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Light Reflected from Colored Mulches to Growing Turnip Leaves Affects Glucosinolate and Sugar Contents of Edible Roots

George F. Antonious[1], Michael J. Kasperbauer*[2], and Matthew E. Byers[1]

[1]Kentucky State University, Atwood Research Facility, Frankfort, KY, USA and
[2]USDA-ARS, Coastal Plains Research Center, Florence, SC, USA

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ABSTRACT

Plastic mulches are widely used to conserve water and control weeds with less applied herbicides in production of food crops. Both yield and quality are important and can be affected by reflected blue (B), red (R) and far-red (FR) light combinations received during growth and development. Photosynthate allocation among growing plant parts and flavor of edible roots were studied in turnip (*Brassica rapa* L.) grown in trickle-irrigated field plots with blue, green and white mulches. The blue and green mulches reflected different amounts of B, but they both reflected FR/R ratios higher than the ratio in incoming sunlight. The white mulch reflected more photosynthetic light and a lower FR/R ratio than the blue or green mulches. Plants grown with blue and green mulches did not differ significantly in leaf length, root size and shoot/root biomass ratio. Those grown with white had shorter leaves and larger roots. Taste testers found that plants grown with blue mulch developed roots with a sharp flavor, and roots from plants grown with green mulch had a mild flavor. Those grown with white had a less distinct flavor. Roots grown with blue mulch had the greatest concentrations of total glucosinolates (GSL) and ascorbic acid. Reducing sugar concentrations were higher in roots grown with green than in those grown with blue mulches. The comparison of chemical composition of roots from plants grown with blue *versus* green mulches is important because the main difference was the amount of reflected B, suggesting that B influenced an enzyme involved in the pathway from glucose to GSL. We conclude that the spectrum of light reflected from mulch on the soil surface can influence not only shoot/root biomass ratio but also flavor-related chemical composition of field-grown food crop plants.

INTRODUCTION

The quantity and quality of plant products are influenced by the light environment that exists during their growth and development. Canopy interception of photosynthetic light has received considerable attention for many years. However, photomorphogenic light plays a major role in regulation of how and where the photosynthates are used within the developing plant. This is important in plant adaptation and survival in the field and may affect yield and chemical composition of edible plant parts.

Morphological and biochemical responses to red (R),† far-red (FR) and blue (B) light have been studied extensively under controlled environments. These wavebands can be influenced in the field by plant population density and row orientation (1–6). Because of absorption and reflection properties of the nearby plants, close-spaced plants generally receive less B and higher FR/R ratios than wide-spaced plants (2,6). The adaptive response to a higher FR/R ratio is development of longer stems (or longer leaves) that have a greater probability of keeping some leaves in sunlight above the competing plants (7). Field plants also respond to quantity and spectral distribution of light reflected from the soil surface and from plant residues or plastic mulches placed on the soil surface (8,9).

Black plastic mulches over trickle-irrigation systems are frequently used to conserve water and control weeds (with less applied herbicides) in production of high value food crops. Mulches with other surface colors also conserve water and control weeds, and colors can be selected to reflect different quantities of B as well as different FR/R ratios to the developing shoots. The quantity of B and the FR/R ratio affect leaf chemistry including esters and fatty acids in surface waxes, chlorophyll, carotene and light-harvesting chlorophyll protein (9,10). We hypothesized that spectra of light received by growing leaves might also affect root chemistry.

Turnip is a crop plant whose roots are used as human food. Hence, information is needed on relationships among different wavelengths of light received by leaves during plant development, biomass distribution among shoots and roots and concentrations of flavor-related compounds in the edible roots.

Glucosinolates (GSL) are present in varying amounts in many members of the Cruciferae family, of which turnip is a member. They are responsible for the sharp or biting taste of condiments (horseradish, mustard) and contribute to the characteristic flavors of plants whose leaves (brussels

*To whom correspondence should be addressed at: USDA-ARS, Coastal Plains Research Center, 2611 W. Lucas Street, Florence, SC 29501-1241, USA. Fax: 803-669-6970.

