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Seasonal Variation in Glucosinolate Accumulation in Turnip Cultivars Grown with Colored Plastic Mulches

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***Abstract*. Glucosinolates (GSLs) are thioglucosides with important properties for plant defense and human health. The objective of this study was to quantify yield and GSL concentration in turnip (*Brassica rapa* subsp. *rapa)* roots and shoots as influenced by colored plastic mulches. Four turnip cultivars (‘Just Right’, ‘Purple Top’, ‘Royal Crown’, and ‘Scarlet Queen’) were grown over five mulch treatments: white, yellow, silver, red, blue, and a bare soil control in both a May and an August planting in 2006 and 2007. Yield varied by variety; however, there was no consistent relationship between mulch treatment and yield. Glucosinolate concentrations and profiles varied with tissue type, genotype, and environmental factors, including temperature and planting date. Mulch-dependent increases in GSL concentrations were not consistent across tissue types, cultivars, planting dates, and years of the study, possibly as a result of differences in climatic factors and mulch-dependent changes in soil temperature between planting dates and years of the study.**

Glucosinolates are thioglucosides found in brassica vegetables that when hydrolyzed at the S-glucose bond create a suite of products involved in plant defense, flavor, and human health. Glucosinolates react with myrosinase [thioglucoside glucohydrolase, electrical conductivity (EC) 3.2.1.147], which hydrolyzes the thioester bond, releasing glucose and creating unstable byproducts such as isothiocyanates, thiocyanates, and nitriles (Bones and Rossiter, 1996). Identifying vegetable production systems that further enhance GSL concentrations and myrosinase activity could have a significant impact on human health, particularly cancer prevention, because GSL hydrolysis products have potent chemopreventive benefits (Neave et al., 2005; Pham et al., 2004; Plate and Gallaher, 2006).

Glucosinolate concentrations and profiles differ between brassica species (Mithen et al., 1987) and cultivars within a species (Carlson et al., 1987; Rosen et al., 2005). Glucosinolate concentration is also influenced by environmental factors including temperature and light quality (Engelen-Eigles et al., 2006). Light quality and temperature can be altered in a field setting by changing planting date (Charron et al., 2005; Charron and Sams, 2004) and by growing plants with colored plastic mulches (Antonious et al., 1996). Several studies have shown that colored plastic mulches influence the photosynthetic photon flux (*PPF*) and amount of red, far-red, and blue light reflected into crop canopies in the field, providing a means to potentially influence processes regulated by phytochrome and cryptochrome mechanisms (Kasperbauer, 1992). Colored plastic mulches can alter secondary metabolite concentrations and flavor profiles in several crops including basil (*Ocimum basilicum* L.; Loughrin and Kasperbauer, 2001) and strawberry *(Fragaria ×ananassa*; Atkinson et al., 2006). Antonious et al. (1996) found that turnips grown with different colored mulches (black plastic mulch painted blue, white, or green) resulted in differential increases in total GSLs. In that study, the blue mulch treatment reflected the greatest amount of blue light among the mulch treatments and also resulted in the highest concentrations of total GSLs (Antonious et al., 1996). However, the experimental conditions included hand-painted plastic mulches and a plant spacing of 30 cm, which is not representative of a typical population for production. In addition, the study did not examine the influence of colored plastic mulches on individual GSL concentrations or on shoot GSLs. Understanding the effect of specific field production strategies on the GSL profile of shoot and root tissues rather than just total GSL concentration in roots is important because shoots and roots inherently have different GSL profiles (van Dam et al., 2009), and individual GSLs have different effects on plant defense and human health (Neave et al., 2005; Plate and Gallaher, 2006). Additionally, regulation of GSL biosynthesis may occur in a tissue-specific manner (Hirai et al., 2007).

We wanted to better understand the effect of reflected light quality and soil temperature on yield and GSL accumulation in vegetable tissues both above and below the soil surface. In this study, we quantified yield and both total and individual GSL concentrations in turnip shoots and roots of four cultivars grown with different colored plastic mulches at two planting dates.

