Light Reflected from Colored Mulches Affects Aroma and Phenol Content of Sweet Basil (*Ocimum basilicum* L.) Leaves

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Basil (*Ocimum basilicum* L.) is an herb the leaves of which are used to add a distinct aroma and flavor to food. It was hypothesized that the size and chemical composition of sun-grown basil leaves could be influenced by the color of light reflected from the soil surface and by the action of the reflected light through the natural growth regulatory system within the growing plants. Leaf morphology, aroma compounds, and soluble phenolics were compared in basil that had been grown over six colors of polyethylene row covers. Altering the ratios of blue, red, and far-red light reflected to growing plants influenced both leaf morphology and chemistry. Leaves developing over red surfaces had greater area, moisture percentage (succulence), and fresh weight than those developing over black surfaces. Basil grown over yellow and green surfaces produced significantly higher concentrations of aroma compounds than did basil grown over white and blue covers. Leaves grown over yellow and green mulches also contained significantly higher concentrations of phenolics than those grown over the other colors. Clearly, the wavelengths (color) of light reflected to growing basil plants affected leaf size, aroma, and concentrations of soluble phenolics, some of which are antioxidants.

Keywords: *Basil; Ocimum basilicum; aroma; colored mulches; reflected light; terpene; terpenoid; phenolics*

INTRODUCTION

Basil (*Ocimum basilicum* L.) is an aromatic herb the leaves of which are used extensively in Italian and Indian style cooking. It is grown commercially and by home gardeners and gourmet cooks. The leaves can be used fresh, or they can be dried, processed, and stored prior to use as a spice. Aroma compounds are also extracted and used in a wide variety of products such as cosmetics and natural flavors. Thus, both yield and chemical composition of basil leaves are important.

Basil plants grow best in full sunlight and well-drained soil (*1*). Weed growth among basil plants is undesirable. For these reasons, the use of raised beds with plastic row covers is a logical choice for the culture of basil. Black plastic (over trickle irrigation tubes) is widely used in raised-bed culture of high-value crops. These practices can conserve water, improve soil drainage, reduce the need for weed control, and keep soil from splashing onto leaves.

Use of plastic mulches with other surface colors can keep these benefits and add the benefit of reflected morphogenic light, which acts through the natural growth regulating system within plants during their growth and development to affect yield and chemical composition (*2*, *3*). For example, a red mulch that reflects a far-red to red (FR/R) photon ratio higher than the ratio in incoming sunlight has been developed to regulate allocation of more growth to developing fruit, such as tomato (*Lycopersicon esculentum* Mill.) (*4*). Reflection of some other colors from mulch surfaces have influenced plant biochemical characteristics such as concentrations of photosynthetic pigments and leaf protein (*5*); the quantity and composition of epicuticular waxes on leaves (*6*); and concentrations of sugars and glucosinolates in turnip (*Brassica rapa* L.) (*7*). We hypothesized that reflected blue (BL), R, and FR may also alter the morphology and flavor components in edible leaves of herbs, such as sweet basil.

The objectives of the present study were to determine whether the color of light reflected from plastic mulches to sun-grown basil could affect (a) the size and weight (yield) of leaves and (b) the concentrations of aroma compounds and nonvolatile phenolics in those leaves.

MATERIALS AND METHODS

Plant Material and Growing Conditions. Sweet basil (cv. Italian Sweet) plants were grown over plastic mulches in irrigated field plots of Norfolk loamy sand (Typic Kandidults) at the Coastal Plains Soil, Water and Plant Research Center near Florence, SC, in 1998. The plots were fertilized, and 90 cm wide by 15 cm high raised beds were prepared at 1.8 m intervals. Trickle 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 BL, R, and FR. The sequence of colors was randomized within each plot. Exterior enamels were used because they provide an economical and repeatable method to obtain the desired reflection spectra for small plot studies. However, it must be noted that different batches of a color of paint may appear identical to human vision but reflect quite differently in the FR region, which is beyond human vision but affects plant growth. Therefore, it was necessary to measure the reflection spectrum for each color of paint used in this study.

