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Field studies of nitrogen application on Greek oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart) essential oil during two cultivation seasons

Andreas J. Karamanos*, Dimitra E.K. Sotiropoulou

Faculty of Crop Science, Agricultural University of Athens, Greece

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ABSTRACT

The effects of four levels of fertilizer nitrogen application (0, 40, 80 and 120 kg N ha^{-1}) on oil yield, concentration, and composition of Greek oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart) during the second and third seasons from its field establishment were examined. Oil concentration per plant increased significantly from about 1.5% to 2.0% in the second to about 5.5% (v/w) in the third season and it was higher in the inflorescences when compared with leaves. No significant effects of fertilizer application on oil concentration were detected. Oil yield showed significant peaks at nitrogen rates of 80 kg ha^{-1} in both seasons and was significantly higher in the third (between 57 and 83 l ha^{-1}), when compared with the second season (between 17 and 27 l ha^{-1}). Such a response was ascribed to the positive nitrogen effects on herbage yield. As regards oil composition, nitrogen fertilization exhibited some significant effects only in the third, more humid, season. Thus, it positively affected linalool content in inflorescences at the rate of 80 kg N ha^{-1} and carvacrol content in leaves at the rates of 40, 80 and 120 kg N ha^{-1}. On the other hand, π-cymene, caryophyllene, α-pinene, thymol, and camphene were observed at higher levels in the unfertilized plots. Carvacrol was the dominant constituent of the essential oil content ranging from 56.46% to 84.88% among organs, treatments and seasons. It was followed by π-cymene (4.19–21.4%) and α-pinene (0.11–1.88%). Thymol was detected at low levels (0.20–1.44%). Carvacrol percentage was higher in the drier and warmer season (70.75–84.88%) in both leaves and inflorescences, whereas a number of compounds (α-thujene, α-pinene, camphene, myrcene, α- and γ-terpinene, π-cymene, *cis*-hydrosavinene, linalool, α-terpineol, α-caryophyllene, and β-disavolene) tended to accumulate at higher levels during the wetter and colder season.

1. Introduction

Greek oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart) is native in the islands, in Crete, in southern Greece, and in coastal areas of the mainland Greece at altitudes up to 1500 m (Kokkini et al., 1994). It is a perennial shrub exhibiting high oil concentration (1.1–8.2%, v/w, depending on its habitat), about 10 times higher in comparison with its relative species (*O. vulgare* ssp. *vulgare*, *O. vulgare* ssp. *viridulum*, *O. vulgare* ssp. *gracile*, *O. vulgare* ssp. *viride*) (Baser et al., 1993; Franz and Novak, 1997; Kokkini et al., 1994; Kokkini and Vokou, 1989; Sezik et al., 1993).

Numerous works examining the effects of nitrogen on growth and yields of plants of the Lamiaceae family have been carried out. The majority deals with *Mentha* species (e.g., Clark and Menary, 1980, and others), and fewer with basil (Sifola and Barbieri, 2006), thyme (Baranauskien et al., 2003), and sage (Karioti et al., 2003). As regards *Oregano* species, Omer (1999) noticed positive nitrogen-effects on Egyptian oregano (*Origanum syriacum*) grown in pots, and Ozgüven et al. (2006) a significant increase in fresh and dry weight of the same species at a rate of 40 kg ha^{-1} in the field. Barreyro et al. (2005) reached similar conclusions working with an oregano hybrid (*Origanum* × *applii*) cultivated in Argentina. Field studies on Greek oregano have shown that nitrogen application significantly affected a number of vegetative traits thus inducing both biomass and oil yield peaks at a rate of 80 kg N ha^{-1} (Sotiropoulou and Karamanos, 2010).

Studies on Greek oregano have shown that a differentiation in oil concentration is associated with different climatic conditions during growth (D'Antuono et al., 2000; Kokkini et al., 1994; Maffei et al., 1993; Novak et al., 2003; Russo et al., 1998; Sangwan et al., 2001; Vokou et al., 1993). Oil quality is determined by its composition, which varies with genotype, climate, soil type, orientation, and plant development (Baydar et al., 2004; Kokkini et al., 1997; Russo et al., 1998). Carvacrol, the main component of the Greek oregano essential oil (Kokkini and Vokou, 1989), is considered to impair to it antioxidant (Mastelic et al., 2008; Shan et al., 2005; Zheng and Wang, 2001), antibacterial (Mastelic et al., 2008; Unlu et al., 2007), antifungal (Adam et al., 1998; Esen et al., 2007), and

* Corresponding author. Tel.: +30 2105294481; fax: +30 2106812052.
E-mail address: akaram@aua.gr (A.J. Karamanos).

