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Aroma Content of Fresh Basil (*Ocimum basilicum* L.) Leaves Is Affected by Light Reflected from Colored Mulches

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Sweet basil (*Ocimum basilicum* L.) is an herb that is used to add a distinct aroma and flavor to food. Volatile compounds emitted from fully expanded fresh leaves grown in drip-irrigated plots that were covered with six colors of mulch were compared. The colors reflected a range of photosynthetic photon flux, far-red, red, and blue light from the soil surface to developing leaves. Our objective was to determine whether reflection from the different colors could influence concentrations of volatile compounds emitted from the fresh leaves. Volatile compounds were isolated by headspace sampling and quantified by gas chromatography. Twenty-six compounds were identified, of which the terpenoids linalool and 1,8-cineole comprised more than 50% of the total yield. Concentrations of volatile compounds from leaves that developed over green, blue, yellow, white, and red mulches followed the same patterns as they did for air-dried leaves of the same cultivar. However, the concentration of volatile compounds from fresh leaves was about 50-fold higher than those found in the previous study of air-dried leaves.

KEYWORDS: **Basil; *Ocimum basilicum*; aroma; colored mulch; herb; photomorphogenesis; phytochrome; terpenoid; volatile**

INTRODUCTION

Basil (*Ocimum basilicum* L.) is an aromatic herb that is used extensively to add a distinctive aroma and flavor to food. The leaves can be used fresh or dried for use as a spice. Essential oils are also extracted from the fresh leaves and flowers for use as an aroma additive in food, pharmaceutical, cosmetic, and household products. Thus, the yield of volatile compounds from basil is important. We hypothesized that the color (wavelengths) of light received by developing leaves could influence the concentration of volatile compounds in fully expanded fresh basil leaves.

Controlled environment studies of the 1960s and 1970s documented phytochrome involvement (as demonstrated by red/far-red photoreversible control) in regulation of leaf size, shape, thickness, and photosynthetic efficiency (*1*, *2*). Starting in the late 1960s, it was shown that far-red reflected from leaves of growing plants could increase the far-red/red photon ratio enough in outdoor-grown plants to act through phytochrome and modify the shoot and leaf size on nearby plants (*1*, *3*−*5*). The far-red/red ratio received by growing plants can also be altered by reflection from natural soil, residue on the soil surface, and artificially colored mulches on the soil surface (*4*, *6*, *7*). This finding contributed to the development of colored mulch technology, an emerging strategy in field crop production that allows us to affect both yield and quality of plant products (*7*). It relies on colored mulches to alter the light environment of sun-grown plants so as to act through photomorphogenic pigments and thereby regulate the allocation of photosynthate within the developing plant. The most influential portions of the electromagnetic spectrum are far-red (FR), red (R), and blue (BL). Because of absorption and reflection characteristics, some colors may have additional influence on growth and concentration of some compounds via temperature effects.

Recently, we compared the size of June-harvested fresh leaves and the chemical composition of dried basil that had been grown over six colors of polyethylene mulch (*8*). The colors used allowed us to compare response to different amounts of FR, R, and BL reflected to the developing plants. Basil grown over yellow and green surfaces produced significantly higher amounts of volatile compounds and soluble phenolics than basil grown over white and blue, respectively. The white surfaces reflected more BL than the yellow surfaces, and the blue surfaces reflected more BL than the green surfaces. In the case of both volatile compounds and soluble phenolics, therefore, it appeared that blue light reflected from the soil surface suppressed the accumulation of these compounds. This inference was drawn by comparing the wavelengths of light reflected from the different colored surfaces in the range of 400−800 nm.

That study was conducted using air-dried leaves. As mentioned, basil is also consumed fresh, and fragrance compounds are extracted from fresh leaves and flowers. Air-drying of herbs

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can result in the loss of volatile compounds (*9*, *10*). To gain a more complete appreciation of how photomorphogenic light affects aroma composition of basil, it was important to measure these compounds from fresh plant parts. Our objective in the present study was to measure the effects in fresh basil leaves that received different combinations of FR, R, and BL during development.