Plants were started in 300 mL pots in mid-April on a greenhouse bench. On May 11, plants were selected for

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uniformity and transplanted to the field plots. They were transplanted through 7.5 cm diameter holes that were cut 60 cm apart in the plastic along the ridge of the raised beds. In this system the holes allowed heat to escape from below the plastic, and there was a high probability that all of the developing leaves received light reflected from the mulch color over which they were grown. On May 21, all leaves longer than ~5 mm in length were removed so that the leaves to be analyzed included only those that developed outdoors over the indicated mulch colors. There were 10 plants per color for each replicate.

Reflected Light. The spectral distribution and quantity of upwardly reflected light was measured ~15 cm above the colored mulch surfaces using a Li-Cor LI-1800 spectroradiometer (Li-Cor Inc., Lincoln, NE) equipped with a remote hemispherical light collector on a 1.5 m fiber optic probe. Measurements were made at 5 nm intervals between 400 and 800 nm. A reference spectrum was obtained by measuring incoming sunlight at the same wavelengths. Light measurements were taken at solar noon ± 15 min on a cloudless day. The reflected light values were then calculated as percentages of incoming sunlight at each measured wavelength. We measured R at 645 ± 5 nm because that is the approximate phytochrome action peak in green plants due to competitive absorption by chlorophyll at 660 nm, which is the absorption peak for the red-absorbing form of phytochrome (P_R) in vitro. Our values for FR were measured at 735 ± 5 nm, which is the absorption peak for the far-red absorbing form of phytochrome (P_{FR}) in vitro (*8*), and at 755 ± 5 nm, which is the beginning of the reflection plateau (percentagewise) from green leaves (*9*). The 755 nm measurement is important morphologically because prolonged exposure (as occurs in the field) to FR at 735 nm results in an R response due to the overlapping of P_R absorption into that waveband (*10*). At 755 nm the P_R absorption is extremely low, resulting in the FR response to prolonged reflection of that waveband from nearby plants as is observed in nature (*2*, *9*). The values in upwardly reflected light were expressed relative to the values in incoming sunlight. The rationale for this approach was that field plants normally grow in sunlight and they might be able to sense and respond morphologically and chemically to a light environment that differs in spectral distribution from incoming sunlight.

Leaf Morphology and Sample Preparation. On June 27, five replicates of 40 recently expanded leaves were collected from plants growing over each of the six colors. All of the collected leaves developed 15−25 cm above the respective colored mulches. The samples were collected at 1100 h ± 30 min. Areas of the 40-leaf samples were determined with a Li-Cor LI-3100 area meter (Li-Cor Inc.). The leaves were weighed fresh, oven-dried at 60 °C, and weighed. The dried samples were stored in darkness in sealed plastic bags at 4 °C until analyzed. Each 40-leaf sample was ground with a mortar and pestle and thoroughly mixed before chemical analyses.

Aroma Compounds. Collections were performed on a "push−pull" apparatus similar to that described in Loughrin et al. (*11*). It had six chambers for samples, each consisting of a 500 mL sidearm Erlenmeyer flask (18 cm tall) closed at the top with a no. 6 rubber stopper adapted for 0.635 cm o.d. (0.25 in.) copper tubing, which served as an air-inlet line. Air was supplied to each inlet via a manifold connected to a 500 mL charcoal filter and continuous duty diaphragm air compressor. The copper tubing extended 4 cm into each flask and had an air diffuser (Reagent Pet Products, Moorpark, CA) attached to its end (total extension into flask was 12 cm). On the sidearm of each flask was attached via Tygon tubing (Norton Performance Plastic Corp., Akron, OH) a 0.635 cm o.d. copper tube with Swage-lock fittings to accommodate 0.635 cm o.d. glass collection traps. Each trap contained 50-mg of Super Q absorbent (Supelco Inc., Bellefonte, PA) enclosed within glass wool plugs. Vacuum was applied to each chamber by another manifold constructed of 0.635 cm o.d. copper tubing and attached to a separate continuous duty diaphragm pump. Vacuum to each chamber was controlled by a separate flow controller, and during collections air was pulled through each chamber at 250 mL/min. The pressure of the entire system was kept slightly above that of atmospheric as measured by a separate flow controller connected to a vent attached to the head of the air-inlet manifold.

