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Full Length Research Paper

Enhanced UV-B radiation alters basil (*Ocimum basilicum* L.) growth and stimulates the synthesis of volatile oils

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Exposure of basil plants (*Ocimum basilicum*) in a controlled environmental room temperature to supplementary UV- B light for 3 h per day in the early morning over a period of two weeks resulted in shorter plants with higher dry matter, thicker leaves and more axillary shoots. Supplementary UV-B did not affect plant leaf area or number of leaf- pairs; however, specific leaf area was significantly increased due to increase in leaf thickness. Analysis of volatile oils by TD-GC/MS in fresh leaf samples harvested after one and two weeks of treatment showed that UV-B also stimulated the synthesis of the phenyl-propanoid, eugenol and the terpenoids 1, 8-cineole and linalool. There was no effect on volatile oil composition.

Key words: *Ocimum basilicum*, basil, growth, essential oil composition, 1, 8-cineole, linalool, eugenol, UV-B.

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.), a member of the Labiatae family, is a tender summer and herbaceous annual plant, which originates from tropical and warm areas, such as India, Africa and southern Asia (Hiltunen and Holm, 1999). The minimum temperature for the grow-th of sweet basil has been determined to be 10.9^{o}C (Chang, 2004). It is cultivated for the use of pharmaceuti-cals, cosmetics, aroma additives in food and other house-hold purposes (Hiltunen and Holm, 1999). Basil has been shown to produce high levels of phenyl-propanoids, e.g. eugenol and methyleugenol, and terpenoids, e.g. linalool and 1, 8-cineole. Both oil yield and herb yield vary greatly under different environmental conditions (Gang et al., 2001).

Due to the decreasing trends in stratospheric ozone concentration, ultraviolet-B (UV-B) radiation has increase-ed at the surface of the earth, which has led to much research on the effects of enhanced UV-B radiation on some changes in plants (Karousou et al., 1998; Kakani et al., 2003). UV-B can reduce plant growth and yield, reduce plant height and leaf area, increase tillering, change plant geometry (Barnes et al., 1988), reduce photosynthesis and increase plant secondary metabolites (Caldwell et al., 1989; Renger et al., 1989; Teramura and Sullivan, 1994).

There have been a few reports of the effects of UV-B radiation on aromatic plants in the Lamiaceae family. In peppermint, UV-B radiation resulted in taller plants with bigger leaf area and the quantity of volatiles oils was increased, however, the composition of the oils was not changed (Maffei and Scannerini, 2000). This supported an earlier report by Karousou et al. (1998). Ioannidis et al. (2002) found no differences in the quality or the quan-tity of essential oils in oil glands on the leaves of basil plants four days after supplementary UV-B treatment. In contrast, Johnson et al. (1999) reported that UV-B not only enhanced the total oil content fourfold but also alter-ed the oil composition in fresh basil leaves.

This paper reports changes in the growth and volatile oil content of basil leaves induced by the provision of supplementary UV-B light in controlled environment rooms, which lacked any natural UV-B radiation. It was hypothesized that supplementary UV-B light would reduce plant size but increase the volatile oil content in leaves

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Table 1. Growth parameters of basil plants following 2 weeks of supplementary UV-B treatment applied at the 3 and 4 leaf-pair growth stages (n = 6).

Growth parameter	Growth stage	Control	UV-B treated	SED	Probability
Plant height (cm)	3	49.7	46.6	1.25	0.037
	4	60.6	57.5	1.48	0.037
Plant dry weight (g)	3	4.3	5.3	0.27	0.006
	4	5.7	7.6	0.39	< 0.001
Leaf dry weight (g)	3	2.6	3.4	0.22	0.005
	4	3.0	3.9	0.23	0.005
Number of	3	7.6	8.0	0.40	0.347
axillary shoots	4	8.6	10.6	0.50	< 0.001
Leaf area (cm^2)	3	775.0	771.0	50.70	0.940
	4	941.0	981.0	92.80	0.172
Specific leaf area	3	303.9	227.4	11.20	< 0.001
($cm^2 g^{-1}$)	4	310.6	249.6	15.00	< 0.001

SED: Standard error of the difference.