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†*Abbreviations*: B, blue light; FR, far-red light; GSL, glucosinolate; PPF,photosynthetic photon flux; R, red light; Rt, retention time.

sprouts, cabbage), floral buds (broccoli, cauliflower), stems (Kohlrabi) or roots (radish, turnip) are consumed by humans (11). When consumed in moderate amounts some GSL or their enzymatically released products are beneficial. Anticancer effects of *Brassica* in the human diet have been covered in recent reviews (12–14). Mode of action of GSL and their derivatives as protective agents against carcinogens has been reported (15,16). On the other hand, animals fed high levels of *Brassica* were found to develop goiter (17,18) and other undesirable physiological effects (19).

Concentrations of ascorbic acid and sugars are also important components of human food and animal feed. Sugar concentrations have been significantly altered in leaves by the FR/R ratio received in controlled environments (20,21). The present study was done to evaluate: (a) effect of light reflected from different colored mulches on biomass distribution among field-grown turnip shoots and roots and (b) effects of different spectra reflected to the leaves on concentration of flavor-related compounds in edible roots.

MATERIALS AND METHODS

Plant material and growth conditions. Turnip (*Brassica rapa* L. cv. Purple top) plants were grown in irrigated field plots of Norfolk loamy sand (Typic Kandidults) at the Coastal Plains Soil, Water, and Plant Research Center near Florence, SC, in 1991, 1992 and 1993. Each year, plots were fertilized according to recommendations of the Clemson University Cooperative Extension Service and 90 cm-wide 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. There were three such plots each year. Each plot contained three 6.5 m-long subplots that were painted with blue, green or white exterior enamel to provide different combinations of reflected B, R and FR. The sequence of colors was randomized within each plot. Exterior enamels were used because they provided an economical and repeatable method to obtain the desired reflection spectra for small plot studies.

The plants were started on a greenhouse bench in 5 cm-diameter pots in April, each year. Two weeks after seeding (when the largest leaf per plant was about 1 cm in diameter), plants were selected for uniformity and transplanted to the field plots. 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. The fleshy roots developed in the soil below the 7.5 cm holes and leaves extended outward over the mulch surfaces. Temperatures near the developing roots of plants grown with blue *versus* green mulches differed less than 0.5°C. Those grown with white mulch were usually 1–2°C cooler. There were 20 plants per color per replicate each year.

Reflected light. The quantity and spectral distribution of upwardly reflected light were measured 10 cm above the colored mulch surfaces using a LiCor LI-1800 spectroradiometer (LiCor 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 on a cloudless day at solar noon $\pm$ 30 min. The reflected light values were then calculated as percentages of incoming sunlight at each measured wavelength. The FR/R ratios in upwardly reflected light were expressed relative to the FR/R ratio 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.

Morphology and root flavor. Each year, the largest leaf per plant was measured for length, and the specific leaf weight (mg dry wt per cm^2) was based on leaf discs taken with a cork borer and freeze dried. Total leaf weights and root weights were determined on a per plant basis. In 1991, after it was evident that shoot and root morphological characteristics were affected by reflection from different colored mulches, flavor of roots was evaluated subjectively by 25 staff members at the Center. Additional taste tests were done in 1992 and 1993. Root samples were coded and cut into small pieces, about 0.5 cm^3. Each person was asked to answer three questions: (a) can you taste a difference among the coded samples, (b) which sample has the sharpest flavor, and (c) which sample has the mildest flavor? The tasters also added their descriptions of the flavors.

