JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 7361–7367 7361
DOI:10.1021/jf9046072

Ultraviolet-B and Photosynthetically Active Radiation Interactively Affect Yield and Pattern of Monoterpenes in Leaves of Peppermint (*Mentha* × *piperita* L.)

Helen Behn,[†,∥] Andreas Albert,*[,‡] Friedhelm Marx,[#] Georg Noga,[†] and Andreas Ulbrich[§,⊥]

[†]Section Crop and Horticultural Sciences, Institute of Crop Sciences and Resource Conservation, University of Bonn, 53121 Bonn, Germany, [‡]Department of Environmental Engineering, Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, 85764 Neuherberg, Germany, [#]Department of Nutrition and Food Sciences, University of Bonn, 53115 Bonn, Germany, and [§]ICG-3 Phytosphere, Forschungszentrum Jülich, 52425 Jülich, Germany. [∥] Current address: ICG-3 Phytosphere, Forschungszentrum Jülich, 52425 Jülich, Germany. [⊥] Current address: Faculty of Agricultural Sciences and Landscape Architecture, University of Applied Sciences Osnabrück, 49090 Osnabrück, Germany.

Solar radiation is a key environmental signal in regulation of plant secondary metabolism. Since metabolic responses to light and ultraviolet (UV) radiation exposure are known to depend on the ratio of spectral ranges (e.g., UV-B/PAR), we examined effects of different UV-B radiation (280−315 nm) and photosynthetically active radiation (PAR, 400−700 nm) levels and ratios on yield and pattern of monoterpenoid essential oil of peppermint. Experiments were performed in exposure chambers, technically equipped for realistic simulation of natural climate and radiation. The experimental design comprised four irradiation regimes created by the combination of two PAR levels including or excluding UV-B radiation. During flowering, the highest essential oil yield was achieved at high PAR (1150 μmol m^{-2} s^{-1}) and approximate ambient UV-B radiation (0.6 W m^{-2}). Regarding the monoterpene pattern, low PAR (550 μmol m^{-2} s^{-1}) and the absence of UV-B radiation led to reduced menthol and increased menthone contents and thereby to a substantial decrease in oil quality. Essential oil yield could not be correlated with density or diameter of peltate glandular trichomes, the epidermal structures specialized on biosynthesis, and the accumulation of monoterpenes. The present results lead to the conclusion that production of high quality oils (fulfilling the requirements of the Pharmacopoeia Europaea) requires high levels of natural sunlight. In protected cultivation, the use of UV-B transmitting covering materials is therefore highly recommended.

KEYWORDS: ***Mentha* × *piperita* L; essential oil; monoterpenes; UV-B radiation; PAR**

INTRODUCTION

Various species of the genus *Mentha* belong to the economically most important essential oil bearing herbal plants (*1*). Peppermint essential oil is a common ingredient of foods (*2*), cosmetics, and medicinal products (*3*). It is mainly composed of monoterpenes (*4*). Qualitative and quantitative composition of monoterpenoid constituents determines quality and commercial value of the essential oil (*5*). High quality oils consist predominantly of menthol (30−55%), moderate proportions of its precursor menthone (14−32%), and low levels of the minor compounds pulegone (< 4%), menthofuran (1−9%), and menthyl acetate (2.8−10%) (*6*). Relative quantities of these compounds are mainly determined by the final steps of the biosynthetic pathway, proceeding from the branch point metabolite pulegone (**Figure 1**). Monoterpene biosynthesis is initiated by cyclization of the universal monoterpene precursor geranyl diphosphate to (−)-(4*S*)-limonene, which undergoes a sequence of transformation reactions (hydroxylation, oxidation, reduction, and isomerization) finally leading to pulegone. According to environmental conditions, this central intermediate can be oxidized to menthofuran by menthofuran synthase or reduced to menthone by pulegone reductase (*7*). Reduction of menthone by menthone reductase finally leads to menthol. As menthone is mainly represented by the stereoisomere (−)-menthone, which is primarily reduced to (−)-menthol (*8*), these two compounds are below referred to as menthone and menthol.

Formation and deposition of monoterpenoid essential oil is exclusively localized to peltate glandular trichomes, specialized epidermal structures, exhibiting an apical disk of eight secretory cells overarched by a subcuticular storage cavity (*9*, *10*). The exposed location of monoterpene accumulation on the leaf surface has contributed to the common assumption of an ecological role as constitutive barrier against herbivore or pathogen attack (*11*). Further functions for example in allelopathy are being discussed, as well (*12*).

*To whom correspondence should be addressed. E-mail: andreas.albert@helmholtz-muenchen.de. Phone: +49 89 31874636. Fax: +49 89 31874431.

