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Substantial UV-B-mediated induction of essential oils in sweet basil (*Ocimum basilicum* L.)

Christopher B. Johnson[a,b,*], John Kirby[b], George Naxakis[a], Simon Pearson[b]

[a]*Department of Natural Products, Mediterranean Agronomic Institute of Chania, Alsyllion Agrokepion, P.O. Box 85, Chania, Greece*
[b]*School of Plant Sciences, The University of Reading, Whiteknights P.O. Box 221, Reading, Berkshire, RG6 6AS, UK*

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Abstract

The effect of supplementary UV-B treatment on the essential oils of glasshouse-grown sweet basil (*Ocimum basilicum* L.) was examined. Two weeks treatment with supplementary UV-B given in the early morning was found to enhance the levels of most of the major volatiles, both phenyl-propanoids (eugenol, methyl eugenol) and terpenoids, notably linalool, 1,8-cineole and *trans*-β-ocimene. The phenyl-propanoids were sensitive to UV-B at an earlier developmental stage than the terpenoids. Overall, the effect of UV-B was a nearly fourfold stimulation in the oldest plants examined.

Keywords: *Ocimum basilicum*; Lamiaceae; Basil; Essential oils; UV-B; Light

1. Introduction

The market for fresh aromatic herbs is expanding, with a significant shift in the use of plant condiments away from the traditional use of dried herbs towards fresh leaf material, either pot herbs or cut and packed fresh material. Many such herbs are grown under glass or plastic in order to maximise yield and in order to obtain a regular supply of material all the year round. Although this approach guarantees a regular supply of herbs not available with traditional approaches to herb production, the aromatic quality of glasshouse-produced herbs often falls far short of that of 'traditionally' grown herbs.

An important characteristic of glasshouse growing regimes is the virtual absence of UV-B from the radiation environment. It is well-known that plants contain specific UV-B absorbing photoreceptors in addition to the UV-A/Blue photoreceptors and phytochromes (Ensminger & Schäfer, 1992; Van der Staaij, Ernst, Hakvoort, & Rozema, 1995). Ultraviolet light (and specifically UV-B) has effects on secondary compounds of the phenyl-propanoid pathway via action on key regulatory enzymes such as phenylalaline ammonia-lyase (Kuhn, Chappell, Boudet, & Hahlbrock, 1984) and chalcone synthase (Batschauer, Rocholl, Kaiser, Nagatani, & Schäfer, 1996; Christie & Jenkins, 1996). There are many published reports of UV-B stimulation of phenolic compounds, including surface flavonols and flavonoids (Cuadra & Harborne, 1996; Cuadra, Harborne, & Waterman, 1997), anthocyanins (Yatsuhashi, Hashimoto, & Shimizu, 1982; Oelmüller & Mohr, 1985) and betacyanins (Rudat & Goring, 1995) and these compounds have been implicated both in plant defence (Chappell & Hahlbrock, 1984; Guevara, Perez-Amador, & Herrera, 1997) and as protection against UV-light (Fiusello, Codignola, & Maffei, 1985; Ziska, Teramura, & Sullivan, 1992; Lois, 1994).

Much less is known on effects of UV-B on essential oils, most of which are terpenoids. However, there is evidence for control by light acting via phytochrome on monoterpene levels in *Satureja douglasii* (Peer & Langenheim, 1998) and in *Thymus vulgaris* (Tanaka, Yamaura, Shigemoto, & Tabata, 1989). In each case red light stimulates the level of monoterpenes.

* Corresponding author.

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Table 1
Effect of UV-B on plant height[a]

	UV-B treated	Control
Two-leaf stage	5.50±0.28	4.37±0.29
Three-leaf stage	9.48±0.41	10.76±0.48
Five-leaf stage	15.01±0.38	14.94±0.50

[a] Plant heights at the end of the treatment period (±SE) are given in cm.

In this paper we have examined the effects of supplementary UV-B radiation on greenhouse-grown sweet basil (*O. basilicum* L.). Basil was selected because its characteristic essential oils include both phenyl-propanoid-derived compounds (typically one or more of; methyl chavicol, methyl cinnamate, eugenol, methyl eugenol (Sheen, Ying-Hsiu, & Shun-Jen, 1991; Hao, Charles, & Simon, 1995; Grayer et al., 1996; Lachowicz et al., 1997) and terpenoids (especially 1,8-cineole, limonene, linalool, β-ocimene Sheen et al., 1991; Grayer et al., 1996; Lachowicz et al., 1997). The volatile components in all treatments were analysed by headspace gas chromatography.