Prior to collection of the aroma compounds, 400 mg of dried basil was placed in each chamber along with 20 mL of deionized H_2O in order to disperse the sample evenly. Collection traps were rinsed with 2 mL of high-purity hexane prior to attachment. Collections were of 4 h duration, and samples from basil grown over each of the colored mulches were included in every collection.

Retained compounds were eluted from the traps with 320 μL of an 80:20 mixture of high-purity hexane/CH_2Cl_2. One microliter aliquots were injected onto a gas chromatograph (GC) operated in splitless mode for 1 min, and the GC was equipped with a 60 m × 0.32 mm Supelcowax 10 (Supelco Inc.) column with a 0.5 μm film thickness. Gas chromatographic operating conditions were as follows: injector, 220 °C; oven temperature, 50 °C for 1 min programmed at 3 °C/min to 180 °C; flame ionization detector, 240 °C; helium carrier linear flow velocity, 21 cm/s.

Compounds were quantified by the injection of external standards at various concentrations to develop calibration curves. Standards of some of the aroma compounds found in basil leaves were not available to us in sufficiently high purity for the purpose of calibration. We therefore quantified compounds within relative retention time windows using high-purity standards of limonene, 1,8-cineole, linalool, and eugenol. Collections were replicated seven times for samples grown over each of the six different colored mulches. Data were subjected to analyses of variance using the SAS System for Windows version 6.12 (*12*) and means for each color contrasted by least significant difference (LSD) tests at $p = 0.05$.

Soluble Phenolics. Soluble phenolics were measured by an adaptation of the method of Slinkard and Singleton (*13*). One hundred milligram samples of ground basil leaves were extracted overnight with 8 mL of 80% MeOH. Four hundred microliters of the extract was diluted to 5.0 mL with deionized water, the solution mixed, and 1.0 mL of 2.0 N Folin−Ciocalteu reagent (Sigma, St. Louis, MO) added. After 2−3 min, 4.0 mL of 15% (w/v) Na_2CO_3 was added, and the samples were again mixed. After the samples had stood overnight, their absorbance was measured at 765 nm. Results were expressed as gallic acid (3,4,5-trihydroxybenzoic acid) equivalents determined by measuring the absorbance of stock solutions of gallic acid. These experiments were replicated 10 times, and data were subjected to analyses of variance using SAS. Mean values for each color were compared by LSD tests at $p = 0.05$.

Compound Identification. Gas chromatography−mass spectroscopy was performed on a GC equipped with a 30 m × 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 40−450 amu. Chromatograph operating conditions were as follows: injector, 220 °C; column oven, 40 °C for 1 min programmed at 3 °C/min to 180 °C. Authentic samples of compounds were obtained from commercial sources.

RESULTS AND DISCUSSION

Reflected Light Characteristics. Quantities and ratios of upwardly reflected light received 15 cm above the different colored mulches are summarized in Table 1. Black reflected ~5%, or less, across the visible and far-red spectrum. The other colors were used to reflect various combinations of photosynthetic photon flux (PPF), BL, R, and FR. For example, red versus black allowed comparison of responses to more reflected R and FR when both colors reflected about the same amount of BL. Green and blue reflected different amounts of BL but about the same PPF, R, and FR. Yellow and white mulches were used because they reflected higher PPF