MATERIALS AND METHODS

Plant materials

Seeds of basil cv, basil sweet Genovese were sown on the surface of Levington F2S Compost (Fisons Horticulture Ltd, Ipswich, UK) in plastic trays. Seedlings with one pair of unfolded leaves were transplanted to 12 cm diameter plastic pots containing Levington M2A compost with one plant per pot. The mean daily temperature in the glasshouse was set at 21ºC for seed germination and plant growth.

Supplementary UV-B treatments

Plants for supplementary UV-B irradiation and control plants were placed on benches in controlled environment rooms at 25±1^{o}C and 16 h daytime photoperiod. 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 for each treatment (Ioannidis et al., 2002 and Johnson et al., 1999).

Plants at the 3 and 4 leaf-pair growth stages were placed for two weeks in one of the two environments, that is control (no supplementary UV-B light) and 3 h (between 04:00 and 07:00) supplementary UV-B light each day. The UV-B treatments were given in July 2003 and repeated in a second experiment in April 2004, using two replicates of 60 plants for each treatment. There was no difference in PAR between the two controlled environment rooms with values of 444±25 mol m^{-2} s^{-1} and 450±25 mol $m^{-2}s^{-1}$ in the supplementary UV-B treatment room and control room respectively. The actual UV-B densities were 222.6 (supplementary UV-B) and 42.4 W/m^2 (control).

Plant growth parameters measured

Plant height, plant weight (fresh and dry), leaf weight (fresh and dry), number of shoots, number of leaf-pairs on the main stem, leaf area and specific leaf area were measured at the end of the two week treatments.

Analysis of leaves for volatile oil content and composition

The volatile oils from fresh samples (5 g) of the fifth pair of leaves of randomly selected plants were collected and analyzed by TD-GCMS as described by Chang (2004) and Chang et al. (2005). Each analysis was performed in triplicate. The relative contents of individual molecules were used to compare the overall composition of the volatile oil, and the sum of the peak areas was used to compare total oil contents in the basil leaves from the different conditions. The TD/GC was calibrated by external standards for molecule quantification (Chang, 2004; Chang et al., 2005).

Statistical analysis

Data for each growth parameter measured and volatile oil content and composition were subjected to an analysis of variance (ANOVA) using GenStat. Differences between treatments were assessed using the F-test, and the least significant difference (LSD) was calculated at 0.05 probability level (P = 0.05).

RESULTS

Growth

Following two weeks of UV-B treatment, there were differences in plant height, specific leaf area, plant and leaf dry weights and number of axillary shoots. Compared with the control, plant height was reduced by approximately 3 cm when supplementary UV-B light had been applied at both the 3 and 4 leaf-pair growth stages.

Although differences in plant leaf area were not signifycant, specific leaf areas were significantly reduced by UV-B treatment, consequently the thickness of these leaves was increased. This increased thickness resulted in increased plant and leaf dry weights following two weeks of UV-B treatment applied at both the 3 and 4 leaf-pair growth stages (Table 1).

Volatile oils

Twenty one chemical molecules were identified, most of which were terpenoids, e.g. 1, 8-cineole, linalool, pinenes and camphor, and the phenyl - propanoid, eugenol (Ta-

Table 2. Volatile molecules identified in basil leaf extracts and the effect of two weeks of supplementary UV-B at the 3 leaf-pair growth stage on the oil composition (n=3).

