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EFFECTS OF ENHANCED UV-B RADIATION ON *MENTHA SPICATA* ESSENTIAL OILS

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Key Word Index—*Mentha spicata*; Labiatae; UV-B radiation; essential oil content; carvone; dihydrocarvone; piperitenone oxide; piperitone oxide.

Abstract—*In vitro* propagated plantlets representing two distinct chemotypes of *Mentha spicata, viz.* plants producing essential oils rich in piperitone oxide and piperitenone oxide (chemotype I) and rich in carvone and dihydrocarvone (chemotype II), were grown in the field under ambient or ambient plus supplemental UV-B radiation, biologically equivalent to a 15% ozone depletion over Patras (38.3°N, 29.1°E), Greece. Enhanced UV-B radiation stimulated essential oil production in chemotype II substantially, while a similar, non-significant trend was observed in chemotype I. No effect was found on the qualitative composition of the essential oils, whereas the quantitative composition was slightly modified in chemotype I. This is the first investigation reporting an improved essential oil content under UV-B supplementation in aromatic plants under field conditions.

INTRODUCTION

The risk of increasing UV-B radiation on the surface of the earth due to the decreasing trends in stratospheric ozone concentration [1] has prompted much research on the effects that this enhanced UV-B radiation may have on plants. Due to their obvious economic importance, cultivated plants were initially the main targets of these investigations [2], while the interest on wild plants and ecosystem functions is more recent [3, 4]. The main outcome from these investigations is that, as far as growth is concerned, UV-B radiation effects are species or even variety-specific, distinguishing between sensitive and tolerant plants, while in some cases UV-B radiation was beneficial.

Concerning aromatic plants, the only relevant study is that of Roth [5], investigating in short-term experiments the effects of the presence or absence of UV-B radiation on oil content and composition in *Thymus vulgaris, Majorana hortensis* and *Ocimum basilicum*, grown in a greenhouse, under non-realistic balance between UV-B, UV-A and visible radiation. Since spectral balance can highly modify the UV-B radiation effects, the results of this study cannot be extrapolated to field conditions [6]. However, it is implied that essential oil production can be increased by UV-B radiation. This may have significant consequences, as terpenoids of essential oils play a variety of ecological roles [7].

A number of studies on wild-growing Labiatae species, have shown that their essential oil content varies according to the climatic gradient found along Greece. In particular, it has been found that within a single species, the richest in essential oil populations are those grown in areas where a typical Mediterranean climate occurs [8–10]. However, it is difficult to conclude from these field studies for the specific effects of each separate climatic factor.

In the present investigation, we have studied the effects of a realistic, additional UV-B radiation dose in two chemotypes (cf. Ref. [11]) of the commercially important aromatic plant, *Mentha spicata*, growing to maturity under Mediterranean field conditions. We describe herein the effects on oil production and composition, while the effects on growth, physiology and reproduction have been reported elsewhere [12].

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Table 1. Essential oil content of *Mentha spicata* leaves under ambient (control) and ambient plus supplemental UV-B radiation (UV-B)

	Units	Control	UV-B	% change	*P*
Chemotype I	ml 100 g^{-1} dry wt	0.49 ± 0.23	0.51 ± 0.08	+4.08	0.87
	ml $(m^2)^{-1}$	0.27 ± 0.14	0.35 ± 0.12	+29.65	0.24
Chemotype II	ml 100 g^{-1} dry wt	0.48 ± 0.13	0.73 ± 0.24	+52.08	0.03
	ml $(m^2)^{-1}$	0.56 ± 0.16	0.99 ± 0.40	+77.00	0.02

Results are means ± SD from 10 plants.

RESULTS AND DISCUSSION

In all cases, stems produced negligible amounts of essential oil; therefore, all the subsequent results refer to the oil produced by the leaves. UV-B supplementation stimulated essential oil production in plants of both chemotypes (Table 1). In particular, in chemotype II there was a significant increase in oil production, either expressed on a dry mass or on a leaf area basis. The estimation of essential oil content per unit surface area is used, to avoid any influence of leaf mass differences between the two radiation conditions. In plants of chemotype II for example, UV-B radiation had no effect on total leaf surface area, but the leaves were heavier, i.e. the biomass invested per unit surface area was increased [12]. In plants of chemotype I exposed to supplemental UV-B irradiation, an increase of essential oil content, yet not statistically significant, was also found (Table 1). In spite of the increase in oil production, the number of glands per unit surface area remained unchanged. Their structure was not examined.