Table 1. Characteristics of Upwardly Reflected Light from Colored Mulches Relative to Those of Incoming Sunlight

	mulch surface color					
characteristic[a]	black	red	green	blue	yellow	white
$PPF_{(400-700\ nm)}$, %	5	12	11	13	32	40
$BL_{(450\ nm)}$, %	5	5	7	21	11	39
$R_{(645\ nm)}$, %	5	24	9	9	40	41
$FR_{(735\ nm)}$, %	5	28	13	13	40	41
$FR'_{(755\ nm)}$, %	5	28	16	15	40	41
FR/R	—[b]	1.2	1.5	1.4	1.0	1.0
FR′/R	—	1.2	1.8	1.7	1.0	1.0

[a] Values are means of at least three measurements taken 15 cm above the respective color. Means for BL, R, FR, and FR′ are expressed as percentage of incoming sunlight at the same wavelengths. [b] FR/R photon ratios were not determined over black because reflection was ~5% across the spectrum and minor differences could result in a large, but meaningless, ratio. The FR/R and FR′/R ratios over the other colors were calculated before rounding off reflection values, and they are expressed relative to the ratios in incoming sunlight at the same time.

than the other colors and FR/R photon ratios similar to those in the incoming sunlight. However, yellow reflected less BL. Because of many documented controlled-environment studies on the effects of color on enzyme activity, photosynthate allocation, and chemical composition, we hypothesized that differences in color of light reflected to field-grown basil leaves could affect their size and content of flavor-related compounds.

Leaf Morphology. Leaf area, weight per area, and percent moisture (succulence) of sungrown fresh basil were significantly affected by growing the plants over different colors of mulch (Table 2). Several comparisons suggest that the quantity of BL and the FR/R photon ratio reflected from the mulch surface to the developing leaves influenced the yield (and chemistry) of those leaves. For example, leaves that developed over red and received higher R/BL and higher FR/R photon ratios (see Table 1) had significantly greater area, moisture percentage, and fresh weight than those that developed over the standard black plastic mulch. In contrast, leaves that developed over white had smaller area, similar fresh weight, greater dry weight, much greater dry weight per area, and lower moisture percentage than leaves grown over red. Clearly, the color of reflected light influenced the yield of basil leaves and their succulence as a fresh herb. The physical differences among basil leaves grown over different colors of soil covers are consistent with those reported for cotton (*Gossypium hirsutum* L.) by Bradburne et al. (*5*). They also found that leaves developing ~15 cm above red and green surfaces had lower dry weight per square centimeter and different chemical composition from those grown over white surfaces. Because dried basil leaves are used to add flavor to food, we analyzed our samples for aroma compounds and for concentrations of soluble phenolics.

Aroma Compounds. Twelve aroma compounds were identified, most of which were monoterpenoids (Table 3). In addition, the aliphatic compounds hexanal and octyl acetate were found as well as the phenylpropanoid eugenol. A number of sesquiterpene hydrocarbons were evident on the chromatograms but not identified due to low concentrations and lack of authentic reference compounds.

Significant differences were evident in the levels of various aroma compounds emitted by basil leaves that received different colors of reflected light during development. For most of the monoterpenoids, the highest levels of emission were obtained from leaves grown over yellow and green, whereas those grown over white and blue emitted the lowest concentrations. The amount of BL (see Table 1) reflected to developing leaves appears to have been involved in the suppression of terpenoids. For example, leaves grown over yellow emitted 68% more 1,8-cineole than leaves grown over white and leaves grown over green emitted 42% more than those grown over blue. For linalool, leaves grown over yellow emitted 45% more than those over white, and leaves grown over green emitted 21% more than those grown over blue. It is obvious from these results that some of the aroma compounds emitted by this widely used herb can be enhanced, or diminished, by manipulating the color of light reflected upward to the developing leaves from colored mulches on the soil surface.

In contrast to the monoterpenoids, the concentration of the aliphatic compound hexanal was numerically highest over red. The concentrations of octyl acetate, however, followed a trend similar to that of the monoterpenoids, that is, relatively high levels from leaves grown over yellow and green and lower levels from leaves grown over blue. The emission of the phenylpropanoid eugenol also followed this trend.