Published on Web 05/19/2010

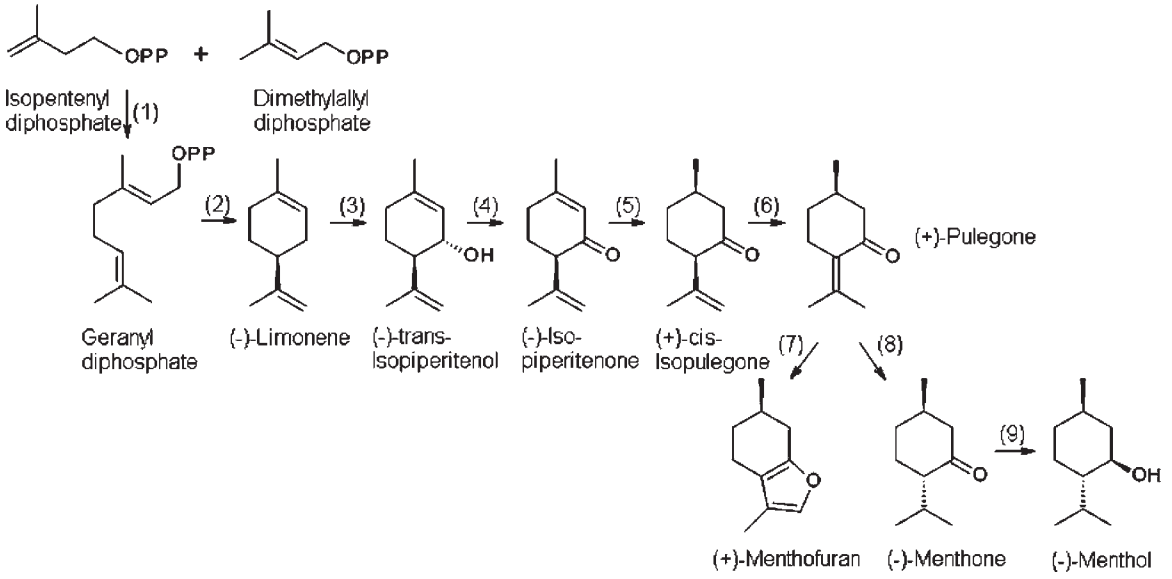

Figure 1. Monoterpene biosynthetic pathway in peppermint. Participating enzymes are geranyl diphosphate synthase (1), (−)-limonene synthase (2), cytochrome P450 (−)-limonene-3-hydroxylase (3), (−)-trans-isopiperitenol dehydrogenase (4), (−)-isopiperitenone reductase (5), (+)-cis-isopulegone isomerase (6), (+)-menthofuran synthase (7), (+)-pulegone reductase (8), (−)-menthone reductase (9). OPP indicates the diphosphate moiety.

Monoterpene biosynthesis is regulated by interaction of developmental and environmental factors. Individual leaf age as well as the stage of plant development exert a modifying effect on yield and composition of monoterpenes (*13*, *14*). During leaf ontogeny biosynthetic activity was found to be highest between day 12 and 20 after leaf emergence (*15*). In addition to this pronounced endogenous control, monoterpene metabolism underlies regulation by changing environmental conditions. These include biotic factors such as pathogens and herbivores (*16*) as well as abiotic factors such as nutrient availability (*17*), temperature (*18*), photoperiod (*19*), and global radiation. Global radiation comprises direct and diffuse solar radiation reaching the earth's surface including ultraviolet-B (UV-B, 280−315 nm), ultraviolet-A (UV-A, 315−400 nm), and photosynthetically active radiation (PAR, 400−700 nm) as well as infrared radiation (IR, > 700 nm).

The role of defined wavelength ranges of global radiation in monoterpene metabolism has been studied earlier revealing effects of UV-B, UV-A radiation, and PAR on monoterpene content and composition in different herbal plant species (*20*−*24*). Even though UV-B, UV-A radiation, and PAR are known to interact in the induction of diverse photomorphogenic (*25*) and metabolic (*26*) responses, and the ratio of UV-B radiation to PAR is of particular significance (*27*), detached interaction of different wavelength ranges has not been examined so far. Our integrated approach was therefore based on the hypothesis that PAR and UV-B radiation interactively affect monoterpene metabolism and thereby content and composition in peppermint leaves. Since monoterpene content was reported to be correlated with glandular trichome density and diameter in thyme (*Thymus vulgaris* L.) (*28*), we furthermore postulate that changes in essential oil accumulation go along with an altered density and/or size of peltate glandular trichomes. In order to test these hypotheses, plants were grown in exposure chambers at four different irradiation regimes composed of two PAR levels including or excluding ambient UV-B radiation. Amount and composition of monoterpenes as well as the density and diameter of peltate glandular trichomes were determined. Essential oil analysis was carried out throughout plant development, from early stage of vegetative growth until flowering. The flowering stage is of particular interest as it represents the common harvesting stage of peppermint (*29*), ensuring high oil quality (*13*, *30*) and improving further processing of plant material.

Table 1. Irradiation Conditions in the Sun Simulators Resulting from the Combination of Two PAR and Two UV−B Levels (In the Following Termed High or Low PAR and Presence or Absence of UV-B Radiation, Respectively)

	PAR	
UV-B	550 μmol m^{-2} s^{-1}	1150 μmol m^{-2} s^{-1}
0	low PAR/−UV-B	high PAR/−UV-B
0.6 W m^{-2}	low PAR/+UV-B	high PAR/+UV-B

MATERIALS AND METHODS

Plant Material and Cultivation. Peppermint (*Mentha* × *piperita* L. cv. BLBP 02, selected by the Bayerische Landesanstalt für Bodenkultur und Pflanzenbau) (*5*) was propagated vegetatively, resulting in genetically identical plants. Cuttings were raised in a greenhouse for six weeks before transfer to the phytotron facility of the Helmholtz Zentrum München. The experiments were arranged in two exposure chambers, each subdivided into two compartments. Thus, four treatments were performed (see **Table 1**), which contained 18 pots with one plant each. Growth conditions were a photoperiod of 16 h with a temperature regime of 20 °C during the day and 14 °C during the night. The relative humidity was 60 and 80%, respectively. Plants were cultivated in pots with soil as substrate and rinsed daily with a nutrient solution (N:P:K, 9.7:1:6.2).

The exposure chambers provided an irradiance spectrum very close to the natural global radiation from the ultraviolet through to the infrared spectrum. They are therefore commonly referred to as "sun simulators" (*31*). The natural photobiological environment was simulated using a combination of four types of lamps (metal halide lamps, quartz halogen lamps, blue fluorescent tubes, and UV-B fluorescent tubes). The lamp types were arranged in several groups to obtain the natural diurnal variations of solar irradiance by switching appropriate groups of lamps on and off. The short-wave cutoff was achieved by selected borosilicate filters, sodalime glass filters, and foils. The experimental setup, shown in **Table 1**, combined two PAR levels with two levels of UV-B radiation. This was achieved by separating the two sun simulators, one with 550 μmol m^{-2} s^{-1} PAR and the other with 1150 μmol m^{-2} s^{-1} PAR, by UV-B absorbing acrylic glass into two zones of UV-B radiation: 0 and 0.6 W m^{-2}. **Figure 2** shows the spectra of our four exposure treatments measured by a double monochromator system (Bentham, Reading, UK).