A number of experiments report an increase in oil content of various aromatic plants with increasing photon fluence rate [13–15]. However, the contribution of the UV-B spectral region to this increase had not been investigated, although the greenhouse, short-term experiments of Roth [5] had pointed out such a possibility.

In order to find out if the supplemental UV-B radiation had any effect on the essential oil qualitative and quantitative composition, we focused on the *p*-menthane monoterpenes of the two *M. spicata* chemotypes. These are distinguished by the position of oxygenation on the *p*-menthane ring. Plants of chemotype I produce exclusively monoterpenes bearing an oxygen function at C-3, whereas those of chemotype II produce monoterpenes bearing an oxygen function at C-6 [14]. UV-B radiation had no qualitative effect on the two main biosynthetic pathways that lead to the accumulation of either C-3- (pulegone, menthone and their relative alcohols and esters, piperitenone, piperitone and their epoxides) or C-6- (carvone and its relative compounds) oxygenated *p*-menthane derivatives (Tables 2 and 3).

Table 2. Percentages of *p*-menthane essential oil components from *Mentha spicata* leaves (Chemotype I), under ambient and ambient plus supplemental UV-B radiation

Compound	Control	UV-B
Limonene	1.48 ± 1.36	2.01 ± 1.63
Isomenthone	0.11 ± 0.13	0.24 ± 0.24
Menthone	0.29 ± 0.20	0.42 ± 0.24
Menthyl acetate	0.05 ± 0.16	0.18 ± 0.24
Neomenthole	0.15 ± 0.17	0.29 ± 0.22
Menthol	6.49 ± 2.45	4.75 ± 1.48
Pulegone	0.03 ± 0.08	0.13 ± 0.14
cis-Piperitone oxide	5.56 ± 1.74	4.71 ± 2.27
trans-Piperitone oxide	44.95 ± 8.40	41.19 ± 11.11
Piperitone	0.28 ± 0.24	0.27 ± 0.21
Isopiperitenone	2.81 ± 0.90	2.07 ± 1.16
Piperitenone	0.27 ± 0.17	0.26 ± 0.11
Piperitenone oxide	15.68 ± 7.13	19.94 ± 10.58
Total	**78.15**	**76.46**

Results are means ± SD from 10 plants. Differences are not statistically significant.

Remainder of essential oil consists of 25 compounds. Among them the most prominent is 1,8-cineole (concentration in control and UV-B 8.35 ± 4.31 and 7.74 ± 4.65 of the total oil, respectively).

Table 3. Percentages of *p*-menthane essential oil components from *Mentha spicata* leaves (Chemotype II), under ambient (control) and ambient plus supplemental UV-B radiation (UV-B)

Compound	Control	UV-B
Limonene	9.04 ± 2.00	8.54 ± 2.29
trans-Dihydrocarvone	0.54 ± 0.24	0.56 ± 0.16
cis-Dihydrocarvone	7.56 ± 3.55	7.08 ± 1.83
Neodihydrocarveol	0.11 ± 0.09	0.10 ± 0.11
Neoisodihydrocarvyl acetate	0.22 ± 0.18	0.11 ± 0.11
Carvone	59.58 ± 3.37	59.51 ± 4.45
Dihydrocarveol	0.16 ± 0.17	0.23 ± 0.17
Neoisodihydrocarveol	0.33 ± 0.12	0.32 ± 0.06
trans-Carvyl acetate	0.12 ± 0.11	0.15 ± 0.10
trans-Carveol	0.41 ± 0.05	0.45 ± 0.02
cis-Carveol	0.03 ± 0.05	0.03 ± 0.04
Total	**78.10**	**77.08**

Results are means ± SD from 10 plants. Differences are not statistically significant.

Remained of essential oil consists of 21 compounds. Among them the most prominent are 1.8-cineole (concentration in control and UV-B 6.31 ± 2.84 and 7.52 ± 2.34 of total oil, respectively) and β-bourbonene (concentration 3.27 ± 1.02 and 3.61 ± 0.98, respectively).