Total levels of aroma compounds varied significantly among leaves that developed over the different colors (Figure 1). Leaves that developed over yellow and green surfaces emitted greater amounts of aroma compounds than those that developed over white, blue, or red (LSD test, $p = 0.05$). The total amount of aroma compounds emitted by leaves grown over black was intermediate.

Emission from leaves that developed over yellow versus white and from those that developed over green versus blue follow similar patterns. In both comparisons, BL reflected from the soil surface to developing leaves appears to have suppressed accumulation of these compounds. Both the yellow and white surfaces reflected ~40% of the incoming R and FR, and they both reflected about the same FR/R ratio as was present in the incoming sunlight. However, yellow reflected less BL than did the white surfaces. In the case of the green versus blue surfaces, they reflected about the same amount of photosynthetic light and similar FR/R photon ratios, but the green surface reflected less BL than did

Table 2. Characteristics of Basil Leaves Grown over Different Colored Mulches[a]

	mulch color					
characteristic	black	red	green	blue	yellow	white
leaf area (cm^2)	608 bc	785 a	700 b	591 c	666 b	687 b
fresh wt (g)	19.921 c	26.275 a	22.654 b	19.375 c	22.722 b	26.978 a
% water	86.3 c	88.4 a	87.8 b	87.5 b	87.5 b	86.2 c
dry wt (g)	2.728 bc	3.038 b	2.756 bc	2.423 c	2.847 bc	3.717 a
mg of dry wt/cm^2	4.49 b	3.87 c	3.94 bc	4.10 bc	4.27 b	5.41 a

[a] Data are the means of five 40-leaf samples. Within each line, values followed by the same letter do not differ significantly at the 5% level.

Table 3. Headspace Concentrations of Volatile Compounds (Nanograms per Gram of Dry Weight) from Basil Leaves Grown over Different Colors[a]

	mulch color					
compound	black	red	green	blue	yellow	white
			Terpenoids			
α-pinene	56.0 ab	52.6 b	58.5 ab	56.4 ab	66.2 a	57.6 ab
β-pinene	39.2 b	38.0 bc	39.2 b	34.8 cd	42.9 a	33.8 d
myrcene	103 abc	89.7 bc	108 ab	87.6 c	112 a	87.9 bc
1,8-cineole	457 b	375 cd	480 ab	350 d	556 a	358 d
camphor	10.5 abc	7.9 cd	6.7 d	9.5 cd	12.1 ab	12.6 a
linalool	757a	607 b	753 ab	627 b	849 a	585 b
terpinen-4-o1	4.1 ab	2.1 d	4.2 ab	3.2 bc	4.6 a	2.8 cd
bornyl acetate	8.0 b	7.1 bc	6.9 c	3.2 d	9.3 a	9.5 a
α-terpineol	5.7 bc	4.2 bc	6.5 ab	4.3 bc	8.6 a	3.7 c
			Miscellaneous Compounds			
hexanal	14.1 a	17.5 a	14.3 a	12.9 a	14.5 a	13.4 a
octyl acetate	3.1 b	2.2 b	4.1 a	3.1 b	4.0 a	3.4 ab
eugenol	60.6 a	48.3 a	51.0 a	45.5 a	55.8 a	38.1 a

[a] Data represent the mean of seven determinations. Within each line, values followed by the same letter are not significantly different by a least significant difference test at $p = 0.05$.