MATERIAL AND METHODS

Plant Material and Growing Conditions. Sweet basil (cv. Italian Sweet) plants were grown in drip-irrigated field plots of Norfolk loamy sand (Typic Kandidults) at the Coastal Plains Soil, Water and Plant Research Center near Florence, SC in 2001. The plots were fertilized, and 90 cm wide by 15 cm high raised-beds were prepared at 1.8 m intervals. Drip irrigation tubes were placed on top of the beds, and the plots were covered with 1.5 m wide black polyethylene mulch. Each plot contained six 6 m long subplots that were left unpainted (black), covered with red plastic (SRM-Red, Ken-Bar Agricultural Plastics, Reading, MA), or painted with green, blue, yellow, or white exterior enamel to provide different combinations of reflected FR, R, and BL. The sequence of colors was randomized within each plot. These colors were the same as those used in the previous study on the affect of reflected light on chemical composition of air-dried basil (*8*).

Plants were started in 300 mL pots on a greenhouse bench in early April, 2001. The plants were selected for uniformity and transplanted to the field plots about 1 month later. They were transplanted through 7.5 cm diameter holes that were cut 30 cm apart in the plastic along the ridge of the raised beds. There were six plants per color for each of three replicate plots. In this system, there was a high probability that all of the developing leaves received light reflected from the mulch color over which they were grown. After the plants were allowed to become established, all leaves longer than about 5 mm in length were removed so that the samples used in the present study included only leaves of the same age that developed outdoors over the indicated mulch colors.

Aroma Analyses. Basil leaves were harvested during August, 2001. Each day samples were analyzed, leaves growing about 25 cm above the mulch surface were collected at 11:00 am and 10 g samples weighed within 15 min of removal from the plants. The leaves were homogenized in 50 mL of deionized water using a high-speed tissue homogenizer and poured into 500 mL Erlenmeyer flasks, which served as sample vessels for dynamic headspace sampling.

Collections of volatile compounds were performed by dynamic headspace sampling using an apparatus, which has been described (*8*). It had six sample vessels and used 0.635 cm o.d. glass collection traps. These traps contained 50 mg of Super Q absorbent (Supelco Inc., Bellefonte PA) enclosed within glass wool plugs. Collections were of 4 h duration, and a flow rate of 250 mL min^{-1} was used. The pressure of the system was kept slightly above that of atmospheric, as measured by a flow tube attached to a vent at the head of the inlet manifold.

At the end of the collection period, the traps were removed from the apparatus and retained compounds were eluted from the traps with 320 μL of high purity pentane. Ten micrograms of γ-hexalactone was added as an internal standard, and 1.0 μL aliquots were injected onto a gas chromatograph (GC; Varian model 3800, Varian Associates, Walnut Creek, CA) equipped with a 60 m by 0.32 mm SPB-5 column with a film thickness of 1.0 μm (Supelco, Inc.). Injections were made in splitless mode for 1 min with an injector temperature of 220 °C, column initial temperature of 50 °C for 4 min, and column oven programming at 2 °C min^{-1} to 100 °C. The column oven was then programmed at 5 °C min^{-1} to 230 °C. Other GC operating conditions were as follows: flame ionization detector, 260 °C; air flow rate, 300 mL min^{-1}; hydrogen, 30 mL min^{-1}; helium linear flow velocity, 17 cm sec^{-1}; injector split ratio, 75:1; and helium makeup gas flow rate of 25 mL min^{-1}.

Data for individual peaks were quantified relative to the γ-hexalactone internal standard. The values obtained were subjected to analysis of variance using the SAS System for Windows version 6.12 (*11*), and means for each color were compared by a Duncan's multiple range test at $P = 0.05$. Eight analyses for each color of mulch were performed.

Leaf and mulch surface temperatures were recorded during the period in which leaves were harvested for aroma analyses on a sunny day around 11:00 am when the air temperature was 27.6 °C and wind velocity was about 3.4 m s^{-1}. They were taken with an infrared thermometer (model 410, Cooper Instrument Corp., Middlefield CT).

Compound Identification. GC/mass spectroscopy was performed on a GC equipped with a 30 m by 0.25 mm HP-5 column (Hewlett-Packard, Palo Alto, CA) interfaced to a Hewlett-Packard GCD Plus mass selective detector. Injections were made onto the GC in splitless mode for 1 min, and the mass ion detector used a scanning range of 40−250 amu. Chromatograph operating conditions were as follows: helium linear flow velocity, 21 cm sec^{-1}; injector, 220 °C; column oven, 40 °C for 1 min and then programmed at 3 °C min^{-1} to 180 °C. Compounds were identified by comparisons of mass spectral data and retention time matches with those of authentic samples of compounds. Authentic samples of compounds were obtained from commercial sources (Bedoukian Research Inc., Danbury, CT; Sigma-Aldrich Co., St. Louis, MO).