Molecule	Relative content (%)		SED	Probability
	Control	UV-B treated		
Thujene	0.11	0.10	0.066	0.845
Alpha-pinene	2.08	2.20	0.256	0.660
Camphene	0.62	0.62	0.309	0.986
Sabinene	1.56	1.84	1.030	0.804
Beta-pinene	2.87	3.00	1.009	0.906
Beta-myrcene	2.74	2.63	0.087	0.299
Alpha-phellandrene	0.35	0.33	0.062	0.808
Delta-3-carene	0.31	0.23	0.247	0.769
Alpha-terpinene	0.46	0.34	0.392	0.779
1,8-cineole	19.60	22.50	2.550	0.340
Cis-ocimene	7.46	7.26	0.954	0.084
Alpha-terpinolene	1.69	1.40	1.035	0.798
Linalool	21.80	23.20	4.020	0.758
Beta – ocimene	1.17	1.71	0.384	0.267
Camphor	1.13	1.52	0.463	0.464
Nerol	0.28	0.43	0.068	0.114
eugenol	11.68	11.02	0.310	0.123
Trans-alpha-Bergamotene	4.62	5.43	0.607	0.273
Germacrene-D	2.92	2.55	0.113	0.049
Germacrene-B	1.32	1.10	0.072	0.052
Gamma-cadinene	1.33	1.30	0.129	0.817

SED: Standard error of the difference.

Table 3. Total peak area of volatile oils in 5 g fresh leaves of basil after one and two weeks of UV-B treatment applied at the 3 and 4 leaf-pair growth stages (n=3).

Growth stage	Duration of treatment	Total peak area ($x10^6$)		SED	Probability
		Control	UV-B treated		
3 leaf-pair	1 Week	395.0	640.0	65.30	0.033
	2 weeks	1262.0	1712.0	45.80	0.010
4 leaf-pair	1 week	1262.0	1490.0	51.10	0.020
	2 weeks	1095.0	1351.0	41.00	0.020

SED: Standard error of the difference.

ble 2) . Comparing the integration peaks, there was signi-fIcant differences in total volatile oil content between treatments applied when plants were at the 3 and 4 leaf-pair growth stages (Table 3). Overall, supplementary UV-B light effectively increased the total volatile oil content of basil leaves.

Differences in the relative content of these twenty one chemicals between the supplementary UV-B light and control treatments, both in plants treated at the 3 and 4 leaf-pair growth stages, were not significant (Table 2). However, there were differences in the yields of three ma-jor volatile oils, that is 1, 8-cineole, linalool and eugenol, with the same trend observed in plants treated at both growth stages and for both durations. Supplementary UV-B enhanced the levels of these major volatiles signifi-cantly (Table 4), in line with the increase in total content of volatile oils.

DISCUSSION

Enhanced UV-B radiation reduced elongation of the main stem of basil plants, resulting in more compact plants, possibly due to changes in the phytohormones, especial-ly IAA that has been shown to play a role in stem elonga-tion in sunflower (Mark and Tevini, 1996). This reduction in plant height associated with supplementary UV-B light supports previous reports for other plants, such as pep-permint (Maffei and Scannerin, 2000), corn, cotton pea,

Table 4. Content of selected volatile oils in 5 g fresh leaves of basil following two weeks of treatment applied at the 3 and 4 leaf-pair growth stages (n=3).

Growth stage	Volatile oil	Content (μg)		SED	Probability
		Control	UV-B treated		
3 leaf-pair	1,8-cineole	270.4	404.4	23.10	0.035
	Linaloo	308.6	444.7	44.60	0.010
	Eugenol	110.0	139.2	9.13	0.050
4 leaf-pair	Cineole	270.7	365.8	21.30	0.028
	Linalool	308.0	414.0	44.80	0.020
	Eugenol	110.0	130.0	8.17	0.045

SED: Standard error of the difference.

and rice (Kakani et al., 2003). The apparent increase in the number of lateral shoots could be a consequence of the elimination of apical dominance due to the decrease in IAA concentration in the apex of the main stem, since some studies have indicated a breakdown of IAA on exposure to UV-B radiation (Ros and Tevini, 1995; Huang et al., 1993).