Materials and Methods

The study was conducted at the Southern Research and Outreach Center in Waseca, MN (lat. 44.07° N, long. 93.52° W) on a Webster Clay loam soil (fine-loamy, mixed superactive typic endoaquoll with a pH of 6.8 and soil phosphorus and potassium of 40 ppm and 158 ppm, respectively) using a randomized complete block design with four replications. Plots consisted of 7.6-m rows on raised beds spaced at 1.5 m on center. Plots were fertilized with 67 $kg \cdot ha^{-1}$ nitrogen preplant. Planting dates were 6 May and 7 Aug. 2006 and 10 May and 28 Aug. 2007. The trial included four turnip cultivars: ‘Just Right’ (JR; Jordan’s Seed, Woodbury, MN), ‘Purple Top’ (PT; Jordan’s Seed), ‘Royal Crown’ (RC; Sakata Seed America, Inc., Morgan Hill, CA), and ‘Scarlet Queen’ (SQ; Johnny’s Seeds, Winslow, ME). These genotypes were chosen based on their epidermal and internal pigmentation. JR is white with no pigmentation, PT and RC are mostly white with purple on the shoulder of the root with a white interior, and SQ has a red-skinned root with a white interior and the shoots have red petioles and red venation. JR, RC, and SQ are hybrid turnip cultivars, whereas PT is open-pollinated.

Turnip cultivars were grown with six different mulch treatments: yellow (Yellow/SLT; Ginegar Plastic Products, Ltd., Israel), blue, red, white, silver (Jordan’s Seed), and a bare soil control. Blue and red mulches were infrared-transmitting plastic mulch films. Yellow, silver, and white were coextruded plastic mulch films with black being the color on the opposing side of the mulch. Mulches were chosen based on their spectral properties (Fig. 1). Light quantities reflected by mulches were measured using an Apogee Model Spec-ultraviolet/photosynthetically active radiation spectroradiometer with a reflectance probe attachment (Apogee Instruments, Inc., Logan, UT). Reflected red to far-red light ratios (R:Fr) and *PPF* were measured 10 cm above the

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mulch surface with a Skye red-far red meter (Skye Instruments, Ltd., Powys, U.K.) and an Apogee Quantum Meter, Model QMSW-SS (Apogee Instruments, Inc.), respectively.

Turnip seeds were planted with 5-cm spacing between seeds using a two-row Nibex 500 Precision Planter (Emeryville, CA). Plastic mulches were installed immediately after seeding using a commercial plastic mulch layer (Mechanical Transplanter Co., Holland, MI). Plastic mulches were then slit to allow seedling emergence through the mulch surface. Plots were irrigated as needed with drip irrigation placed under the mulch surface to provide a minimum of 2.5 cm of water each week. Soil temperatures under each mulch type were monitored 5 cm below the soil surface for all planting dates using an Optic StowAway® data logger, Model WTA08 (Onset Computer Corp., Pocasset, MA) (Fig. 2). Seasonal temperature and solar radiation data (Table 1) were obtained from the Southern Research and Outreach Center weather information web site (http://sroc.cfans.umn.edu/WeatherInformation/index.htm).

Whole plants were harvested when root diameter reached 5.0–7.6 cm. Twenty plants were randomly harvested from each plot between 0900 and 1300 HR. Plants were topped, and shoot and root weights were recorded separately for yield measurements. A composite sample of four plants was used for GSL quantification. All samples were stored at 4 °C before processing for GSL quantification and were processed within 2 weeks after harvest.

Glucosinolate quantification. Extraction and GSL quantification were performed as per Hecht et al. (2004) using modifications from Rosen et al. (2005). Briefly, a 150-g leaf sample or 200-g root sample was heated in 700 mL of boiling water for 10 min to deactivate myrosinase. Boiled samples were macerated in a blender for 2 min. A 40–mL aliquot of blended sample was homogenized using a BioSpec M133 Homogenizer (BioSpec Products, Inc., Bartlesville, OK) set at 12,000 rpm for 2 min, then centrifuged for 10 min at 5000 *g*, 4 °C.