RESULTS AND DISCUSSION

We identified 26 compounds from fresh basil leaves, the greatest yield of which was terpenoids (**Table 1**). Of these, linalool and 1,8-cineole comprised over 50% of the total yield of volatile compounds. In addition, we found a number of aliphatic aldehydes, alcohols, and esters as well as the aromatic compound eugenol. The compounds hexanal and (*Z*)-3-hexenal were not separated on the SPB-5 column and are reported as one peak. In agreement with our study of air-dried basil (*8*), we did not find evidence for other aromatics such as methyl chavicol, methyl eugenol, and methyl cinnamate, compounds that have been identified as important aroma compounds in some basil cultivars (9, *12*). Some other compounds that we identified from dried basil such as *n*-octanol and octyl acetate occurred in relatively minor amounts in the fresh leaves and are not reported herein. The sesquiterpene hydrocarbon α-bergamotene was tentatively identified based on its mass spectral fragmentation pattern and reports of its occurrence in basil (*13*, *14*).

The total yield of volatile compounds from fresh basil leaves was in the range of 100 μg g^{-1} (**Figure 1**). This was approximately 50-fold higher than the yield of volatile compounds that we found for air-dried basil leaves (*8*). This concentration difference in fresh vs dry leaves is consistent with previous research wherein it was found that air-drying of basil leaves resulted in significant loss of volatile compounds (*9*, *10*).

Significant differences were apparent in the concentrations of volatile compounds emitted by basil leaves that developed over different colors of mulch (**Table 1**, **Figure 1**). For most compounds, the highest concentrations were obtained from leaves grown over black, green, and yellow surfaces while the lowest levels were obtained from those grown over red and blue surfaces. Clearly, the color of the mulch on the soil surface during leaf development affected their content of volatile compounds. Comparisons of the relative concentration of volatile compounds isolated from fresh leaves that developed over green vs blue and yellow vs white mulches followed the same patterns as they did in air-dried leaves that were harvested in June (*8*). However, the concentration of volatile compounds found in fresh leaves that developed over black relative to the other colors of mulch was higher than was found for air-dried basil.

The characteristics of light reflected from the colored mulches relative to those of incoming sunlight were similar to those in the study of air-dried basil leaves (*8*). Black reflected about 6% of sunlight across the visible and far-red spectrum. The other colors were chosen to allow evaluation of plant responses to various combinations of photosynthetic photon flux (PPF), FR,

Table 1. Concentrations (Nanograms per Gram of Fresh Weight) of Aroma Compounds from Basil Leaves Grown over Six Colors of Polyethylene Mulch[a]