Determination of Plant Morphological Parameters. Evaluation of leaf morphology included determination of leaf area as well as fresh and dry weight of 12 mature leaf pairs collected at the onset of the generative

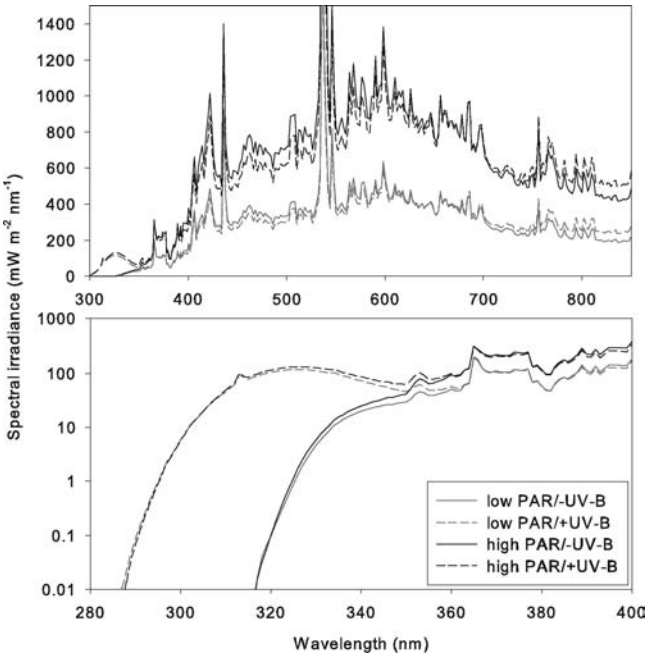

Figure 2. Simulated irradiance spectra of the four treatments on a linear scale from 300 to 850 nm (top) and on a logarithmic scale showing the UV range from 280 to 400 nm (bottom).

phase (9 weeks after transfer of plants into the sun simulators). Leaf area was examined by scanning of leaves and subsequent calculation using Sigma Scan Software (Sigma Scan Pro 5.0, Aspire Software International, Ashburn, USA). Shoot growth was investigated by determination of mean internode length (internodes 2−5) of 35 shoots.

Monoterpene Extraction and Gas Chromatographic Analysis. Essential oil extraction and analysis was carried out at three defined stages of development, during vegetative growth (at the age of 9 weeks), bud formation (at the age of 10 weeks), and flowering (at the age of 12 weeks). Newly formed leaves were collected at maturity (between day 21 and 30 of leaf development, leaf pair 4 and 5 from apex) in order to ensure a specific leaf onthogenic stage and thereby stable monoterpene composition. Sampling time was 9:30. In each stage of plant development, 8−10 samples consisting of 5 g of fresh leaves were pooled from shoots of five plants. Extraction was performed by steam-distillation in a clevenger apparatus (Faust GmbH, Meckenheim, Germany) for 2 h. As receiving solvent 1 mL of hexane containing 2.5 g L^{-1} of the internal standard undecane (Merck KGaA, Darmstadt, Germany) was injected. The final solution of monoterpenes in hexane was analyzed using a Hewlett-Packard 5890 gas chromatograph equipped with a BPX 5 (SGE Analytical Science, Australia) capillary column (length 25 m, i.d. 0.25 mm, film thickness 0.25 μm) and a flame ionization detector (FID, 280 °C). Nitrogen at 500 hPa was used at a flow rate of 1 mL min^{-1}. The injection volume was 1 μL, the split ratio was 1:50, and the temperature program increased from 60 to 240 °C at a rate of 3 °C min^{-1}. Compounds were identified by comparison of retention times with the following standard substances and their purities: (±)-menthol ≥99%, menthone ≥97%, (*R*)-(+)-limonene ≥96%, 1,8-cineole Ph Eur (Fluka AG, Buchs, Switzerland); isomenthone ≥75%, menthofuran ≥91%, (+)-pulegone ≥98% (Roth GmbH, Karlsruhe, Germany); (1*R*)-(−)-menthylacetat 98% (Sigma-Aldrich GmbH, Munich, Germany). Use of undecane as internal standard allowed calculation of a recovery rate for each sample. Total monoterpene content was quantified based on the molecular weight of the two main compounds menthone and menthol (≈155 g mol^{-1}) which comprise more than 70% of total monoterpenes. In addition, compounds were previously identified by comparison of retention times with standard compounds and gas chromatography−mass spectrometry (GC-MS) analysis with a Delsi-Nermag AUTOMASS 120 system. The GC separation was performed with a BPX 5 (SGE Analytical Science, Australia) equipped with a capillary column (length 25 m, i.d. 0.25 mm, film thickness 0.25 μm). Helium at 500 hPa was used at a flow rate of 1 mL min^{-1}. The injection volume was 1 μL, the split ratio was 1:50, and the temperature program increased from 60 to 240 °C at a rate of 3 °C min^{-1}. The MS was operated in EI mode at 70 eV. Compounds were identified by comparison of the MS with those of the National Institute of Standards and Technology (NIST) mass spectral library and with those of standard compounds mentioned above.