Chemical analyses. Turnip roots were harvested after about 5 weeks of growth in the mulch-covered field plots. Representative samples of five roots per replicate were collected from the different colored mulches in 1992 and 1993. They were stored in darkness at 4°C. Within 4 days of harvesting, roots were cut into 1–3 cm cubes, and 100 g subsamples were dropped into boiling methanol (300-mL analytical grade) on a steam bath in 1-L wide-mouthed Erlenmeyer flasks (covered with a watch glass) for 15 min (22). After cooling, the material was blended and vacuum filtered. The residue was washed with 100 mL methanol, then resuspended in 7:3 methanol/water (to precipitate protein from the sample extracts). The homogenate was heated again for 10 min, cooled and filtered a second time. The combined filtrates were concentrated using rotary evaporator at 40°C until the aqueous residue contained no organic solvent (methanol). The concentrates were centrifuged (5000 rpm for 10 min at 0°C), supernatants were combined, and volume was adjusted to 40 mL with redistilled water. The extracts were kept frozen until analysis for total GSL.

Separation of total GSL was accomplished by adsorption on Sephadex A-25 (2-[Diethylamino] ethyl ether) of 40–125 μm bead size, an anion-exchange resin. Extracts were applied to ion-exchange glass columns (20 × 1.4 cm) containing Sephadex (preswelled with 2 *M* ammonium acetate) to give a settled resin bed height of 15 cm, then eluted with a stepwise gradient of 1 *M* and 2 *M* aqueous ammonium acetate using the procedure described by Hanley *et al.* (23). A portion of the GSL pure extract was subjected to an enzymatic hydrolysis procedure (24). Quantitation of total GSL was based on measurement of enzymatically released glucose (25). Moles of glucose released into the aqueous medium are equivalent to the moles of total GSL.

A calibration curve was determined with each group of samples by using 10, 20 and 30 μg/mL glucose standards. The activity of myrosinase was optimized using sinigrin as substrate. A 1 mL solution of sinigrin (5 m*M* in phosphate buffer, pH 7) plus 1 mL (5 mg/mL) enzyme solution were mixed. The solution was incubated 30 min at 37°C. One milliliter of the hydrolyzed mixture was used for glucose determination. Standards of sinigrin monohydrate (allylglucosinolate), myrosinase and pure glucose were obtained from Sigma Chemical Co. (St. Louis, MO). The homogenizations were performed in boiling extraction solution to ensure complete inactivation of the myrosinases in the sample extracts. The hot solvent treatment allowed GSL to be extracted completely without hydrolysis by endogenous myrosinase. To determine the performance of the method, endogenous thioglucosidase was first heat inactivated and the free glucose was measured colorimetrically by the same procedure except that no thioglucosidase was added. The actual amount of glucose in each sample was obtained by subtracting the amount of free glucose detected in the sample and then adjusting the value according to the percent recovery. Recovery for added glucose was 83.2% using the same analytical procedure used to determine GSL in turnip samples.

Representative samples (100 g) were blended with 0.4% oxalic acid solution for ascorbic acid extraction (26). Extracts were filtered through 1.2 μm Millipore filters prior to HPLC analysis using a Waters Millipore high performance liquid chromatograph (Waters Associates, model 441, Milford, MA) equipped with a UV detector (Varian model 9050, Sunnyvale, CA) set at 254 nm. The eluting solvent was 30:70 methanol/buffer solution (0.04 *M* H_3PO_4–KH_2PO_4, pH 3.5) at a flow rate of 1.0 mL/min and pressure of 2220 psi. The column used was μBondapak C_{18} (30 cm × 0.39 cm). Alternative runs of a standard solution of ascorbic acid between sample extracts were made. Retention time (Rt) was 3.4 min.