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antiinflamatory properties (Ocana-Fuentes et al., 2010). Thymol, γ-terpinene, π-cymene, and linalool were also identified at relatively high proportions in Greek oregano oil (D'Antuono et al., 2000; Skoula and Harborne, 2002).

It has been shown that a number of soil physical and chemical characteristics affect both oil concentration and composition in Greek oregano and other related species (Panagopoulos, 2012). Nevertheless, there is no information on the effects of fertilization on the levels of bioactive compounds and of secondary metabolites in oregano species (Alizadeh et al., 2010). In a previous work (Sotiropoulou and Karamanos, 2010), the effects of different levels of fertilizer nitrogen application on growth and yields of a Greek oregano plantation were described. In this work, the effects of nitrogen application on oil concentration and composition of the same Greek oregano plantation during the second and third year from its establishment were investigated as a part of the same project. In this way, the effects of both nitrogen and growth season were assessed. In addition, separate analyses on leaves and inflorescences were carried out in order to understand the patterns of the allocation of the oil components among plant organs.

2. Materials and methods

A Greek oregano plantation was set up in a field located at Throfari (Northern Peloponnese, 130 km southwest of Athens) at an altitude of 790 m above sea level in spring 2001. The field had been cultivated with wheat up to 1995 and was set aside thereafter. The experiment was carried out according to the randomized complete blocks design consisting of 5 replicates and 4 treatments of nitrogen application (at rates of 0, 40, 80 and 120 kg of N ha^{-1}) for two cultivation periods (2002 and 2003). The soil was a clay loam (10.4% sand, 34.2% silt and 55.4% clay) with pH 6.5, 0.13% available $CaCO_3$, 1.087% organic matter, 0.106% total nitrogen, 22.4 ppm available phosphorus (Olsen), and 0.248 mg g^{-1} available potassium. The dimensions of each plot were 3 m × 2 m.

8–10 cm-cuttings were taken from a mother plantation of Greek oregano plants of Cretan origin (*Origanum vulgare* sp. *hirtum*) grown at the Agricultural University of Athens experimental field on 7 January 2001. The bottoms of the cuttings were initially dipped in water, then in dust of "Radicin" (0.2% indolylobutyric acid) and finally planted in small pots containing a 1:1 mixture of peat (pH 4–4.5) and perlite enriched at a rate of 3 g l^{-1} with "Complesal" fertilizer (12% N, 12% P_2O_5, 17% K_2O, 3% MgO, 1670 ppm Fe, 320 ppm Mn, 10 ppm Zn, 91 ppm Cu and 9 ppm B). The pots were placed in benches within a glasshouse (maximum temperature 25 °C and minimum 13 °C) and covered with a transparent plastic sheet for the first 3 weeks after planting. Then, the sheet was removed and 4 weeks after planting the leafy cuttings were placed outdoors to be hardened under normal conditions.

The field was sprayed with the herbicide "Roundup" (Glyphosate 30%, w/w) at a rate of 18 g l^{-1} of water on 21 January 2001 to control winter weeds, and rotavated on 18 February 2001. The cuttings were planted in the field on 3 March 2001 at a spacing of 50 cm between and 30 cm within lines. Each plot contained 5 rows. The average density was 6.7 plants m^{-2}. Ammonium nitrate (34.5–0–0) fertilizer was used as nitrogen source. In the first season, the total amount required was applied on 28 April 2001. In the second season, 2/3 of the fertilizer was applied on 23 March 2002 and the rest after harvesting (22 June 2002). In the third season, 2/3 of the fertilizer was applied on 3 May 2003 and the rest on 6 September 2003.

The plants were watered twice a week at a dose of 1 l water per plant from planting up to the end of March 2001 to help plant establishment. Then, watering was reduced to 0.5 l per plant once a week. Water was also applied at a dose of 1 l per plant immediately after harvest (22 June 2001), and once in July and August 2001 with the same dose. In the second season, plants were watered twice: on 23 March 2002 following fertilization and on 22 June 2002 immediately after harvest at a dose of 1 l water per plant. In the third season, plants were irrigated three times with 1 l water each time: on 3 May 2003 following fertilization, on 22 June 2003 (immediately after harvest), and on 6 September 2003 following the second fertilization.

Weeds were controlled by hand-hoeing when necessary. "Benlate" (Benomyl 50%, w/w) was applied at a rate of 0.4 g l^{-1} in May 2002 to control a fungus attack.

The plantation was harvested by cutting at a height of 5 cm above ground during the 80% flowering stage in all three seasons (22 June). Herbage yield was determined from the inner 3 rows of each plot after air-drying in the laboratory for 1 week. The dry weights of leaves, stems and inflorescences were also separately determined.