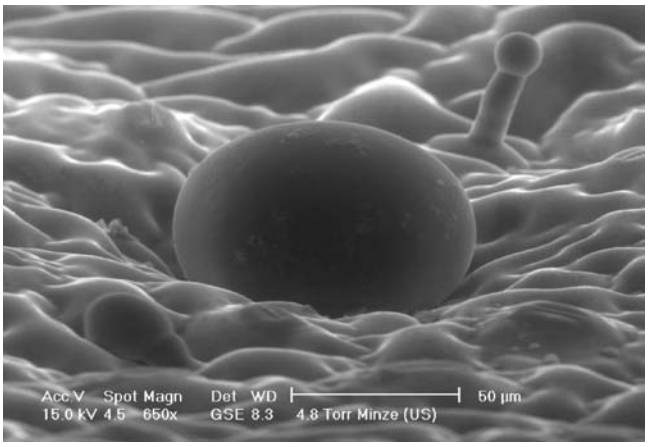

Figure 3. SEM image of leaf surface with peltate (foreground) and capitate trichome (background), 650-fold magnification.

Trichome Analysis by Scanning Electron Microscopy (SEM). For SEM examination, leaves were sampled at the age of 21 days after emergence (fourth leaf pair from apex) in order to ensure that leaf expansion was completed and trichome density was stable. Ten leaf pairs were collected from each treatment during bud stage (corresponding to 30 day old leaves in the flowering stage). For conservation and transport leaves were placed in Petri dishes containing wet tissue. Each of the two leaves served for examination of either the adaxial or the abaxial side by means of scanning electron microscopy (SEM, XL30 ESEM, FEI-Philips, Hillsboro, USA). Samples were always cut from the same position in the middle of the leaf on either side of the main vein and mounted on a peltier element. Sixteen images were taken of each sample at a magnification of 150 resulting in a total area of 8.17 mm^2. Images were evaluated by counting all types of trichomes individually (nonglandular, capitate, and peltate glandular trichomes, **Figure 3**) and measuring the diameter of peltate glands. Calculation of trichome density required determination of leaf area by measuring and multiplying maximum leaf length and width, and considering a corrective factor as described by Knoche and Noga (*32*).

RESULTS

Dry weight of mature leaves was significantly increased at high PAR (independent of UV-B radiation), whereas total leaf area was not affected by irradiation conditions. Consequently, specific leaf weight was significantly enhanced at high PAR. Shoot morphology was affected in terms of an elevated mean internode length at low PAR; this difference was only significant in the presence of UV-B radiation (**Table 2**).

The data obtained from essential oil analysis revealed a significant influence of PAR and UV-B level on yield and composition of monoterpenes (**Table 4**). Quantitative differences were strongly dependent on the developmental stage of the plant. During vegetative growth total monoterpene content of mature leaves was significantly higher at low PAR and ambient UV-B radiation compared to high PAR in the absence of UV-B radiation (**Figure 4**). No differences in monoterpene content were detected during bud formation. Finally, at the stage of flowering total monoterpene content was significantly enhanced at high PAR in the presence of UV-B radiation compared to the treatments without UV-B radiation (**Figure 4**).

Quantitative composition of monoterpenes, particularly, the ratio of menthone to menthol content, was endogenously controlled by the plants' stage of development. The onset of the generative phase led to a decline in menthone and an increase in menthol content (**Table 3** and **Figure 5**).

Table 2. Leaf and Shoot Morphological Parameters for the Four Treatments: LDW = Leaf Dry Weight, TLA = Total Leaf Area, SLW = Specific Leaf Weight, IL = Internode Length[a]

	low PAR/−UV-B	low PAR/+UV−B	high PAR/−UV-B	high PAR/+UV−B
LDW (g)	0.09 ± 0.03 a	0.10 ± 0.03 ab	0.11 ± 0.02 bc	0.13 ± 0.04 c
TLA (cm^2)	22.96 ± 3.00 n.s.	23.26 ± 2.90 n.s.	20.37 ± 3.84 n.s.	22.17 ± 5.62 n.s.
SLW (mg cm^{-2})	3.59 ± 1.45 a	4.16 ± 0.97 a	5.64 ± 1.05 b	6.07 ± 1.06 b
IL (cm)	6.14 ± 1.09 a	6.08 ± 1.42 a	5.47 ± 0.83 ab	5.06 ± 0.88 b

[a] Mean values ± standard deviation for $n = 24$, except for IL: $n = 35$; Tukey Test, $p < 0.05$, different letters denote significant differences or no significance (n.s.).

Table 3. Relative Amounts of Essential Oil Constituents (Given in Area Percent) As Affected by UV-B Radiation and PAR at the Stage of Flowering, Mean Values ± Standard Deviation, Tukey Test, $n = 8-10$, $p < 0.05$, Different Letters Denote Significant Differences, n.d., Means Not Detected (< 0.1%)

	low PAR/−UV-B	low PAR/+UV-B	high PAR/−UV-B	high PAR/+UV-B
menthol	27.0 ± 3.30 a	32.4 ± 1.93 b	32.5 ± 1.40 b	31.7 ± 2.00 b
menthone	43.1 ± 4.33 a	35.9 ± 2.18 b	35.3 ± 1.88 b	35.6 ± 2.40 b
isomenthone	3.30 ± 0.17 a	3.03 ± 0.11 b	2.84 ± 0.10 c	2.90 ± 0.09 bc
menthofuran	0.40 ± 0.04 a	0.36 ± 0.04 ab	0.31 ± 0.03 c	0.33 ± 0.02 bc
pulegone	n.d.	n.d.	n.d.	n.d.
limonene	7.44 ± 1.06 a	8.40 ± 0.80 ab	9.10 ± 0.92 b	9.01 ± 0.85 b
menthyl acetate	1.10 ± 0.61 ab	1.13 ± 0.45 ab	0.86 ± 0.42 a	1.59 ± 0.78 b
1,8-cineole	7.33 ± 0.42 a	8.11 ± 0.38 b	7.99 ± 0.41 b	7.73 ± 0.35 ab