Total and reducing sugars were extracted with 80% ethanol and purified using the method developed by Antonious and Abdel-All (27). Quantitation was carried out colorimetrically using the method of VanEtten *et al.* (25) for reducing sugars and the phenol–sulfuric

Table 1. Quantities and ratios of upwardly reflected light measured 10 cm above different colored mulches

Light characteristic*	Mulch surface color		
	Blue	Green	White
PPF (400–700 nm) (%)	13	12	42
Blue (B, 450 ± 5 nm) (%)	25	7	41
Green (550 ± 5 nm) (%)	10	24	42
Red (R, 645 ± 5 nm) (%)	8	9	43
Far-red (FR, 735 ± 5 nm) (%)	12	13	43
Far-red′ (FR′, 755 ± 5 nm) (%)	18	22	43
FR/R (ratio)	1.5	1.4	1.0
FR′/R (ratio)	2.3	2.4	1.0

*Values are expressed as percentages of incoming sunlight in the same wavebands; FR/R, photon ratios calculated relative to ratio in incoming sunlight, which was assigned a value of 1.00.

acid reaction for total sugar determination (28). Total and reducing sugars were calculated from glucose concentrations.

Data were analyzed statistically using analysis of variance (ANOVA) (29). Means and standard errors (SE) were also calculated for the combined 2 year data for GSL, ascorbic acid, reducing sugar and total sugar concentrations (30).

RESULTS

Reflected light

Quantities and ratios of reflected light received 10 cm above the different colored mulches are summarized in Table 1. The blue and green surfaces reflected about the same photosynthetic photon flux (PPF), R, FR and FR/R ratio; but the amounts of reflected B differed. White surfaces reflected greater PPF, more R and a lower FR/R ratio than the blue or green surfaces. Because of earlier controlled environment studies on effects of light color on photosynthate partitioning among shoots and roots (6,31) and on leaf and stem chemistry (20), we hypothesized that differences in color of light received by the leaves could also affect size and chemistry of field-grown turnip roots.

Morphological

Length of the largest leaf, leaf thickness (specific leaf weight), shoot weight and root weight of plants grown with the various mulch colors in 1991 are shown in Table 2. Significant differences for all measured characteristics occurred among plants grown with the different mulch colors even though all of the plants received the same quantity and spectral distribution of incoming sunlight in the field. Leaf lengths and root weights were about the same when grown with the blue and green surfaces (which reflected about the same FR/R ratios, as shown in Table 1). However, plants grown with white surfaces received lower FR/R ratios and they had shorter leaves, higher specific leaf weights, heavier roots and lower shoot/root ratios. The same pattern of results among mulch colors was also observed in 1992 and 1993 (data not shown).

After it was apparent that turnip shoot/root ratios could be influenced by color of mulch, representative roots were tasted to determine whether mulch color affected root flavor. Of the 25 participants in the 1991 taste test, 24 indicated that roots from plants grown with blue mulch had a "sharp" flavor. The majority of panelists also indicated that roots from the green mulch treatment had the mildest ("almost sweet") flavor. Roots of plants grown with white mulch had a less distinct flavor. Additional taste tests were conducted in 1992 and 1993 (data not shown) with the same pattern of results (*i.e.,* roots grown with blue mulch developed a sharp flavor and those grown with green developed a mild flavor). Results of the subjective taste test indicated the need for objective chemical analyses of compounds that might contribute to the sharp or mild flavor.

Table 2. Morphological characteristics of turnip plants grown from 25 April to 23 May with different colored mulches in trickle-irrigated field plots near Florence, SC

Character	Mulch surface color		
	Blue	Green	White
Longest leaf (mm)	360 a*	371 a	334 b
Specific leaf wt (mg/cm^2)	4.8 b	4.9 b	5.7 a
Total shoot wt (g)	141 a	145 a	128 b
Total root wt (g)	75 b	74 b	84 a
Shoot/root wt (ratio)	1.88 a	1.95 a	1.52 b

*Each value is the mean for 20 plants per color per replicate. Values in the same row followed by different letters differ significantly at the 5% level (29).