Furthermore, limonene, the precursor of both *p*-menthane oxygenated series [16] is present in almost similar amounts under ambient and ambient plus supplemental UV-B radiation. Biosynthetic studies have shown that the oxygenation pattern of *Mentha* monoterpenes is determined by regiospecific cytochrome P450-catalysed hydroxylation of the common olefinic precursor (−)-limonene [17] (and references therein). Thus, the results of the present study suggest that the UV-B radiation applied in both chemotypes of *M. spicata* had no effect on the C-3- and C-6-limonene hydroxylases.

A slight decrease of the total amount of the C-3-oxygenated monoterpenes under supplemental UV-B radiation was found in chemotype I (76.67% vs 74.45%), but as in the case of single component quantitative fluctuation, this difference is not statistically significant. However, it is worth noting the opposite trend found in the amounts of the two main oil components, *trans*-piperitone oxide and piperitenone oxide. Lawrence [18] proposed that 1,2 epoxy-*p*-menthane components of *Mentha* essential oils, are formed from the conversion of piperitenone as following:

(i) piperitenone → (+)-piperitone → (−)-*trans*-piperitone oxide, and

(ii) piperitenone → (+)-piperitenone oxide → (−)-*cis*-piperitone oxide

Therefore, the decrease of *trans*-piperitone oxide and the increase of piperitenone oxide observed when supplemental UV-B radiation is applied (Table 2), may suggest that the second pathway is favoured. Similar opposite trends in the quantitative participation of these two compounds have been found in the essential oils obtained from *M. spicata* plants grown wild in climatically different areas [10]. Since there are no measurements of UV-B radiation from these areas, no correlation can be made with the findings of the present work.

The quantitative composition of the essential oils obtained from chemotype II under the two radiation conditions show no significant differences in the percentages of limonene, carvone and the rest of the carvone-relative components (Table 3). In particular, the amount of carvone, the main essential oil component, is almost the same in both radiation conditions (mean value 59.58% and 59.51% of the total oil, respectively). The same is true for the sum of the C-6-oxygenated compounds (69.06% vs. 68.54%).

Our results show that a mild, above ambient enhancement of UV-B radiation under field conditions can improve the essential oil content of both chemotypes of the commercially important *M. spicata*, without changing their quality and without having any adverse effects on the growth and physiology of the plant [12]. At the moment, there is no evidence to explain the milder effect on oil production observed on chemotype I. However, differential effects of UV-B radiation on growth of varieties within a species have been reported for some cultivated plants [19, 20].

It is well documented that the phenylpropanoid biosynthetic pathway can be accelerated by exposing plants to UV-B radiation [21]. Concerning the other major pathway of secondary plant metabolism, i.e. the mevalonic acid pathway, leading to terpenoids which constitute the main components of essential oils, investigations are very scarce. To the best of our knowledge, apart from the already mentioned dissertation of Roth [5], the only other work reporting on UV-B radiation effects on terpenoids is that of Lydon *et al.* [22], who found an increase in cannabinoids of the drugtype (but not of the fiber-type) chemotype of *Cannabis sativa*. More work is needed in order to confirm the above observations with additional plant species and to elucidate the underlying mechanisms.

A multiplicity of ecological roles has been ascribed to essential oils and their constituents. There is a general consensus that these compounds constitute a basic armament in the defensive potential of the plants against excessive water loss [23, 24] or biotic attack; antiherbivore, antibacterial, antifungal and allelopathetic functions have been reported in many cases [7, 25] (and references cited therein). We may, therefore, suggest that the expected UV-B radiation increase on the surface of the earth would not be detrimental for *M. spicxta*. On the contrary, the increase of its essential oil content, together with the amelioration of its reproductive effort by enhanced UV-B radiation [12] may strengthen its defensive potential and improve its fitness.