Table 4. ANOVA *p*- and *f*-Values for Monoterpene Content (mol per leaf), Relative Menthone and Menthol Content (area percent) at the Stage of Flowering, Trichome Density (number per leaf, abaxial leaf side) and Trichome Diameter (μm, abaxial leaf side) as Affected by PAR and UV-B Radiation As Well As by the Interaction of PAR and UV-B Radiation

	PAR	UV-B	PAR × UV-B
monoterpene content	$p = 0.021$	$p = 0.001$	$p = 0.041$
	$f = 5.716$	$f = 13.12$	$f = 4.428$
menthone content	$p < 0.001$	$p < 0.001$	$p < 0.001$
	$f = 23.50$	$f = 17.37$	$f = 20.72$
menthol content	$p = 0.001$	$p = 0.001$	$p < 0.001$
	$f = 13.34$	$f = 11.89$	$f = 22.02$
trichome density	$p = 0.815$	$p = 0.247$	$p = 0.951$
	$f = 0.055$	$f = 1.383$	$f = 0.004$
trichome diameter	$p = 0.080$	$p = 0.197$	$p = 0.168$
	$f = 3.063$	$f = 1.667$	$f = 1.904$

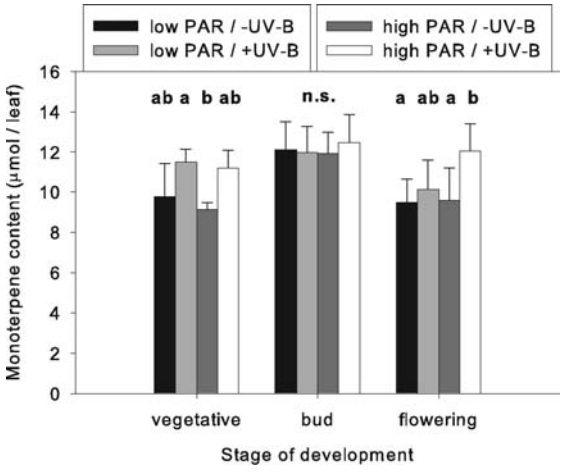

Figure 4. Total monoterpene content per leaf during vegetative development, bud and flowering stage; mean values ± standard error, Tukey Test, $n = 8-10$, $p < 0.05$, samples pooled from five plants each, different letters in the bars denote significant differences or no significance (n.s.).

The impact of PAR and UV-B radiation on quantitative monoterpene composition was most striking during flowering (**Table 3**). The main compounds menthone and menthol showed a marked response to irradiation conditions. Low PAR and exclusion of UV-B radiation led to increased menthone and reduced menthol contents (**Figure 5**), indicating an attenuated conversion of menthone to menthol. Under these irradiation conditions, the menthofuran content was the highest of all treatments.

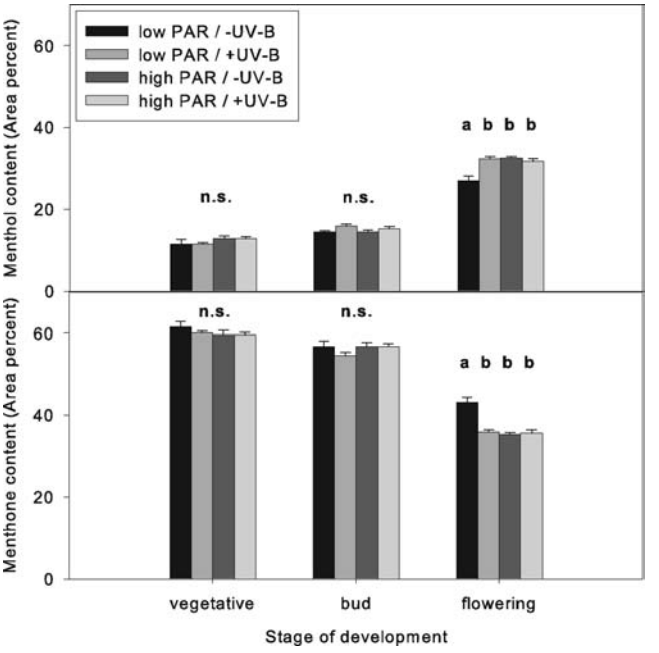

Figure 5. Relative content of menthol (top) and menthone (bottom) during vegetative growth, bud stage, and flowering as affected by the four different radiation regimes, mean values ± standard error, Tukey Test, $n = 8-10$, $p < 0.05$, mixed samples from five plants each; significant difference is indicated by different letters in the bars or no significance (n.s.).

The presence of UV-B radiation led to an increase in the number of peltate glandular trichomes per leaf (abaxial side, not statistically significant, **Table 4**, **Figure 6**, top). The adaxial leaf side (exhibiting a 10 times lower density of peltate glands) did not show any difference. Diameters of peltate trichomes were not affected at all, consistently ranging around 98 μm independent of radiation treatment (**Figure 6**, bottom).

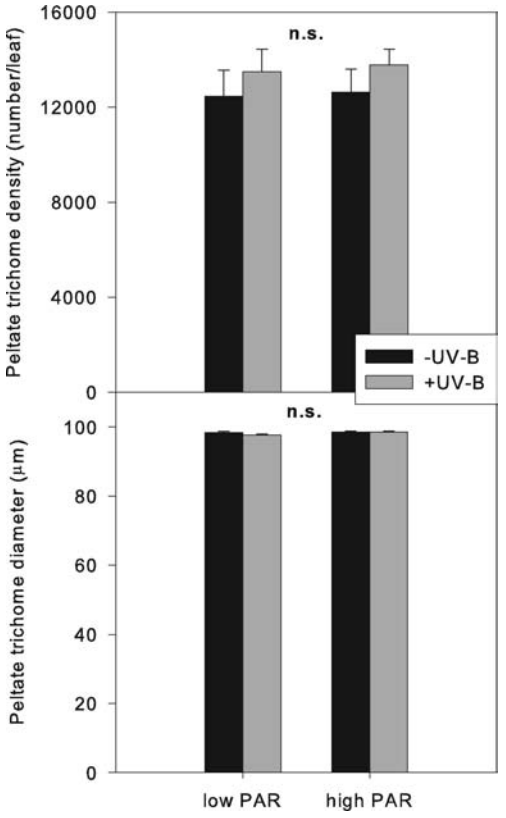

Figure 6. Density (top) and diameter (bottom) of peltate glandular trichomes on abaxial side of peppermint leaves, mean values ± standard error, Tukey Test, $n = 10$ for density, $n = 160$ for diameter, $p < 0.05$, no significance (n.s.) was detected.