	mulch color					
compound	black	red	green	blue	yellow	white
			A. aliphatic compounds			
hexanal and (*Z*)-3-hexenal	10 731 a	4991 c	9521 ab	8964 ab	7623 abc	6481 bc
(*E*)-2-hexenal	1960 a	1594 a	1991 a	1744 a	1519 a	1461 a
(*Z*)-3-hexen-1-ol	2219 a	1412 b	2005 ab	1878 ab	1727 ab	1436 b
(*E*)-2-hexen-1-ol	110 a	144 a	80 a	120 a	75 a	93 a
n-hexanol	121 a	175 a	73 a	89 a	110 a	73 a
1-octen-3-ol	2689 a	1610 b	2498 a	1979 ab	2654 a	2354 ab
(*Z*)-3-hexenyl acetate	99 ab	54 b	134 a	72 b	96 ab	86 b
total	17 929 a	9980 d	16 302 ab	14 846 abc	13 804 bc	11 984 cd
			B. terpenoids and eugenol			
α-pinene	1198 a	698 b	996 ab	875 ab	1243 a	903 ab
β-pinene	2771 a	1780 b	2291 ab	1985 ab	2745 a	2427 ab
camphene	230 ab	153 b	242 ab	201 b	295 a	243 ab
myrcene	3030 a	1741 c	2825 ab	2099 bc	3014 a	2770 ab
2-carene	136 a	42 a	142 a	107 a	163 a	90 a
3-carene	46 a	41 a	40 a	44 a	48 a	36 a
limonene	870 a	574 a	994 a	712 a	772 a	827 a
1,8-cineole	52 799 a	26 640 b	49 035 a	36 766 ab	49 455 a	47 609 a
linalool	65 033 a	31 125 c	64 006 a	42 726 bc	60 044 ab	53 241 ab
camphor	337 ab	342 ab	346 ab	164 b	463 ab	511 a
tepinen-4-ol	364 a	185 c	360 ab	250 bc	371 a	332 ab
bornyl acetate	1163 a	304 c	971 ab	720 b	962 ab	332 c
α-terpineol	267 a	159 a	265 a	267 a	226 a	310 a
eugenol	1882 a	945 b	1808 a	1610 a	1948 a	1795 a
caryophyllene	1175 ab	641 c	1279 ab	950 bc	1432 a	1287 ab
α-humulene	194 a	538 a	141 a	202 a	260 a	184 a
α-bergamotene[b]	384 abc	202 d	265 cd	288 bcd	477 a	406 ab
(*E*,*E*)-α-farnesene	47 ab	32 b	34 b	40 b	65 a	48 ab
total	131 926 a	66 142 c	126 040 a	90 006 bc	123 983 a	113 351 ab

[a] Data represent the mean of eight determinations. Within each line, values followed by the same letter are not significantly different at $P = 0.05$. [b] Identification tentative.

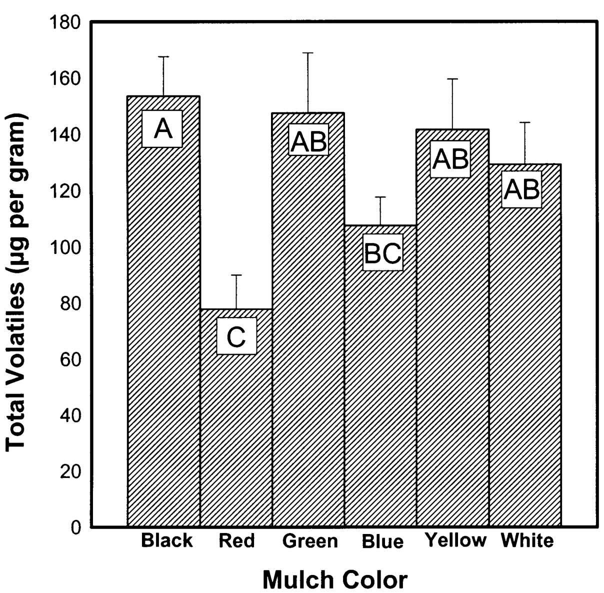

Figure 1. Total aroma compounds entrained per gram of fresh basil leaves grown over six colors of polyethylene mulch. Bars represent the means of eight determinations ± standard error of the mean. Bars labeled with different letters are significantly different by a Duncan's multiple range test at $P = 0.05$.

R, and BL. Green and blue reflected different amounts of BL but similar PPF, R, and FR. Yellow and white reflected high PPF and FR/R ratios similar to that of sunlight. Yellow, however, reflected less BL than white. Red reflected R and FR but very little BL. Black reflected very little of any color and absorbed more than 90% of incident radiation. Therefore, radiated heat from black may have had a greater influence on the concentration of volatile compounds in the August-harvested basil than it did in the June-harvested basil (*8*).

In our previous study on the effects of reflected light during leaf development on the composition of volatile compounds of air-dried basil (*8*), we inferred that BL reflected from the mulch surfaces suppressed accumulation of volatile compounds in comparisons of yellow vs white and green vs blue. Similar results were obtained in the present study. That is, basil grown over yellow and green surfaces produced significantly higher amounts of volatile compounds than did basil grown over white and blue, respectively. The white surfaces reflected more BL than did the yellow surfaces, and blue surfaces reflected more BL than did the green surfaces. If limited to these comparisons, the response to FR/R ratios did not dominate the chemical response to BL.