EXPERIMENTAL

Plant material

Mentha spicata plants representing two distinct chemotypes of the species were used. Musty odoured plants producing oils rich in piperitone oxide and piperitenone oxide (chemotype I) and spearmint-odoured plants, producing essential oils rich in carvone and dihydrocarvone (chemotype II) were collected from two wild populations, i.e. Mt. Minthi and Alfios Dam (Peloponnisos, S. Greece), respectively. Voucher specimens of the collected material were identified by the last author and kept in the Herbarium of the Laboratory of Systematic Botany and Phytogeography, Aristotle University of Thessaloniki (TAU) (herbarium voucher numbers: Kokkini 12340 and Kokkini 12358, respectively). Furthermore, collected plants material was propagated *in vitro* by Vitro Hellas S.A. All plantlets from each chemotype used in this experiment, originated from the same clone. Two-week-old

plantlets were transplanted into 20 cm diameter clay pots (one plant per pot) containing a mixture of local soil and perlite 4:1 (v:v) and transferred in a small open nursery in the vicinity of the field plots. Their growth rate was recorded for 20 days and 10 similar plantlets from each chemotype were selected for further experimentation. Selection was based on leaf number, total stem length and plant height. The experiment started on 25 April 1996 by placing the pots under the appropriate control and UV-B frames (two frames per treatment, 10 plants per frame or, five plants per frame per chemotype) and terminated by harvesting the plants in mid August.

Growth conditions

The UV-B irradiation system has been described previously [26]. In general, UV-B radiation was given by Philips TL 40/12 fluorescent tubes wrapped with 0.1 mm cellulose acetate film (Courtaulds Chemicals, Derby), which was replaced regularly to avoid changes in its transmission properties. Spectral irradiance was measured with an OL 752 Optronic (Orlando, FL) spectroradiometer, calibrated against an OL 752-10 spectral irradiance standard and an OL 752-150 module for wavelength accuracy. In order to determine the supplemental, biologically effective ($UV\text{-}B_{BE}$) daily dose under the required 15% ozone depletion, the absolute spectral irradiance (measured at night) was weighed with the generalised plant action spectrum normalised at 300 nm [27] and used in conjunction with the computer program of Ref. [28]. Accordingly, the required daily lamp duration was calculated. Each day, the supplemental irradiance was increased (and decreased) in two steps centred at solar noon and the lamp duration was modified monthly, in order to follow the natural march of ambient UV-B radiation change. In control frames, the TL 40/12 tubes were replaced by white, plastic tube effigies of the same diameter. In this way, the visible light environment under the control and UV-B frames was the same. With this system, UV-A radiation under the UV-B frames was increased by 1.9% in relation to the ambient, due to the small amount of UV-A radiation emitted by the tubes. This slight increase, however, was valid as long as all the tubes were on (around mid-day), it was reduced to 0.95% when half of the tubes were off and completely abolished when all the tubes were off (early morning and late afternoon, Ref. [26]). Accordingly, the daily integrated percent increase in UV-A is less than 1%. Much higher differences are needed for UV-A radiation to modify the UV-B radiation effects [6].

Plants received 100 ml of water daily (April, May and June) or 200 ml daily (July and August), plus the natural precipitation (37.07 mm of rainfall) for the experimental period. Soil water potential was measured at intervals with a Decagon (Pullman, WA) SC-10 thermocouple psychrometer. With the above irrigation scheme, soil water potential was the same for both treatments and varied between −0.43 and −0.54 MPa during the experimental period.

Essential oils

On August 13, aerial parts of the plants were harvested, separated into stems and leaves and air-dried in dark conditions and room temp. (20–25°) for 10 days. The essential oil of leaves and stems from each individual plant (2.4 to 6.0 g), was isolated after hydrodistillation for 2 h in a Clevenger apparatus. Essential oil content is expressed on a dry wt and on a leaf area basis.

GC was carried out with FID, using a 60 m × 0.25 mm Supelcowax 10 capillary column with the following temp. programme: 70° isothermal for 10 min, then an increasing rate of 4° min^{-1} up to 180°, isothermal for 35 min, then an increasing rate of 4° min^{-1} up to 220°, and isothermal for 5 min. Injector and detector temps were 240°. Carrier gas was He at 0.6 ml min^{-1}. GC-MS analyses were conducted in a system equipped with a capillary column under the same GC conditions. The detector was a quadropolar system with ionisation energy of 70 eV. Oil components were identified by comparing their RR_I and ms with those of authentic samples, the Wiley Registry of Mass Spectral data [29] and literature [30, 31].

Statistics

Since the results in the two replicated plots per treatment were almost the same, we used individual plants as the statistical unit. Accordingly, results are expressed as means ± SD from 10 plants per treatment. One-way ANOVA tests were used to determine statistically significant differences between control and UV-B plants within each chemotype at various levels of significance.

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