DISCUSSION

Results obtained on total essential oil content clearly support our hypothesis of an interactive regulation by UV-B radiation and PAR (**Figure 4**, **Table 4**). At the stage of flowering total monoterpene content calculated on a single leaf basis was significantly increased at high PAR in combination with UV-B radiation. The measured monoterpene content is supposed be closely correlated with the total amount of monoterpenes synthesized in mature leaves since losses by volatilization and degradation are negligible (*33*).

As postulated, the monoterpene pattern was interactively affected by PAR and UV-B level as well (**Figure 5**, **Tables 3** and **4**). Low PAR and the exclusion of UV-B radiation led to a higher menthone and a lower menthol content during flowering, indicating an attenuation of endogenously induced menthone reduction at the onset of the generative phase. Because of this shift in the ratio of menthone to menthol and the enhanced level of the unfavorable constituent menthofuran (*34*), essential oil quality is considerably decreased under low irradiation conditions (*6*). This result is additionally confirmed by experiments conducted in greenhouses (Lehr- und Forschungsstation Marhof, Wesseling, Germany) covered with foils and glasses differing in UV-B transmission at PAR levels comparable to our "low PAR" conditions here (see Supporting Information).

To our knowledge, this is the first time that interactive effects of UV-B radiation and PAR on essential oil yield and pattern of peppermint has been shown. The effect of either PAR or UV-B radiation on essential oils of herbal plants is well documented. PAR was found to modify monoterpene composition in peppermint (*18*) and increase total essential oil content of thyme (*28*). Mediation of PAR effects observed in thyme was attributed to the phytochrome system (*35*). Studies on the role of UV-B radiation reported an enhanced production and an altered composition of essential oils in spearmint (*Mentha spicata* L.) (*21*) and basil (*Ocimum basilicum* L.) (*24*) exposed to supplementary UV-B radiation. Hence, PAR and UV-B radiation were individually shown to affect quantity and quality of essential oils in herbal plants. Nevertheless, it is important to keep in mind that essential oil compositions in thyme and basil differ sustantially from peppermint comprising considerable proportions of higher terpenoids and phenolic compounds. Although responses to UV-B radiation are known to depend on the level of background PAR (*25*, *27*), little attention has been paid to interaction of both spectral ranges in regulation of essential oil production (*36*).

Considering the importance of PAR and UV-B levels as well as their ratio, the mentioned studies, performed under varying irradiation conditions, can hardly be compared. Simulation of solar radiation requires a realistic balance of different spectral ranges, for example, sufficient PAR and no excessive UV-B level (*37*). Therefore, results produced under spectrally unbalanced conditions have to be carefully interpreted.

Besides specific signaling irradiation effects on yield and pattern of monoterpenes can also be mediated by availability of substrate (and cofactors) arising from photosynthesis (*17*). Monoterpene formation requires high amounts of the primary precursor acetyl-coenzyme A (acetyl-CoA) and the cofactors ATP (adenosine triphosphate) and $NADPH+H^+$ (nicotinamide adenine dinucleotide phosphate) (*17*, *38*). Substrate and cofactors must be generated at the site of monoterpene biosynthesis since they are not intercellularly transported. The secretory cells, being nonphotosynthetic, need to be provided with assimilates for production of these metabolites via glycolysis, the TCA cycle, and the oxidative pentose phosphate pathway (*39*). The observed elevation in essential oil content at high PAR therefore may be attributed to an increased availability of assimilates due to enhanced photosynthetic activity (*17*). However, the contribution of UV-B radiation to this effect is not clear.

The monoterpene pattern is to a large extent determined by $NADPH+H^+$-dependent reductive interconversions, such as reduction of menthone to menthol. According to the photosynthesate model, proposed by Burbott and Loomis (*18*), disposability of assimilates is considered the key mediator of environmental effects on essential oil composition. As photosynthate level is basically a result of the balance between photosynthetic production (day) and metabolic utilization (night), highest levels are reached at full light intensity combined with either short or cool nights (due to lower respiration) (*18*). In this respect, the photosynthesate model gives an explanation for the enhanced menthone reduction found at high PAR. At low PAR in contrast, menthone to menthol conversion would be expected to decline (as the factors determining utilization, for example, night temperature and daylength, remain unchanged). This was only the case in the absence of UV-B radiation. Participation of UV-B radiation compensated for low levels of PAR. A possible explanation for the effect of UV-B on both quantity and pattern of monoterpenes might be the induction of supply pathways from primary metabolism (*40*).

The postulated effect of UV-B radiation and PAR on peltate trichome density and/or diameter could not be confirmed (**Figure 6**, **Table 4**). Only a slight trend toward a higher peltate trichome density was found on the abaxial leaf side in response to UV-B radiation. The finding that only the abaxial leaf side showed a weak response to irradiation treatment might be because this is the light-exposed side during peltate gland development and that peltate trichome density is about 10-fold increased on this leaf side. However, the diameter of peltate

glands was not affected at all. Consequently, the observed increase in monoterpene content at high PAR and ambient UV-B radiation could not be correlated with corresponding changes in absolute peltate trichome number (per leaf side) and/or diameter. This discrepancy suggests that the data obtained from these samples might not be representative for entire leaves. Monoterpene content as well as trichome number were calculated per total leaf (instead of leaf area or weight) in order to avoid an influence of leaf morphology, which was significantly affected by irradiation treatment. The most striking morphological response was an increase in specific leaf weight (indicating an increased leaf thickness) at high PAR, which is typically observed (together with decreased leaf area) in "sun leaves" (*41*).