Essential oil concentration of leaves and inflorescences was determined on the air-dried material in the second (2002) and third (2003) seasons, since herbage production in the first season was low. The concentration in shoots was found negligible. Distillation was performed in two Clevenger apparatus using 50 g of ground plant samples diluted in 500 ml deionised water each time. The solution was heated at 100 °C for 3 h and the volume of the essential oil produced was measured and expressed as ml/100 g dry weight.

The analysis of all essential oils was performed using a Hewlett-Packard 55890 II GC, equipped with a HP-5 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) and a mass spectrometer HP 5972 as detector. The carrier gas was helium, at a flow rate of 1 ml min^{-1}. Column temperature was initially 55 °C for 3 min, then gradually increased to 200 °C at 3 °C/min and finally increased to 220 °C at 5 °C/min. For GC–MS detection, an electron ionization system was used with an ionization energy of 70 eV. The extracts were diluted 1:100 (v/v) with acetone and 1 μl of the diluted samples was injected automatically in spitless mode. Injector and detector temperatures were set at 220 and 290 °C, respectively.

A combined ANOVA was performed to detect the significance of the various sources of variance (season, treatment, replicates).

3. Results

There were considerable differences in the weather conditions prevailed during the two cultivation periods (2002 and 2003). In 2002 mean air temperatures were much lower in February and March than 2003; yet, they became slightly higher from May onwards. The last season received higher amount of rainfall (679.8 mm, of which 459.2 mm during the first half of the year) in comparison with the first one (497 mm, of which 29.6 mm during the same spell), In comparison with 2003, air relative humidity in 2002 was lower during February and March but higher from May up to September. More detailed information on the weather conditions during the two seasons is given by Sotiropoulou and Karamanos (2010).

3.1. Oil concentration and yield

Fig. 1 shows oil concentrations per plant and in different plant parts. The average oil concentration per plant increased significantly from about 1.5 to 2.0% in the second to about 5.5% (v/w) in the third season (Fig. 1a). Oil concentration in inflorescences varied between 2.27% and 3.60% in the second to 8.0% and 8.92% in the third season (Fig. 1b). Oil concentration in leaves fluctuated between 0.63% and 1.03% in the second to 3.75 and 4.25 in the third season (Fig. 1c). No significant differences among treatments were detected within either season in all cases mentioned above.

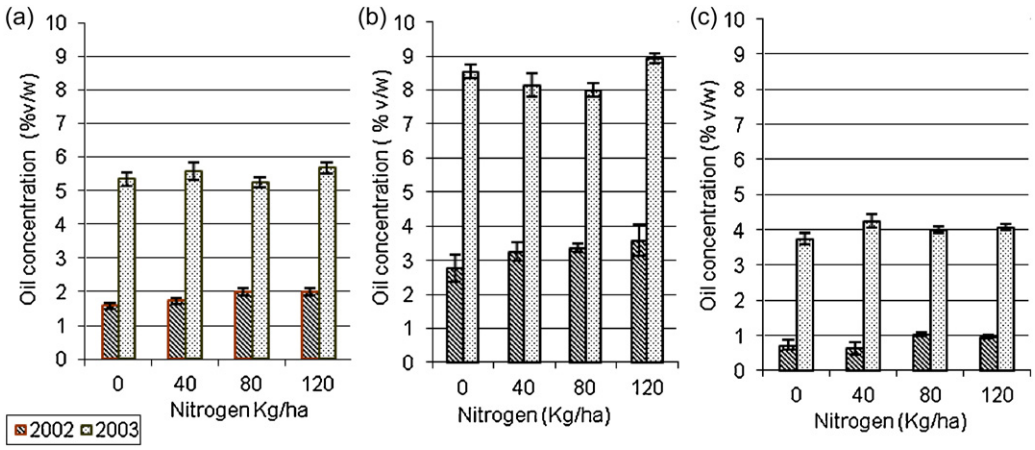

Fig. 1. The average values of oregano oil concentration in whole plants (a), inflorescences (b), and leaves (c) at 0, 40, 80, and 120 kg N ha^{-1} during 2002 and 2003. The bars indicate the standard errors of the means.

Fig. 2 shows the oil yields obtained from plants and different plant parts. Total oil yield showed significant peaks at nitrogen rates of 80 kg ha^{-1} in both seasons and was significantly higher in the third season (between 57 and 83 l ha^{-1}), when compared with the second one (between 17 and 27 l ha^{-1}) (Fig. 2a). Inflorescence oil yield varied significantly between 12 and 19.3 l ha^{-1} in the second to 30.7 and 41.4 l ha^{-1} in the third season without any significant treatment effect (Fig. 2b). On the other hand, significant treatment effects were detected for the oil yield of leaves exhibiting a peak at 80 kg N ha^{-1} in both seasons. The values fluctuated between 3.3 and 7.3 l ha^{-1} in the second to 26 and 42 l ha^{-1} in the third season (Fig. 2c).