Kakani et al. (2003) in their review of UV radiation research during the past 18 years indicated that 40 studies using 23 crop species have revealed the effect of UV-B light on plant dry weight, but no information was presented for basil. Only 5% of the studies demonstrated an increase in dry matter accumulation under supplementary UV-B. Approximately one third of the studies reported no effect on dry weight and more than 50% reported a reduction in dry matter. The differences were probably associated with crop species, genotypes and UV-B doses.

The present study has shown that supplementary UV-B light can increase dry matter of basil following two weeks of treatment applied at the 3 and 4 leaf-pair growth stages. This can be explained if basil plants are more tolerant than other species to UV-B damage, or if the UV-B dose used was not strong enough to reduce dry matter accumulation. Kakani et al. (2003) showed that crop biomass production in response to UV-B radiation was highly dependent on UV-B dose. In the present study, the increase in shoot number and leaf thickness may explain the increase in plant dry weight.

Although differences in plant mean leaf area were not significant, highly significant differences were found in leaf dry matter and specific leaf area. This resulted from the increase in leaf thickness due to UV-B radiation, possibly due to the addition of spongy mesopyll cells as reported in Arabidopsis (Weston et al., 2000). Likewise, changes in leaves of potato have been associated with UV-B radiation (Santos et al., 2004).

One of the mechanisms that plants possess to adapt to enhanced UV-B radiation is to increase the production of secondary metabolites in leaf tissues, and most of these metabolites accumulate in the epidermal layer to absorb or screen UV-B radiation and protect the underlying tissues against this harmful radiation (Kakani et al., 2003).

There have been two reports on the effects of UV-B on volatile oils in basil plants. Johnson et al. (1999) reported that UV-B not only increased the total content but also changed the composition of these oils. In contrast, Ioannidis et al. (2002) stated that neither the quality nor the quantity of the volatiles was affected by UV-B. The present findings represent a compromise between these two reports, that is the total content of volatiles was signifycantly increased by UV-B but there was no effect on composition. It is possible that the effect of UV-B on volatiles in basil depends very much on genotype, because these three studies used three different genotypes. In the present study, seeds of basil cv, basil sweet Genovese were obtained from Nicherson-Zwaan Ltd (Lincoln, UK). Johnson et al. (1999) obtained seeds from France (Vilmorin, La Verpilliere Cedex) and Ioannidis et al. (2002) obtained seeds from Italy (Franchi Sementi S. P. A., Bergamo). In all three studies, UV-B light was supplied by two Philips 20 W/12 UV- B fluorescent tubes placed 1 m apart and 1 m above the bench, but for different durations, that is 1.0 h, (Ioannidis et al. 2002) 1.5 h, Johnson *et al.* 1999 and 3.0 h (in the present study).

In addition to using different genotypes, differences in the time of year and in the duration of the UV-B treatment in these three studies also could have led to different results. The temperature conditions used also may have importance; however the two previous studies did not provide details. Temperature could be important because Mark and Tevini (1996) found that it alleviates the effects of UV- B in sunflower and corn. Thus, future studies should report genotypes, UV-B dose, as well as abiotic factors, such as water stress, CO_2 concentration, nutrients, light intensity and temperature. Future studies to understand the mechanism of UV-B light at the cell and molecular levels should also be pursued.

Further experiments should apply UV- B light for a range of durations in the day and the night. In the present study, the supplementary UV-B light was applied for 3 h in the early morning in order not to expose operators to the UV light. Since there is a lack of UV-B radiation inside glasshouses (Johnson et al., 1999), this suggests the potential for safe application of UV-B radiation to basil plants in commercial glasshouses.

Conclusion

This research suggested that supplementary UV-B significantly reduced plant height but increased the number of shoots and plant dry matter. Although it did not affect plant leaf area, leaf thickness was significantly enhanced. The total volatile oil content in basil fresh Ieaves was increased by supplementary UV- B light; however there was no effect on the composition of the compounds.

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