Desulfoglucosinolate (ds-GSL) extraction was performed using conditioned solid phase strong anion exchange (SAX) columns (Sigma-Aldrich, St. Louis, MO). Sinigrin (S-1647; Sigma-Aldrich) was added to the conditioned SAX columns as an internal standard. To desulfate, samples were incubated with two units (0.2 mg/mL) of sulfatase (aryl-sulfate sulfohydrolase; EC 3.1.6.1; S-9626; Sigma-Aldrich) on SAX columns for ≈15 h at room temperature (≈21 °C), then eluted with 3 mL water, and the collected volume was determined by weight. Further washing of the columns yielded no additional ds-GSLs, confirming complete elution. Eluent was stored at –20 °C until high-performance liquid chromatography (HPLC) analysis.

HPLC analysis was performed on an Agilent 1200 Series Quaternary system (Agilent Technologies, Inc., Santa Clara, CA) set at λ = 229 nm using a Luna C18, 5-µm, 250 × 4.6-mm column (Phenomenex, Torrance, CA) maintained at 30 °C. A 50-µL aliquot of the eluent was separated on the system with a flow rate of 1.0 $\text{mL}\cdot\text{min}^{-1}$ using the following gradient: solvent A = water and B = acetonitrile; 0 to 2 min, 95% A, 5% B; 2 to 20 min, 85% A, 15% B; 20 to 23 min, 53% A, 47% B; 23 to 30 min, 0% A, 100% B; and 30 to 33 min, 95% A, 5% B. Peaks were integrated using ChemStation for LC 3D Systems, Revision B.04.01, software. Glucosinolate peak identities were confirmed using retention time and ultraperformance liquid chromatography–mass spectrometry (Waters Corporation, Milford, MA) using a C18 column, a water: acetontirile gradient, and negative electrospray ionization. Ds-GSL concentrations were calculated using relative quantification with an internal standard (sinigrin) and previously published response factors (EU, 1990). Ds-GSL concentrations are reported on a µmol/100 g fresh weight (FW) basis.

Statistical analysis. Data were analyzed with R 2.9.1 (R Foundation for Statistical Computing, Vienna, Austria). The significance of differences among treatments, cultivar, and years was assessed by a fixed-factor analysis of variance. Mean values were considered significantly different at $P < 0.05$ as determined by Tukey's honestly significant difference.

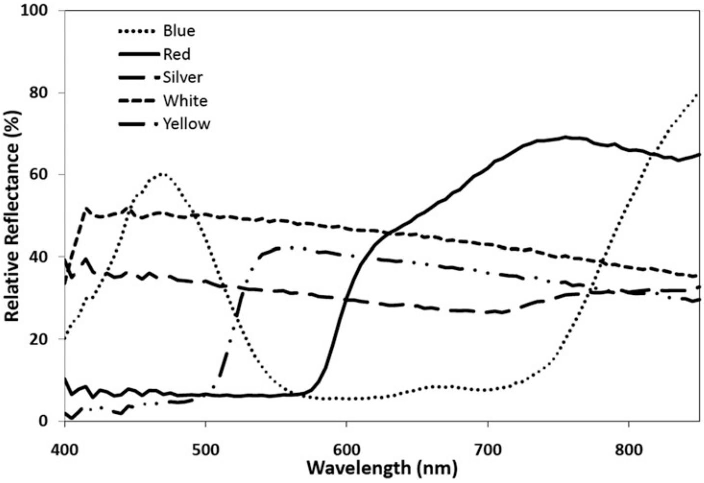

Fig. 1. Reflectance properties of colored plastic mulches. Relative reflectance values were obtained using an Apogee Model Spec-UV/photosynthetically active radiation spectroradiometer with reflectance probe attachement (Apogee Instruments, Inc., Logan UT).