2. Results

Some morphological differences could be observed in basil plants following two weeks of UV-B treatment. Although there were only small effects on plant height (Table 1), there was a reduction in leaf area (except in the youngest plants) with little effect on leaf number (Table 2). After two weeks with the UV-B treatment the leaves were also changed slightly in shape, becoming slightly longer and more pointed, a trait that could still be observed in the mature plants (data not shown).

There were clear effects of UV-B on the quantity and composition of basil essential oils. Table 3 shows the content of volatiles in control and UV-B-treated plants of different developmental stages. Values are shown as total integrated area for each compound as detected by FID. In the broad-leaved variety we used, the principal phenyl-propanoid compounds are eugenol and methyl eugenol. Only a trace of methyl chavicol could be detected. The major terpenoids are 1,8-cineole and linalool, with lesser but significant amounts of α-*trans*-bergamotene, pinenes and *trans*-β-ocimene.

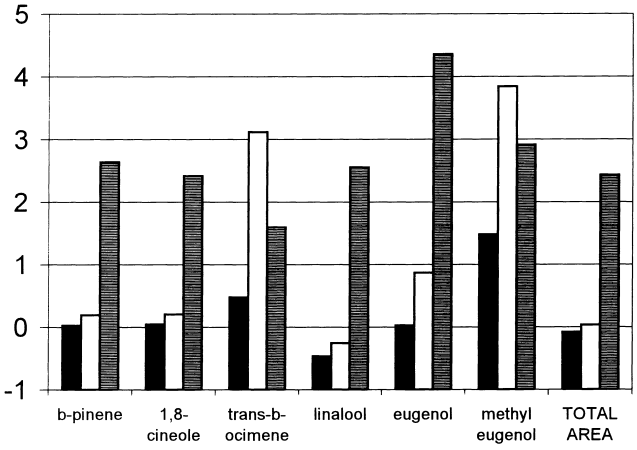

Fig. 1. Ratio of content of selected essential oils and total content in UV-B treated compared with control plants. Symbols: closed bars: two-leaf stage, open bars: three-leaf stage, hatched bars; five-leaf stage. A value of 0 indicates equal level in both; a value of +1 indicates double the level in UV, etc.

It can be seen that, in the leaves of plants at the two- and three-leaf stages, the total volatiles measured show negligible differences between control and UV-B plants, but in the five-leaf plant leaves there is a nearly fourfold enhancing effect of UV-B. However, closer examination of the breakdown of the total (Fig. 1) shows that in the youngest material, UV-B has a negative effect on the major component, linalool, virtually no effect on the other terpenoid compounds and a positive effect on the phenyl-propanoid, methyl eugenol. In the intermediate leaves both phenyl-propanoids are strongly increased in level. In the five-leaf plants, both phenyl-propanoids and terpenoids (including linalool) are strongly enhanced, with again the largest effect (a more than fivefold stimulation) being found in eugenol but most compounds affected rather similarly at between three and fourfold enhancement. It is worth noting that even before analysis the difference in aroma between UV-B and control plants was immedi-

Table 2
Effect of UV-B treatment on leaf area and leaf number[a]

	Total mean leaf area		Mean number of leaves		Mean specific leaf area	
	UV-B	Control	UV-B	Control	UV-B	Control
Two-leaf stage	29.5±3.3	23.2±3.1	8.2±0.8	6.0±0.0	3.74±0.50	3.86±0.52
Three-leaf stage	43.2±1.6	88.8±1.8	13.0±0.5	15.2±0.7	3.38±0.46	5.87±0.41
Five-leaf stage	116.4±5.8	147.4±5.8	21.3±1.5	19.5±1.6	5.63±0.29	7.88±0.39

[a] Leaf areas are in cm^2 per plant (±SE).

Table 3
Effect of UV-B treatment on essential oil content in *Ocimum basilicum*. DB5 values from Adams (1995) and our own observed retention times are shown. Content is expressed as integrated peak FID readings for each compound and for the total. Since most compounds were affected by UV-B treatment, these values are more informative than % composition