Red surfaces also produced basil with a low content of volatile compounds. Similarly to the green and yellow used in this study, red surfaces reflected a low percentage of BL, but unlike yellow surfaces, a high FR/R ratio. A high FR/R ratio serves as a signal of competition in higher plants causing the allocation of photosynthate to above ground portions of the plant (*1*, *4*, *5*). The red mulch employed in this study was formulated to reflect a FR/R ratio that favors above ground plant growth (*7*), and basil leaves that were grown over red surfaces had significantly higher leaf areas and lower dry weight per area relative to those grown over other colors of plastic mulch (*8*). Basil grown over this red mulch, therefore, had a higher yield of leaf but a lower volatile content on a per weight basis.

In general, differences in the concentration of aliphatic compounds were less pronounced than for other volatile

Table 2. Temperatures Recorded at Solar Noon from Leaf or Mulch Surfaces on a Sunny Day

	mulch color					
surface (°C)	black	red	green	blue	yellow	white
leaf	25.8 ± 0.2 a	25.4 ± 0.4 a	25.5 ± 0.7 a	25.4 ± 0.8 a	25.3 ± 0.4 a	25.9 ± 0.8 a
mulch	64.1 ± 5.0 a	48.9 ± 3.5 cd	53.1 ± 5.3 c	58.6 ± 2.2 b	44.8 ± 2.6 d	32.3 ± 2.1 e

[a] Ambient temperature was 27.6 °C. Data represent the mean of eight determinations ± standard deviation. Within a row, values followed by the same letter are not significantly different by a Duncan's multiple range test at $P = 0.05$.

compounds. Furthermore, it did not appear that BL reflected from the mulch surface suppressed their accumulation. Unlike the terpenoids and eugenol, however, volatile aliphatics in basil are not sequestered in glands but rather are synthesized de novo by hydroperoxidation and cleavage of fatty acids liberated in response to mechanical damage (*15*). Similarly to the terpenoids and eugenol, basil leaves grown over red mulch also tended to produce lower levels of aliphatic compounds. Total identified concentrations of aliphatic compounds and putative gland-sequestered compounds from leaves that developed over the various mulches are presented in **Table 1**.

In our previous study with air-dried June-harvested basil leaves, we found that those which had developed over black contained amounts of volatile compounds intermediate between yellow/green- and red/blue-grown basil (*8*). However, in the present study, we found August-harvested fresh basil leaves that developed over black mulch contained the highest concentrations of volatile compounds on a weight basis (**Table 1**, **Figure 1**). A possible explanation for this difference involves temperature during shoot (especially leaf) development. The black reflects very little of the incoming sunlight and absorbs much of the incident energy, so some of that energy might be conducted to roots or radiated to leaves where the altered temperature might affect concentrations of volatile compounds. Thus, the results of the present study with leaves that developed in August might be influenced by high temperature more than those of the previous study where the sampled leaves developed during June. Because volatile emissions by plants have been shown to vary due to season and temperature (*16*−*18*), we measured leaf and mulch surface temperatures to determine the variation among the various mulch treatments.

Significant differences were found in the mulch surface temperatures (**Table 2**). The black mulch was hotter than any of the other colors while the white mulch was significantly cooler than any of the other colors. Leaf temperatures as measured with an infrared thermometer, however, varied less than 1 °C between plants grown over the various colored mulch surfaces, indicating leaf themoregulation by transpiration and air movement (wind). Elevated temperatures have been shown to increase the rate of volatile emission in *Quercus ilex* (*17*). Perhaps the elevated concentration of volatile compounds in basil leaves that developed over black mulch in August, as compared to concentrations from June-grown leaves (*8*), was due to temperature stress induced by the higher temperature of the black mulch.

While basil leaves are often dried and used as a spice, a major use for basil and other aromatic herbs is for the preparation of essential oils for use in the food and fragrance industries. In addition, basil is marketed fresh and grown by home gardeners to add flavoring to foods. As interest in traditional herbal medications and aromatherapy grows, the hectarage of aromatic herbs is likely to increase and adaptation of science-based production procedures will become increasingly important in maximizing yield and quality of plant products.

Raised bed culture with drip irrigation and plastic mulch is a logical option for the culture of basil for its water conservation and weed control benefits. Colored mulch adds the additional benefit of photomorphogenic light and, to a lesser extent, the benefit of temperature effects that can enhance its content of volatile compounds.

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