Results and Discussion

Yield. Shoot and root yields differed by cultivar (C) and were inconsistently influenced by mulch treatments (M) across planting dates and years (Y) (Table 2). Despite the

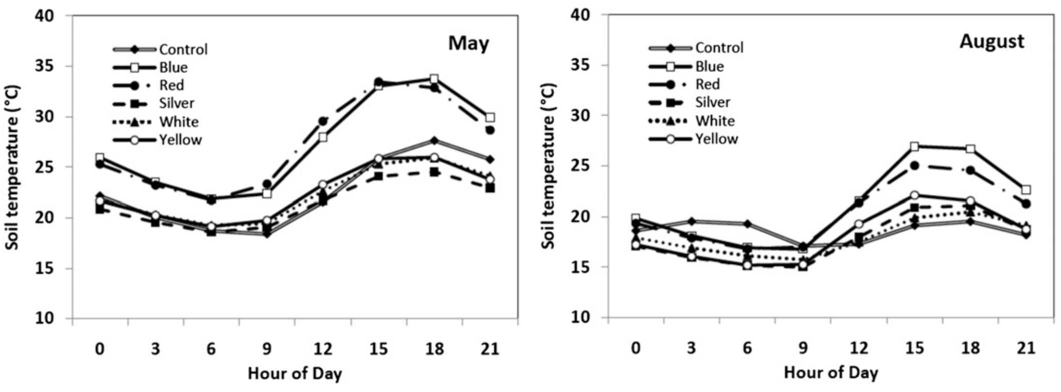

Fig. 2. Mean diurnal soil temperatures of plastic mulch treatments from May and August plantings. Soil temperatures were measured 5 cm below the soil surface and averaged across 2006 and 2007 for each planting date.

Table 1. Climatic data for each growing season and ten days before harvest for each planting date and year.

		Mean daily maximum air temperature (°C)		Mean daily minimum air temperature (°C)		Cumulative growing degree units^{z}		Mean daily solar radiation (mol·m^{-2}·d^{-1})	
Planting	Yr	Season	Harvest	Season	Harvest	Season	Harvest	Season	Harvest
May	2006	24	27	13	14	813	188	39	40
	2007	26	28	13	17	866	213	40	44
August	2006	23	19	13	9	760	99	27	24
	2007	24	22	11	11	632	144	23	12

^{z}Growing degree units were calculated using a base and minimum allowed temperature of 10 °C and a maximum allowed temperature of 30 °C.

Table 2. Fresh weight yield of turnip (*Brassica rapa* subsp. *rapa*) shoots and roots in May and August planting dates in 2006–2007 across years, cultivars, and plastic mulch treatments.

	Fresh wt (Mg·ha^{-1})			
	Shoots		Roots	
Source of variation^{z}	May	August	May	August
Cultivar (C)				
Just Right	23 ± 1	24 ± 1	14 ± 0	15 ± 0
Purple Top	17 ± 0	17 ± 1	13 ± 0	14 ± 1
Royal Crown	20 ± 1	17 ± 1	12 ± 0	12 ± 1
Scarlet Queen	14 ± 0	14 ± 0	11 ± 0	11 ± 0
Significance^{y}	*	*	*	*
Mulch (M)				
Bare soil	18 ± 1 ab	21 ± 1 a	11 ± 0 b	12 ± 1
Blue	18 ± 1 ab	18 ± 1 b	12 ± 0 ab	13 ± 1
Red	17 ± 1 b	18 ± 1 b	13 ± 0 ab	13 ± 1
Silver	19 ± 1 a	17 ± 1 b	13 ± 0 a	13 ± 1
White	19 ± 1 ab	17 ± 1 b	13 ± 1 ab	14 ± 1
Yellow	19 ± 1 a	17 ± 1 b	12 ± 0 a	13 ± 1
Significance^{y}	*	*	*	NS
Year (Y)				
2006	19 ± 1	20 ± 1	13 ± 0	16 ± 1
2007	17 ± 1	16 ± 1	12 ± 0	10 ± 0
Significance^{y}	*	*	*	*
Interactions^{y}				
C × M	NS	NS	NS	NS
C × Y	*	*	*	*
M × Y	NS	NS	NS	NS
C × M × Y	NS	NS	NS	NS

^{z}Means with different letters within each column and source of variation are significantly different at $P \leq 0.05$ as determined by Tukey's honestly significant difference.