Chemical

Concentrations of total GSL, ascorbic acid and sugars in turnip roots grown with blue, green or white colored mulches are shown in Fig. 1. Some differences in total GSL occurred among roots grown with different mulch colors. Roots from plants grown with blue mulch had the highest GSL concentrations. Roots grown with white mulches were larger than those grown with blue or green mulches (see Table 2) and they had lower GSL concentrations (on a fresh weight basis) relative to those grown with blue or green mulches. This relationship between root size and GSL concentration was consistent with previous studies (32,33). However, the greater quantity of total GSL in roots grown with blue relative to green is of biological significance because the blue and green mulch surfaces reflected about the same PPF and FR/R ratios (see Table 1), and they had very similar biomass distribution in shoots and roots (see Table 2).

Roots of plants grown with blue mulch also had the highest ascorbic acid concentrations, and those grown with white mulches had the lowest concentrations on a fresh weight basis. Ascorbic acid concentrations were lower within each mulch color in 1993 than in 1992, but the trends among mulch colors remained the same each year.

Reducing sugar and total sugar concentrations were significantly affected by mulch color. Concentrations of reducing sugars were highest, and total sugar concentrations were lowest in roots from plants grown with green mulch.

DISCUSSION

Colored mulches affected the quantity of reflected B and photosynthetic light as well as the FR/R ratio received by leaves of the field-grown plants (Table 1). Those grown with

