%), 93 (32%), 119 (29%), 81 (27%), 272 (26%).

Table 2
Characterisation of the siliceous and calcareous oils by different ratios between some major components and α-cadinol (minimal value – maximal value)[a]

Compound or ratio	Essential oil from calcareous populations	Essential oil from siliceous populations
α-Cadinol (M)	(0.82–6.18)	0
N/T	> 0.402 (0.486–1.264)	≤ 0.402 (0.077–0.402)
O/R	> 0.137 (0.180–78.939)	≤ 0.137 (0.013–0.137)
O/U	> 1.229 (1.255–414.576)	≤ 1.229 (0.132–1.229)
O/Y	> 0.786 (1.147–97.065)	≤ 0.786 (0.105–0.786)

[a] Correspondence between the letter and the name of the compound is given in Table 1.

major essential oil components vary according to the study site and the sampling month, but the degree of variation is characteristic of the type of oil. These variations are presented in Table 2.

The oils from plants growing on both substrates consist of a wide diversity of compound families. Diterpenes are the major components followed by sesquiterpenes (hydrocarbons and alcohols). The fact that the diterpenes are the major components of the essential oil from *C. monspeliensis* leaves is in agreement with the results given by Gülz et al. (1984). However, their identifications differ greatly from our own. Indeed, among the compounds that we have identified, only six (β-caryophyllene and the five alkanes) were found by Gülz et al. (1984), who instead found numerous monoterpenes, alkanes and some sesquiterpenes that we did not find, such as α-humulene. Plants studied by Gülz et al. (1984) were grown under artificial conditions, whereas our plants are from natural populations growing in the Mediterranean climate. However, the differences between our findings and those of Gülz et al. (1984) are unlikely to be due to environment changes, since their studies showed only differences in amounts, not in the types of compounds present. This would suggest that more than two chemical races (or ecotypes) exist. The existence of chemical races is well known for families such as Lamiaceae (Mártonfi, Grejtovský & Repcák, 1994; Salgueiro et al., 1997), but has not been reported for the Cistaceae. Other studies are needed to verify whether these differences are genetically determined, and investigations on essential oil composition should be carried out on other species of Cistaceae to find out whether infraspecific variability is a general feature for the genus *Cistus*.

3. Experimental

3.1. Plant material

Leaves were harvested from 20 individuals of *C. monspeliensis* L. (Cistaceae), randomly chosen from five wild study areas, located in Provence (South of France) on both calcareous and siliceous soils. Leaves were taken from each leaf level (except for senescent leaves). CaA, CaB and CaC are calcareous sites from near Marseille. SiA and SiB are siliceous sites in the "Massif des Maures", the first one near Bormes les Mimosas and the second near Pierrefeu. The voucher specimens from CaA (95–221 to 95–240), CaB (95–241 to 95–260), CaC (95–261 to 95–280), SiA (95–281 to 95–300), SiB (95–301 to 95–320) are deposited in the Herbarium of the Faculty of Sciences (St Jérôme), University of Marseille, France. *Cistus monspeliensis* leaves were collected in each study area on the same day, approximately every 5 weeks (February–September, November and December 1995, January–March 1996).

3.2. Analysis of the essential oils

The dried leaves of *C. monspeliensis* (about 200 g) were subjected to hydrodistillation for 2.5 h. The essential oils were diluted in hexane and analysed by GC and GC–MS using a capillary column FFAP: 50 m × 0.32 mm × 0.52 μm. The column temperature was programmed from: 60 to 155°C at 1°C/min (isotherm at 155°C for 30 min); from 155 to 230°C at 2°C/min (isotherm at 230°C for 30 min). Injector temperature was 270°C (injection split 1/100). The carrier gas was He (flow rate: 3ml/min) and the injection volume for the sample was 2μl. For the GC analysis, oil constituents were detected using a flame ionisation detector (air, H_2) at 270°C. *p*-Cymene was used as an internal standard. For GC/MS analysis, the MS conditions were: temperature ion source: 240°C; energy: 70 eV; electron current: 300 μA.

Identification of chromatographic peaks was made by comparison of their mass spectra with reference mass spectra and, for some of them, by comparison of the retention time values with those of commercial standards.

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