^{y}Mean separations on main effects were not performed as a result of the presence of significant interactions.

Significance: NS, *Nonsignificant or significant at $P \leq 0.05$.

significance of the C × Y interaction, JR consistently yielded the most shoot biomass (23–24 Mg·ha^{-1}) and SQ the least (14 Mg·ha^{-1}) at both planting dates. Root yields were consistently higher in JR (14 Mg·ha^{-1}) than SQ (11 Mg·ha^{-1}) in May plantings, but not for August plantings. Mulch treatments inconsistently influenced shoot and root yield between the planting dates. In May plantings, silver and yellow mulch treatments resulted in the highest shoot yields, whereas bare soil plots resulted in the lowest root yields among the mulch treatments (Table 2). In August plantings, bare soil control plots yielded more shoot biomass (21 Mg·ha^{-1}) than all other mulch treatments, whereas mulch treatment had no effect on root yield.

Influence of genotype and tissue type on glucosinolates. Glucosinolate concentrations were significantly influenced by the interactions between planting dates and years; therefore, data for root and shoot GSL concentrations are presented individually by planting date. Within a cultivar, shoots had slightly less total GSLs (TTL; Table 3) than roots (Table 4), except for JR in which shoots had roughly the same amount of TTL as roots. This is in agreement with previous research reporting GSL concentrations to be higher in root tissues than shoot tissues (Carlson et al., 1987; van Dam et al., 2009). The aliphatic GSLs gluconapin (GNP), glucobrassicanapin (GBN), gluconapoleiferin (GF), and progoitrin (PRO) comprised over 90% of the TTL GSL concentration in shoots on average across all cultivars and planting dates (Table 3). Root GSL profiles contained higher proportions of the aromatic GSL gluconasturtiin (GNS) than shoots. Shoots had on average 3 µmol/100 g FW of GNS (data not shown), whereas roots had on average 54 µmol/100 g FW of GNS (Table 4). These results are consistent with previous research reporting GNS to be high in root tissues of *Brassica rapa* (Zhang et al., 2008) and other members of the order Brassicales (van Dam et al., 2009). These tissue-dependent differences in GSL profiles are also consistent with previous data showing that root tissues contain a more diverse GSL profile than shoot tissues (van Dam et al., 2009). The diversity of GSL profiles in root tissues may have evolved to defend against constant pathogen pressures in soils including the plant parasitic nematode *Pratylenchus neglectus* (van Dam et al., 2009).

Cultivar-dependent differences in shoot GSLs varied between years of the study as indicated by the significance of the C × Y interaction for most of the individual GSLs and TTL. The primary GSLs in JR and SQ shoots across both planting dates were GNP and GBN. In RC and PT shoots, GBN and PRO were the predominant GSLs. Our results are consistent with previous research showing GNP and GBN to be the predominant GSLs in turnip shoot tissue (Carlson et al., 1987; Smetanska et al., 2007). Despite the significance of the C × Y interaction, JR shoots consistently had the highest GNP concentrations, whereas PT and RC shoots consistently had the highest PRO and GF concentrations of the four cultivars examined across both planting dates and years of the study (Table 3).

Cultivar-dependent differences in root GSL concentrations varied across years of the study for both planting dates (Table 4). Despite the C × M × Y and C × Y interactions present for individual root GSLs at both planting dates, some cultivar-dependent trends existed in this study. In many instances, SQ roots had the highest TTL and JR the lowest; however, this trend was not significant across all planting dates and years of the study. JR always had the lowest PRO (≈9 µmol/100 g FW) and the highest GNP (≈62 µmol/100 g FW) across years and planting dates. These values are similar to those observed in JR shoots. SQ always had the highest GBN (43 and 72 µmol/100 g) and the highest concentration of the indolic GSL, 1-methoxyglucobrassicin (1MGB) (25 and 7 µmol/100 g FW) for May and August plantings, respectively. 1-methoxyglucobrassicin comprised 7% of the profile on average in SQ and less than 3% (4 µmol/100 g FW) of the profile in all other cultivars and tissues. These results are inconsistent with other research showing 1MGB to be the primary root GSL in teltower turnip (Brassica *rapa var. rapa pygmaea teltoviensis*) (Smetanska et al., 2007). This discrepancy may be the result of inherent genotype-dependent differences in GSL profiles.