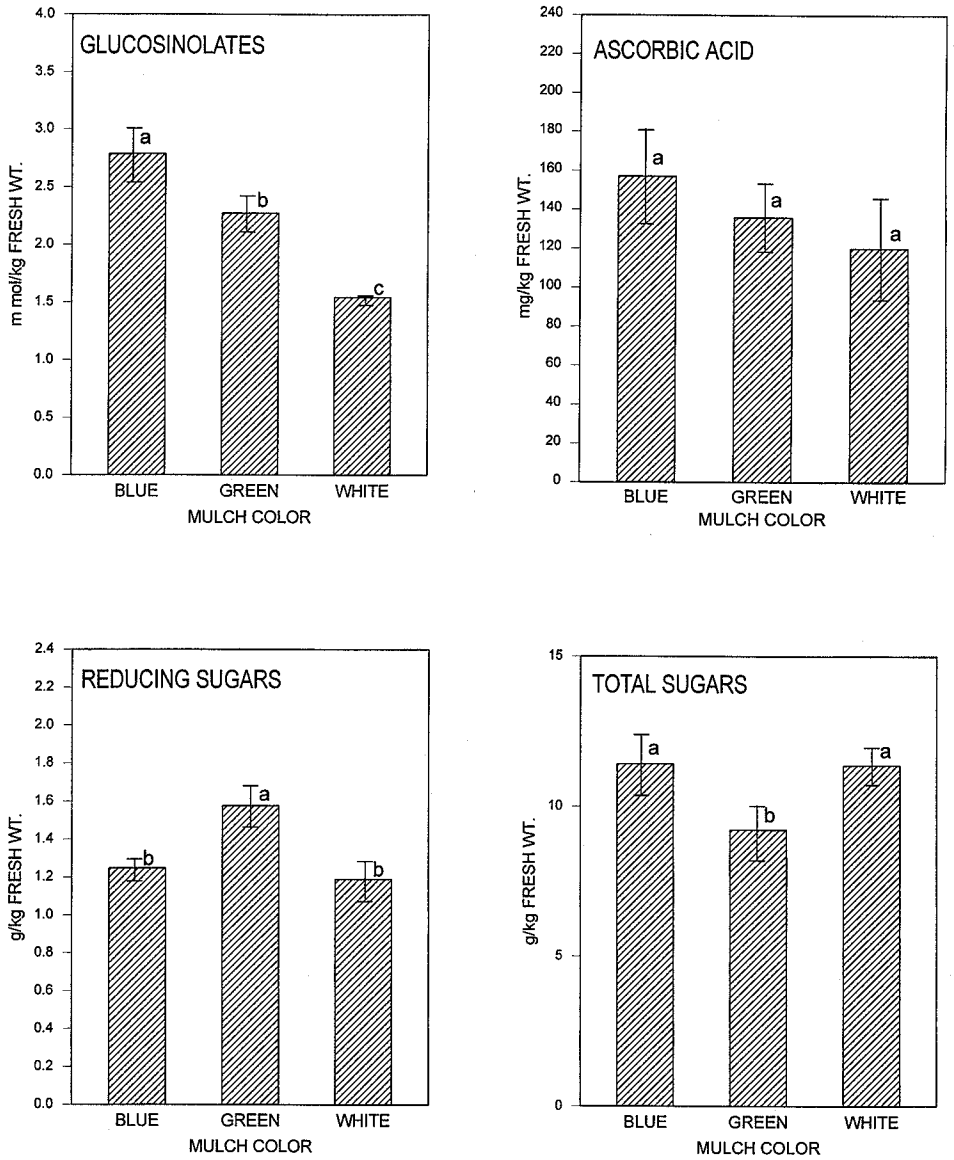

Figure 1. Concentrations of total GSL, ascorbic acid, reducing sugars and total sugars in roots of turnip plants whose leaves grew in sunlight in field plots over different colored mulches. Data plotted in bars are means ± SE for six values, three replicates from each of 2 years for each mulch color. The data were also subjected to analysis of variance and bars accompanied by different letters differ significantly at the 5% level (29).

the blue and green mulches received about the same reflected FR/R ratio, and they did not differ significantly in shoot size, root weight and shoot/root weight ratio (Table 2). They did, however, receive different quantities of reflected B and they differed in root flavor as determined by taste testers and by chemical analyses. Roots grown with blue mulch had the "sharpest" flavor, whereas those grown with green had a mild ("almost sweet") flavor according to the taste testers. Chemical comparison of roots from plants grown with blue (*versus* green) mulches showed that those grown with blue had higher concentrations of total GSL, which contribute to the sharp flavor of members of the Cruciferae family. Roots from plants grown with blue mulch also had higher concentrations of ascorbic acid. Roots grown with green mulches had the highest concentration of reducing (soluble) sugars. The combination of low blue with high FR/R ratio in reflected light may be important in regulating soluble sugar concentrations in field-grown plants. This would be consistent with controlled environment experiments in which soluble sugar concentrations increased in response to higher FR/R ratios when the quantity of blue remained constant (20,21).

The higher concentration of GSL and the lower concentration of soluble sugars (including glucose) in roots developed with blue (*versus* green) mulch in the present study suggest a blue light influence on an enzyme involved in the synthetic pathway from glucose to GSL. However, more detailed study is needed.

Although plants grown with white mulch received much more reflected photosynthetic light (Table 1), they had lower shoot weights, heavier roots and lower shoot-to-root weight ratios (Table 2). This pattern of photosynthate allocation in turnip plants grown with white mulch *versus* colors that reflect less photosynthetic light but higher FR/R ratios is consistent with results obtained with other plant species when insulation panels were used to avoid rhizosphere temperature differences below the different colors (34,35). Hence, we interpret this photosynthate allocation pattern associated with mulch color to be primarily due to FR/R ratio and to a

lesser extent to the combination of FR/R ratio and quantity of B reflected to the growing leaves.

Although effects of PPF, B, R, FR and the FR/R ratio on stem length, leaf shape, shoot/root weight ratio and chemical composition of leaves under controlled environments have been well documented for many years (20,31,36–38); response of field-grown plants to light reflected from neighbor plants (1–4) and from the soil surface (5,9,10) are recent discoveries. To the best of our awareness, the present report is the first documentation of effects of colored mulches on flavor (detected by humans) and concentration of flavor-related chemicals in edible roots of plants whose leaves received different quantities and wavelength ratios of light reflected from different colored soil surface covers (mulches) during plant growth in the field.

We conclude that concentrations of flavor-related components (including GSL, ascorbic acid and sugars) of field-grown edible roots can be affected by the FR/R ratio and the quantity of B reflected to the growing leaves from different colored mulches. This discovery has important implications for consumers, scientists, commercial growers and home gardeners.

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