3.2. Oil composition

The oil composition of leaves and inflorescences in the two seasons is shown in Table 1. The compounds with concentrations higher than 0.01% are presented in the rank of appearance of their peaks in the air-chromatogram.

Carvacrol was the dominant compound ranging from 56.46% to 84.88% among organs, treatments and seasons. It was followed by π-cymene (4.19–21.4%) and α-pinene (0.11–1.88%). Thymol was detected at low levels (0.20–1.44%).

Fertilizer treatments significantly affected the levels of some compounds only during the second season. In particular, the nitrogen rate of 80 kg ha^{-1} significantly increased linalool percentage in inflorescences (0.48%). In leaves, significantly higher percentages of α-pinene (1.88%), camphene (0.39%), π-cymene (21.4%), thymol (0.59%), and caryophyllene (1.48%) were detected in the unfertilized (control) plots, whereas carvacrol levels were significantly higher at the 40, 80 and 120 kg ha^{-1} (61.5–63.78%) in comparison with the control (56.46%).

Significant seasonal effects were detected for many compounds. Thus, in inflorescences, higher values were found for α-thujene, α-pinene, camphene, β-pinene, myrcene, α-terpinene, π-cymene, γ-terpinene, *cis*-hydrosavinene, linalool, α-terpineol, *trans*-hydrocarvone, α-caryophyllene, and β-disavolene during 2003, while higher values were found for carvacrol, 1-octen-3-ol, and borneol during 2002. In leaves, the values were significantly higher for α-thujene, α-pinene, camphene, 1-octen-3-ol, myrcene, α-terpinene, π-cymene, γ-terpinene, *cis*-hydrosavinene, linalool, terpinen-4-ol, α-terpineol, *cis*-dihydrocarvone, bourbonene, caryophyllene, α-caryophyllene, β-disavolene, spathulenol, and α-disavolol during 2003 and for carvacrol, thymol, π-cymenene and eugenol during 2002.

There were some compounds, such as β-pinene, α-phellandrene, terpinolene, ocimene, carvotanacetone and sesciphellandrene, which were detected only in inflorescences and others, such as π-cymenene, *cis*-dihydrocarvone, α- and β-thymicinone, acetic bormyl, eugenol, bourbonene, *cis*-calamenene, spathoulenol, epi-α-disavolol, α-disavolol, and sescicineol detected only in leaves during both seasons. In addition, carvacrol, myrcene, caryophyllene, α- and γ-terpinene were systematically

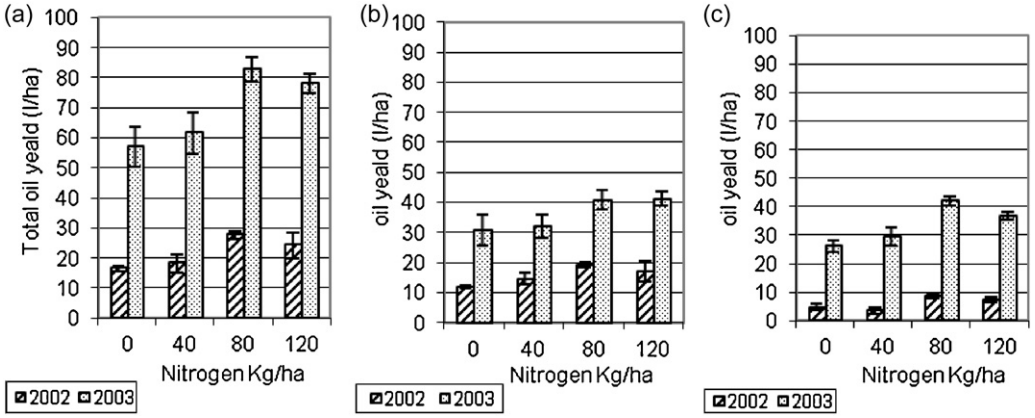

Fig. 2. The average values of oil yield of the oregano crop (a), inflorescences (b), and leaves (c) at 0, 40, 80, and 120 kg N ha^{-1} during 2002 and 2003. The bars indicate the standard errors of the means.

Table 1
The constituents of the Greek oregano (*Origanum vulgare* spp. *hirtum*) essential oil (%, v/v) extracted from leaves and inflorescences on harvest during 2002 and 2003.