The observed interaction between PAR and UV-B radiation in the regulation of monoterpene content and pattern in peppermint requires integration of direct and indirect irradiation effects. Integration of signals might occur within light signaling pathways, which are known to form a well coordinated network (*42*). However, the regulatory mechanisms are not yet entirely elucidated at the molecular level.

The present findings are also of practical relevance, since medicinal plants such as peppermint are increasingly grown in protected cultivation. Our study demonstrates that modulation of growth conditions by the use of UV-B transparent covering materials affects yield and quality of peppermint essential oil. Especially for cloudy conditions (low PAR), oil quality is improved in terms of an enhanced menthone to menthol conversion by UV-B exposure as requested by the Pharmacopoeia Europaea (*6*). On a sunny day (high PAR), UV-B exposure has no effect. Nevertheless, more detailed studies on signaling pathways and regulatory mechanisms mediating radiation effects on monoterpene metabolism are required.

ACKNOWLEDGMENT

The authors thank the staff at the Helmholtz Zentrum München for their help performing the experiments (alphabetical order): D. Arthofer, P. Bader, A. Glaser, P. Kary, R. Kuhnke, H. Lang, A. Richter, B. Rieger, W. Rupprecht, H. K. Seidlitz, J. B. Winkler. We also thank H. Fabricius for help with monoterpene analysis, M. Hunsche and K. Wichterich for trichome analysis, and J.-P. Schnitzler and W. Heller for sharing their great knowledge.

Supporting Information Available: Data from our greenhouse experiment showing the relative content of menthone and menthol during flowering as affected by UV-B transmission of different greenhouse covering material. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) *Crop Production 2008 Summary*; Technical Report Cr Pr 2-1 (09); National Agricultural Statistics Service, USDA: Washington, DC, **2009**.

(2) Burt, S. Essential oils: their antibacterial properties and potential application in food - a review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253.

(3) McKay, D.; Blumberg, J. A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). *Phytother. Res.* **2006**, *20*, 619–633.

(4) Maffei, M.; Chialva, F.; Sacco, T. Glandular trichomes and essential oils in developing peppermint leaves. *New Phytol.* **1989**, *111*, 707–716.

(5) Bomme, U.; Gatterer, M.; Hillenmeyer, G.; Kärner, C. Ergebnisse aus mehrjährigen Leistungsprüfungen mit ausgewählten Herkünften von Pfefferminze (*Mentha piperita* L.). Zweite Mitteilung: Zusammensetzung des ätherischen Öls. *Z. Arzn. Gew. Pfl.* **2005**, *10*, 73–81.

(6) Ph. Eur., *Pharmacopoeia Europaea - Europäisches Arzneibuch, Amtliche Deutsche Ausgabe*; Deutscher Apotheker Verlag: Stuttgart, Germany, 2008; pp 3633−3634.

(7) Mahmoud, S.; Croteau, R. Menthofuran regulates essential oil biosynthesis in peppermint by controlling a downstream monoterpene reductase. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14481–14486.

(8) Croteau, R.; Davis, E.; Ringer, K.; Wildung, M. (−)-Menthol biosynthesis and molecular genetics. *Naturwissenschaften* **2005**, *92*, 562–577.

(9) Amelunxen, F. Elektronenmikroskopische Untersuchungen an den Drüsenschuppen von *Mentha piperita*. *Planta Med.* **1965**, *13*, 457–473.

(10) Turner, G.; Gershenzon, J.; Nielson, E.; Froehlich, J.; Croteau, R. Limonene synthase, the enzyme responsible for monoterpene biosynthesis in peppermint, is localized to leucoplasts of oil gland secretory cells. *Plant Physiol.* **1999**, *120*, 879–886.

(11) Langenheim, J. Higher plant terpenoids: a phytocentric overview of their ecological roles. *J. Chem. Ecol.* **1994**, *20*, 1223–1280.

(12) Maffei, M.; Camusso, W.; Sacco, S. Effect of *Mentha* × *piperita* essential oil and monoterpenes on cucumber root membrane potential. *Phytochemistry* **2001**, *58*, 703–707.

(13) Marotti, M.; Dellacecca, V.; Piccaglia, R.; Giovanelli, E. Effect of harvesting stage on the yield and essential oil composition of peppermint (*Mentha* × *piperita* L.). *Acta Hort.* **1993**, *344*, 370–379.

(14) Rohloff, J. Monoterpene composition of essential oil from peppermint (*Mentha* × *piperita* L.) with regard to leaf position using solid-phase microextraction and gas chromatography/mass spectrometry analysis. *J. Agric. Food Chem.* **1999**, *47*, 3782–3786.

(15) McConkey, M.; Gershenzon, J.; Croteau, R. Developmental regulation of monoterpene biosynthesis in glandular trichomes of peppermint. *Plant Physiol.* **2000**, *122*, 215–223.

(16) Valladares, G.; Zapata, A.; Zygadlo, J.; Banchio, E. Phytochemical induction by herbivores could affect quality of essential oils from aromatic plants. *J. Agric. Food Chem.* **2002**, *50*, 4059–4061.

(17) Gershenzon, J. Metabolic costs of terpenoid accumulation in higher plants. *J. Chem. Ecol.* **1994**, *20*, 1281–1328.

(18) Burbott, A.; Loomis, W. Effects of light and temperature on the monoterpenes of peppermint. *Plant Physiol.* **1967**, *42*, 20–28.