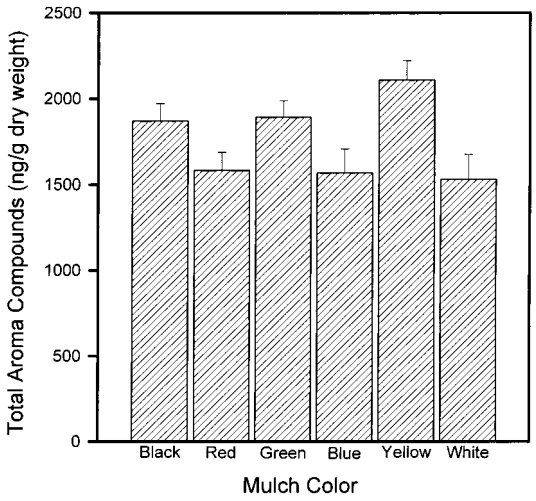

Figure 1. Total aromatic compounds entrained from basil leaves grown over various colors of reflective soil covers. Data are the means ± standard error of the mean.

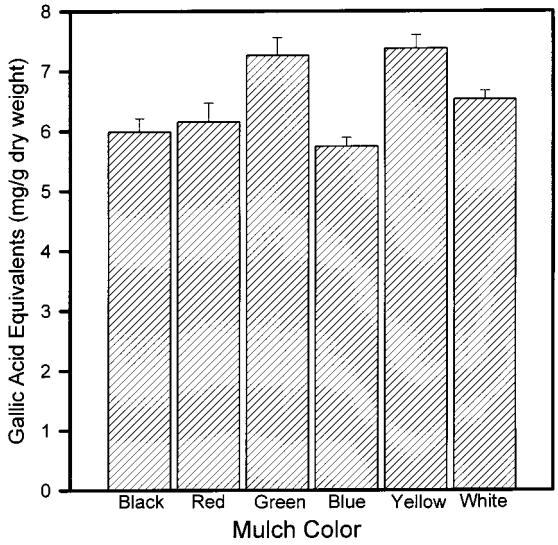

Figure 2. Soluble phenolics extracted from basil samples grown over various colors of reflective soil covers. Data are the means of 10 determinations ± standard error of the mean.

the blue surface. The mechanism of action by which upwardly reflected BL during leaf development influenced the amounts of aroma compounds emitted from the basil leaves is beyond the scope of the present study.

Soluble Phenolics. Basil occurs in a number of chemotypes, in some of which phenylpropanoids constitute a major percentage of the essential oil (*14*). Methylchavicol, methyl cinnamate, and eugenol are all major components of the essential oil in some of these chemotypes. Because the cultivar used in this study produced relatively low levels of these compounds, we performed analyses for total soluble phenolics to gain insight into how the levels of these compounds were affected by the colored mulches.

As was the case with the aroma compounds, basil leaves that developed over yellow and green contained the highest levels of soluble phenolics (Figure 2). The values for these treatments were 7.4 and 7.3 μg/mg, respectively. These values were significantly higher than those obtained for basil leaves grown over the other colors. The concentrations of soluble phenolics in basil leaves grown over blue surfaces were lowest. However, the trends of greater concentrations in leaves grown over yellow versus white and over green versus blue followed the same pattern as noted for aroma compounds. That is, within each of the pairs, the leaves that received the least reflected BL had the greatest concentrations of soluble phenolics.

Preliminary experiments separating the phenolics of the dried basil as their trimethylsilyl derivatives revealed a number of simple aromatic acids including some phenolic acids. Cinnamic, caffeic, sinapic, and ferrulic acids were some of the major compounds analyzable in this manner. Only minor levels of more complex phenols such as flavonoids were detected. However, air-drying of the leaves may have resulted in significant losses of complex phenols. Perhaps future experiments using fresh or lyophilized material will allow greater insight into the composition of phenolics altered by the color of light reflected to developing leaves from mulches on the soil surface.

Plant "secondary metabolites" such as those examined in the present work have numerous effects on mammalian physiology. Many of the naturally occurring phenolics, for example, are well-known and extensively studied antioxidants. Monoterpenoids have been implicated as anti-inflammatory agents (*15*), antimicrobial agents (*16*), and analgesics (*17*). Increasing the levels of these compounds in field-grown plants may increase their efficacy as traditional herbal medications, in

addition to improving their flavor and nutritional value when used as food crops.

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