	Inflorescences		Leaves	
	2002	2003	2002	2003
1. α-Thujene	0.19–0.28	1.10–1.23*	0–0.10	0.90–1.31*
2. α-Pinene	0.60–0.85	1.31–1.47*	0.11–0.50	**1.24–1.88***
3. Camphene	0.03–0.10	0.11–0.15*	0–0.12	**0.26–0.39***
4. β-Pinene	0–0.06	0.26–0.30*	–	–
5. 1-Octen-3-ole	0.28–0.43*	0.09–0.27	0–0.22	0.29–0.45*
6. Myrcene	0.80–1.30	2.17–2.39*	0.07–0.22	0.77–1.31*
7. α-Phellandrene	0.08–0.33	0.19–0.22	–	–
8. α-Terpinene	0.79–1.04	1.31–1.51*	0.11–0.22	0.50–0.93*
9. π-Cymene	4.19–5.76	5.33–6.98*	9.54–20.71	**15.75–21.40***
10. Ocimene	–	0.01–0.05	–	–
11. γ-Terpinene	2.42–3.11	4.80–5.43*	0.41–0.95	1.33–3.51*
12. *cis*-Hydrosavinene	0.06–0.15	0.62–1.06*	0.03–0.13	1.36–1.51*
13. Terpinolene	0.11–1.12	0.11–0.14	–	–
14. π-Cymenene	–	–	0.10–0.19*	0.03–0.11
15. Linalool	0.25–0.30	**0.34–0.48 (80)***	0.13–0.25	0.60–0.61*
16. Borneol	0.24–0.31*	0.09–0.25	0.26–0.45	0.29–0.38
17. Terpinen-4-ol	0.57–0.87	0.93–1.11	0.61–0.84	1.03–1.31*
18. Naphthalene	0–0.07	–	0.11–0.22	–
19. π-Cymen-8-ol	0–0.07	–	0.07–0.16	0.08–0.18
20. α-Terpineol	0–0.06	0.06–0.12*	0.02–0.09	0.11–0.17*
21. *cis*-Dihydrocarvone	–	–	0.03–0.13	0.11–0.19*
22. *trans*-Dihydrocarvone	0–0.03	0.04–0.11*	0–0.07	–
23. *α*-Thymicinone	–	–	0.13–0.36	–
24. *β*-Thymicinone	–	–	0.14–0.34	–
25. Bormyl acetate	–	–	0–0.05	–
26. Carvotanacetone	0.03–0.06	0.01–0.07	–	–
27. Thymol	0.20–0.36	0.23–0.31	0.50–1.44*	**0.44–0.59 (0)**
28. Carvacrol	81.19–84.88*	72.07–75.12	70.75–82.70*	**56.46–63.78 (40, 80, 120)**
29. Eugenol	–	–	0.23–0.38*	0–0.23
30. Bourbonene	–	–	0.02–0.05	0.06–0.09*
31. Caryophyllene	1.13–1.14	1.37–1.67	0.44–0.67	**1.10–1.48***
32. α-Caryophyllene	0.08–0.11	0.14–0.17*	0.02–0.04	0.12–0.15*
33. β-Disavolene	0.87–1.26	1.35–1.62*	1.09–1.80	2.30–3.06*
34. *cis*-Calamenene	–	–	0.02–0.05	–
35. Spathoulenol	–	–	0.14–0.29	0.24–0.45*
36. Caryophyllene oxide	0.03–0.21	0.03–0.05	0.37–0.60	0.42–0.73
37. *epi*-α-Disavolol	–	–	0.02–0.05	–
38. α-Disavolol	–	–	0.02–0.06	0.06–0.14*
39. Sesquicineol	–	–	0.02–0.06	–
40. Sesquiphellandrene	–	0.06–0.08	–	–

Numbers in bold indicate significance within nitrogen treatments ($p < 0.05$) and the number in brackets the treatment exhibiting the highest percentage of the constituent.
* The season exhibiting significantly higher percentage ($p < 0.05$) of each constituent.

found at higher levels in inflorescences, whereas thymol, π-cymene, β-disavolene and caryophyllene oxide at higher levels in leaves.

The concentration of the prevailing phenolic compounds (carvacrol and thymol) in inflorescences fluctuated between 81.48% and 85.08% in 2002 to 72.37% and 75.34% in 2003 without any significant nitrogen-effect. The corresponding values were lower in leaves and ranged between 71.43–84.14% in 2002 and 57.06–64.23% in 2003, also without any significant nitrogen-effect (Fig. 3a). The sum of the concentrations of π-cymene and γ-terpinene, considered as precursors of thymol and carvacrol, fluctuated between 6.94–8.86% during 2002 and 10.77–11.85% during 2003 in inflorescences; in leaves, the sum of π-cymene and γ-terpinene ranged at higher levels (from 10.28% to 21.12% in 2002 and from 17.64% to 23.42% in 2003) without any significant nitrogen-effect (Fig. 3b).