(19) Voirin, B.; Brun, N.; Bayet, C. Effects of daylength on the monoterpene composition of leaves of *Mentha* × *piperita*. *Phytochemistry* **1990**, *29*, 749–755.

(20) Clark, R.; Menary, R. Environmental effects on peppermint (*Mentha piperita* L.). I. Effect of daylength, photon flux density, night temperature and day temperature on the yield and composition of peppermint oil. *Austr. J. Plant Physiol.* **1980**, *7*, 685–692.

(21) Karousou, R.; Grammatikopoulos, G.; Lanaras, T.; Manetas, Y.; Kokkini, S. Effects of enhanced UV-B radiation on *Mentha spicata* essential oils. *Phytochemistry* **1998**, *49*, 2273–2277.

(22) Maffei, M.; Scannerini, S. Photomorphogenic and chemical responses to blue light in *Mentha piperita*. *J. Essent. Oil Res.* **1999**, *11*, 730–738.

(23) Maffei, M.; Canova, D.; Bertea, C.; Scannerini, S. UV-A effects on photomorphogenesis and essential-oil composition in *Mentha piperita*. *J. Photochem. Photobiol. B: Biol.* **1999**, *52*, 105–110.

(24) Johnson, C.; Kirby, J.; Naxkis, G.; Pearson, S. Substantial UV-B-mediated induction of essential oils in sweet basil (*Ocimum basilicum* L.). *Phytochemistry* **1999**, *51*, 507–510.

(25) Krizek, D. Influence of PAR and UV-A in determining plant-sensitivity and photomorphogenic responses to UV-B radiation. *Photochem. Photobiol.* **2004**, *79*, 307–315.

(26) Yatsuhashi, H.; Hashimoto, T. Multiplicative action of a UV-B photoreceptor and phytochrome in anthocyanin synthesis. *Photochem. Photobiol.* **1985**, *41*, 673–680.

(27) Deckmyn, G.; Impens, I. The ratio UV-B/photosynthetically active radiation (PAR) determines the sensitivity of rye to increased UV-B radiation. *Environ. Exp. Bot.* **1997**, *37*, 3–12.

(28) Yamaura, T.; Tanaka, S.; Tabata, M. Light-dependent formation of glandular trichomes and monoterpenes in thyme seedlings. *Phytochemistry* **1989**, *28*, 741–744.

(29) Bomme, U. Kultur, Anbau und Ernte der Pfefferminze. *Z. Phytotherapie* **2004**, *25*, 147–152.

(30) Rohloff, J.; Dragland, S.; Mordal, R.; Iversen, T.-H. Effect of harvest time and drying method on biomass production, essential oil yield, and quality of peppermint (*Mentha* × *piperita* L.). *J. Agric. Food Chem*. **2005**, *53*, 4143–4148.

(31) Thiel, S.; Döhring, T.; Köfferlein, M.; Kosak, A.; Martin, P.; Seidlitz, H. A phytotron for plant stress research: How far can artificial lighting compare with natural sunlight? *J. Plant Physiol*. **1996**, *148*, 456–463.

(32) Knoche, M.; Noga, G. Effect of non-ionic surfactants on ethylene release and leaf growth of *Phaseolus vulgaris* L.. *Sci. Hortic*. **1991**, *46*, 1–11.

(33) Gershenzon, J.; McConkey, M.; Croteau, R. Regulation of monoterpene accumulation in leaves of peppermint. *Plant Physiol*. **2000**, *122*, 205–213.

(34) Mahmoud, S.; Croteau, R. Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran synthase. *Proc. Natl. Acad. Sci. U.S.A*. **2001**, *98*, 8925–8927.

(35) Tanaka, S.; Yamaura, T.; Shigemoto, R.; Tabata, M. Phytochrome-mediated production of monoterpenes in thyme seedlings. *Phytochemistry* **1989**, *28*, 2955–2957.

(36) Nitz, G.; Schnitzler, W. Effect of PAR and UV-B radiation on quality and quantity of the essential oil in sweet basil (*Ocimum basilicum* L.). *Acta Hort*. **2004**, *659*, 375–381.

(37) Caldwell, M.; Flint, S. Uses of biological spectral weighting functions and the need of scaling for the ozone reduction problem. *Plant Ecol*. **1997**, *128*, 67–76.

(38) Davis, E.; Ringer, K.; McConkey, M.; Croteau, R. Monoterpene metabolism. Cloning, expression, and characterization of menthone reductases from peppermint. *Plant Physiol*. **2005**, *137*, 873–881.

(39) Lange, B.; Wildung, M.; Stauber, E.; Sanchez, C.; Pouchnik, D.; Croteau, R. Probing essential oil biosynthesis and secretion by functional evaluation of expressed sequence tags from mint glandular trichomes. *Proc. Natl. Acad. Sci. U.S.A*. **2000**, *97*, 2934–2939.

(40) Logemann, E.; Tavernaro, A.; Schulz, W.; Somssich, I.; Hahlbrock, K. UV light selectively coinduces supply pathways from primary metabolism and flavonoid secondary product formation in parsley. *Proc. Natl. Acad. Sci. U.S.A*. **2000**, *97*, 1903–1907.

(41) Longstreth, D.; Hartsock, T.; Nobel, P. Light effects on leaf development and photosynthetic capacity of *Hydrocotyle bonariensis* Lam. *Photosynth. Res*. **1981**, *2*, 95–104.

(42) Nagy, F.; Schäfer, E. Phytochromes control photomorphogenesis by differentially regulated, interacting signalling pathways in higher plants. *Annu. Rev. Plant Biol*. **2002**, *53*, 329–355.

Received for review January 8, 2010. Revised manuscript received April 29, 2010. Accepted May 1, 2010. The work was funded by the German Federal Ministry of Education and Research, Project 0330724D.