Compound	DB5	R_t	Two-leaf stage		Three-leaf stage		Five-leaf stage	
			UV-B treated	control	UV-B treated	control	UV-B treated	control
α-Pinene	319	8.63	20109	19428	18288	15469	11912	3273
Sabinene	379	10.79	18017	18008	16523	14797	11205	3010
β-Pinene	386	10.89	35340	34396	32548	27333	21060	5783
β-Myrcene	408	12.00	20488	25158	20467	19193	12627	3278
Limonene	481	14.29	11215	10915	10192	10371	8125	2390
1,8-Cineole	485	14.45	274371	262498	252902	209827	158479	46460
trans-β-Ocimene	519	15.94	53590	36249	49486	12035	27123	10474
cis-Sabinene hydrate	560	17.14	7601	4894	6008	5588	3996	1064
α-Terpinolene	608	18.74	5358	5456	5289	3969	3817	1398
Linalool	632	20.08	335290	491503	450679	565417	397116	111923
Camphor	734	23.05	12544	13881	10605	8499	5835	1693
Borneol	789	24.95	7125	6051	6272	8187	4915	1245
α-Terpineol	852	27.25	15190	14468	10558	10701	7776	2019
iso-Bornyl acetate	1099	35.61	13341	6619	9160	8173	6299	2663
Eugenol	1279	41.94	99692	97339	51697	27662	35531	6637
Methyl eugenol	1403	46.34	58371	23549	27453	5674	6519	1669
α-*trans*-Bergamotene	1485	48.44	48907	49652	49397	65908	33421	14761
α-Humulene	1527	49.44	7882	7729	6956	3377	2277	n.d.
trans-B-Farnesene	1502	50.59	21119	21129	19118	8416	8709	4498
Germacrene D	1594	51.80	11006	15366	13030	12965	9967	3580
Bicyclogermacrene	1632	53.10	4922	6285	7930	4600	3033	n.d.
γ-Cadinene	1676	54.61	6860	8127	8951	7776	5221	1441
Total: listed above			1088338	1178700	1083509	1055937	784963	229259
Total of all			1135017	1231587	1134279	1095075	813685	237659
% of total included			95.89	95.71	95.52	96.43	96.47	96.47

ately obvious to the nose. Thus, there is a strong effect of UV-B on the two groups of compounds, such difference as can be distinguished is mainly that the phenylpropanoid volatiles are sensitive to UV-B earlier in development than the terpenoid compounds. These findings support anecdotal evidence that aromatic plants grown in the glasshouse are less aromatic than those found in nature and are, we believe, the first report of an unambiguous demonstration of UV-B effects on the essential oil content of plants.

3. Experimental

3.1. Plant material

Broad leaved sweet basil (*O. basilicum* L.) seed (Vilmorin, La Verpilliere Cedex, France) were sown either in 1% agar at 25°C and transferred to a mixture of peat and perlite (1:3) after 5 days, or else sown directly in the same peat–perlite mixture. The seedlings were grown in a cool glasshouse (mean temperature 15°C) until the start of the experimental light treatments, a period of four to six weeks in March and April 1998 in Chania, Crete. Care was taken to ensure that none of the plants received significant doses of UV-B prior to the onset of the experimental treatment.

3.2. Light treatments

Plants for UV-B irradiation and the control plants were placed on parallel benches in the glasshouse separated by a transparent plastic screen, opaque to UV-B, in a glasshouse with ambient daylight. The experimental period for the UV-treatment was 2 weeks commencing 22 April. The UV-B light was provided by two Philips 20 W/12 UV-B fluorescent tubes placed 1 m apart and 1 m above the bench. The UV-B treatments were given between 04.30 and 07.00 each day, i.e. commencing ca. 1.5 h before dawn.

3.3. Analysis of plant material

Leaves were harvested and dried to constant weight for gas chromatographic analysis. At the start of the experimental treatments indicated above, plants were

divided into groups, the first typically having two leaf pairs, the second three or four leaf pairs and the third five leaf pairs. Headspace sampling for GC analysis was carried out using 100 mg dry leaf material, randomly selected from the plants of the indicated developmental stage. Analysis was carried out using a Hewlett Packard 5890 II gas chromatograph with coupled headspace analyser and equipped with FID. For analysis, the dried leaves were placed in a 20 ml vial. Each sample was retained in the headspace oven for 30 min at 90°C, then it was extracted with carrier gas, retained in the loop at 100°C for 1 min and transferred to the GC at 100°C. The column was a DB5 (length 30 m, 0.25 mm diameter) and the carrier gas He at a velocity of 38 cm/s. The split ratio was 1:28. Injector and detector temperatures were 230 and 260°C, respectively. The initial oven temperature was 45°C, rising at 1.5°C min^{-1} to 150°C, then at 40°C min^{-1} to 220°C and then held for the final 10 min at 220°C. Compounds were identified by comparison of retention times with known standards, and by GC–MS (HP5980 II GC coupled to a VG-TRIO 2000 mass spectrometer with MASS LYNX software). Mass spectra were taken at 70 eV. Scanning speed was 1 scan/s from 35 to 320 m/z. Peaks were identified from retention times (Adams, 1995) and by comparison with mass spectra in two libraries (Wiley, Adams).

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