The total concentration of the oxygen compounds (phenols, oxides, and cetones) was much higher than that of hydrocarbons both in inflorescences and leaves (Fig. 3c and d). In inflorescences, oxygen compounds ranged from 83.66% to 86.67% during 2002 and from 75.64% to 77.99% during 2003, while hydrocarbons ranged from 12.53% to 15.47% during 2002 and from 20.5% to 22.8% during 2003. No significant nitrogen effect was detected. In leaves, the concentration of oxygen compounds ranged from 74.86% to 86.53% in 2002 and from 62.83% to 68.99% in 2003; hydrocarbon concentrations ranged from 12.38% to 24.49% in 2002 and from 25.88% to 33.67% in 2003. No nitrogen effect was detected in 2002, whereas in 2003 a significant increase in oxygen compounds with a concomitant significant decline in hydrocarbons under increasing nitrogen fertilization was observed.

In both inflorescences and leaves the total concentration of monoterpenes (α-thujene, α- and β-pinene, camphene, myrcene, α-phellandrene, α- and γ-terpinene, π-cymene, *cis*-hydrosavinene, terpineol, π-cymenene, linalool, borneol, terpinen-4-ol, naphthalene, π-cymen-8-ol, α-terpineol, *cis*- and *trans*-dihydrocarvone, α- and β-thymicinone, thymol, carvacrol, eugenol, carvotanacetone, and ocimene) was very high: in inflorescences it ranged from 95.9% to 96.7% during 2002 and from 94.64% to 95.42% during 2003; the respective values for leaves were 95.03–96.74% during 2002 and 89.2–92.55% during 2003. No nitrogen effect was detected in either season (Fig. 3e).

On the other hand, the concentration of sesquiterprenes (bourbonene, caryophyllene, α-caryophyllene, β-disavolene, *cis*-calamenene, spathoulenol, α- and epi-α-disavolol, sesquineol, caryophyllene oxide) was very low: in inflorescences it ranged from 2.2% to 2.85% during 2002 and from 2.9% to 3.45% during 2003. In leaves, the values were higher, from 2.2% to 3.7% during 2002 and from 4.3% to 5.9% during 2003. No nitrogen effect was detected. The increase observed during 2003 in

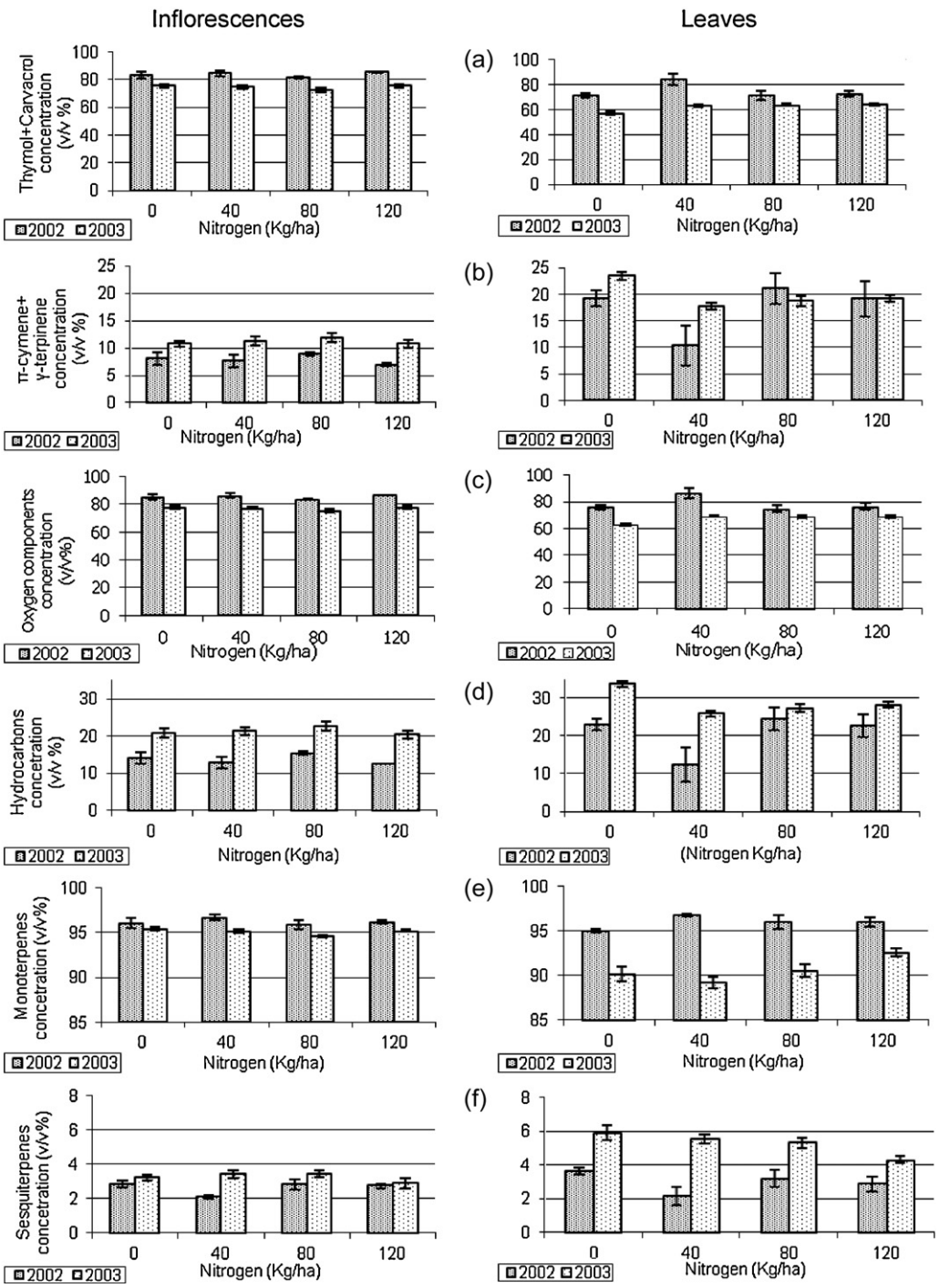

Fig. 3. The average values of the percentages of different groups of chemical constituents of the oregano oil in inflorescences and leaves at 0, 40, 80, and 120 kg N ha^{-1} during 2002 and 2003. (a) Thymol and carvacrol, (b) π-myrcene and γ-terpinene, (c) oxygen compounds, (d) hydrocarbons, (e) monoterpenes, and (f) sesquiterpenes. The bars indicate the standard errors of the means.

both inflorescences and leaves was significant in leaves only (Fig. 3f).

4. Discussion

The average values of oil concentration per plant recorded in this work varied within the range of 1.1–8.2% (v/w) already reported by other investigators for Greek oregano (Baser et al., 1993; Franz and Novak, 1997; Kokkini et al., 1994). These values are much higher than those found for other *Oregano* species in Greece: *O. vulgare* ssp *vulgare* and *O. vulgare* ssp. *viridulum* exhibited concentrations between 0.3% and 0.8% (Kokkini et al., 1994; Kokkini and Vokou, 1989). Because of the higher density of glandular hairs in flowers, oil concentration was found much higher (from 2 to 3.5 times) in inflorescences in comparison with leaves (see also Werker et al., 1985).

No nitrogen-effect on oil concentration was evident in either cultivation period in this work. Similar results were found for other species of the Lamiaceae family (Amr et al., 2003; Baranauskien et al., 2003; Mitchell and Farris, 1996; Omer, 1999; Ram et al., 2006; Ram and Kumar, 1997), whereas an increase in oil concentration by nitrogen application was observed in basil

(Sifola and Barbieri, 2006), *O. syriacum* (Ozgüven et al., 2006), and sage (Karioti et al., 2003). On the other hand, a reduction in oil concentration by nitrogen fertilization in *Origanum vulgare* ssp *hirtum*, *Origanum vulgare* var *samothrake*, and *Origanum vulgare* var. *creticum* has been reported by Azizi et al. (2009).

In contrast to oil concentration, oil yield was significantly increased by nitrogen application. This was caused by the positive nitrogen-effect on biomass production (Sotiropoulou and Karamanos, 2010), as it was also found in numerous other works on related species (e.g., Baranauskien et al., 2003; Omer, 1999; Ram et al., 1995; Sifola and Barbieri, 2006).

A clear effect of the cultivation period on oil concentration was observed. The increased values observed in 2003 in both leaves and inflorescences could be ascribed to the age of the plantation, and/or the environmental conditions prevailed. According to Goliaris (1997, 1988), the oregano plantation reaches its maximum production potential from the third year onwards, whereas Sotiropoulou and Karamanos (2010) showed that a full herbage production can be attained already from the second year. The contrasting climatic conditions prevailed between 2002 and 2003 in this work support the idea of an important environmental effect on oil concentration. In particular, the substantially higher oil concentrations attained in 2003 (Fig. 1) can be related both to the much higher amounts of rainfall during the first half of this year (459.2 mm in 2003 vs. only 29.6 mm in 2002) and the definitely lower average air temperatures prevailed during February, March, and April in comparison to 2002. Higher oil yields of Greek oregano were also associated with higher precipitation and lower temperatures during growth in other works in Greece (Panagopoulos, 2012) and elsewhere (Russo et al., 1998). Similarly, Marzi (1997) reported that dry and warm weather conditions were associated with lower oil concentrations. However, there is also evidence that water shortage and warm weather induce high oil concentrations in *O. vulgare* by means of a higher density of glandular hairs in the plant organs (Azizi et al., 2009; Kokkini et al., 1994).

Carvacrol was the main component of the essential oil in both seasons. The high carvacrol content is a characteristic of the Greek oregano essential oil (Franz and Novak, 1997; Kokkini and Vokou, 1989; Vokou et al., 1993) closely associated with its quality (Exarchou et al., 2002; Kokkini, 1997; Putievsky et al., 1997; Economou et al., 2011). In both years, carvacrol content was higher in inflorescences in comparison with leaves (see also Pizzale et al., 2002, on *Origanum onites* and *Origanum indercedens*). In contrast to carvacrol, thymol content was detected at very low levels (see also Baser et al., 1994; D'Antuono et al., 2000; Esen et al., 2007; Figueredo et al., 2006a,b; Russo et al., 1998). Nitrogen application did not affect the concentration of these phenolic compounds in inflorescences. In leaves, however, nitrogen fertilization significantly increased carvacrol and decreased thymol and π-cymene contents in respect to the unfertilized plots in 2003. It is known that both carvacrol and thymol are biosynthesized through the autooxidation of γ-terpinene to π-cymene followed by the hydroxylation of the latter (Poulose and Croteau, 1978). Accordingly, the increased levels of carvacrol in association with the low levels of thymol and π-cymene in the fertilized plots probably reflect the close biosynthetic relation between these compounds in Greek oregano (see also Toncer et al., 2009). According to Omer (1999), the enhancement of carvacrol biosynthesis by nitrogen fertilization at the expense of π-cymene occurs by activating the enzymatic system responsible for its conversion to carvacrol.

In view of the high levels of carvacrol, it is not surprising that linalool was detected at low levels in both leaves and inflorescences (Vokou et al., 1993). According to Vernet (1976, 1977), linalool accumulation at the expense of phenolic compounds is regulated by a dominant allele found in a genetically independent locus in thyme. Nevertheless, linalool content was positively affected by nitrogen application in inflorescences. However, there is also evidence for a negative effect of nitrogen on linalool content in basil (Sifola and Barbieri, 2006).

Essential oil composition was significantly affected by the cultivation season, considering that 2003 was substantially more humid from January to June and colder from January to April than 2002, when oregano plants grow and develop vigorously. A considerable number of oil constituents, mostly hydrocarbons, were accumulated at significantly higher levels in both leaves and inflorescences in 2003 in comparison to 2002 (α-thujene, α-pinene, camphene, myrcene, α- and γ-terpinene, π-cymene, *cis*-hydrosavinene, linalool, α-terpineol, α-caryophyllene, and β-disavolene), though only carvacrol was detected at higher levels in 2002 (Table 1). An increasing trend in carvacrol concentration with rising temperature and falling precipitation and relative humidity during the 3 months before harvest was also reported for Greek oregano by Panagopoulos (2012). In addition, increasing precipitation and falling temperature trends were associated with higher levels in π-cymene, γ-terpinene and caryophyllene in the same work.

5. Conclusions

Nitrogen application did not affect essential oil concentration in both leaves and inflorescences of Greek oregano in either season. However, it affected positively essential oil yield, exhibiting an optimum at 80 kg N ha^{-1}, through its favourable effects on herbage yield. Oil concentration was significantly higher in the third, more humid, season.

As regards oil composition, nitrogen fertilization exhibited some significant effects only in the third season. Thus, it positively affected linalool content in inflorescences at the rate of 80 kg N ha^{-1} and carvacrol content in leaves at all nitrogen levels. On the other hand, π-cymene, caryophyllene, α-pinene, thymol, and camphene were observed at higher levels in the unfertilized plots.

Carvacrol content was higher in the drier and warmer season, whereas a number of compounds (α-thujene, α-pinene, camphene, myrcene, α-and γ-terpinene, π-cymene, *cis*-hydrosavinene, linalool, α-terpineol, α-caryophyllene, and β-disavolene) tended to accumulate at higher levels during the wetter and colder season.

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