It is possible that cultivar differences in root GSL concentrations could have been the result of a dilution effect, because JR roots have lower GSL concentrations than SQ roots but larger root yields than SQ. We attempted to compensate for dilution effects by using roots of comparable size (5–7.6 cm diameter) for GSL analysis. When root GSL concentrations are adjusted based on yield, JR roots still have lower GSL content (2130 mol TTL/ha)

Table 3. Glucosinolate concentrations of turnip (*Brassica rapa* subsp. *rapa*) shoots in May and August planting dates in 2006 and 2007 across cultivars, plastic mulch treatments, and years.

	Glucosinolate concn (μmol/100 g FW)									
	PRO		GF		GNP		GBN		TTL	
Source of variation^z	May	August	May	August	May	August	May	August	May	August
Cultivar (C)										
Just Right	1 ± 0	1 ± 0	1 ± 0	1 ± 0	101 ± 5	92 ± 5	54 ± 4a	41 ± 2	164 ± 8a	138 ± 6
Purple Top	24 ± 2	31 ± 2	17 ± 1	16 ± 1	18 ± 2	23 ± 1	56 ± 3a	84 ± 4	131 ± 8b	167 ± 7
Royal Crown	24 ± 2	31 ± 2	15 ± 1	14 ± 1	17 ± 2	16 ± 1	36 ± 2b	68 ± 3	114 ± 6b	145 ± 5
Scarlet Queen	6 ± 1	6 ± 1	10 ± 1	7 ± 1	36 ± 3	62 ± 3	57 ± 4a	74 ± 4	117 ± 8b	158 ± 7
Significance^y	*	*	*	*	*	*	*	*	*	*
Mulch (M)										
Bare soil	15 ± 2	20 ± 3	12 ± 2	9 ± 1	43 ± 5	43 ± 6	45 ± 4	53 ± 5	127 ± 9	137 ± 8
Blue	17 ± 3	18 ± 3	12 ± 2	10 ± 2	58 ± 8	54 ± 8	59 ± 4	76 ± 4	158 ± 10	165 ± 7
Red	18 ± 3	19 ± 3	14 ± 2	10 ± 1	46 ± 7	47 ± 6	63 ± 5	72 ± 6	156 ± 10	158 ± 9
Silver	9 ± 2	17 ± 3	8 ± 1	10 ± 1	35 ± 7	45 ± 6	45 ± 4	63 ± 4	107 ± 10	144 ± 6
White	13 ± 3	16 ± 3	11 ± 2	10 ± 1	41 ± 8	51 ± 7	49 ± 4	72 ± 4	125 ± 10	157 ± 7
Yellow	10 ± 2	14 ± 2	7 ± 1	8 ± 1	36 ± 6	51 ± 6	44 ± 3	66 ± 6	114 ± 7	151 ± 8
Significance^y	*	NS	*	NS	*	NS	*	*	*	*
Year (Y)										
2006	14 ± 2	17 ± 2	14 ± 1	10 ± 1	41 ± 4	58 ± 5	53 ± 3	71 ± 3	138 ± 7	167 ± 5
2007	13 ± 1	18 ± 2	7 ± 1	9 ± 1	46 ± 4	39 ± 2	49 ± 2	62 ± 3	124 ± 5	136 ± 4
Significance^y	NS	NS	*	NS	NS	*	NS	*	*	*
Interactions^y										
C × M	*	NS	NS	NS	NS	NS	NS	*	NS	NS
C × Y	NS	*	*	*	*	*	NS	*	NS	*
M × Y	*	*	*	NS	*	NS	*	*	*	*
C × M × Y	NS	NS	NS	NS	NS	NS	NS	*	NS	*

[z] Means with different letters within each column and source of variation are significantly different at $P \leq 0.05$ as determined by Tukey's honestly significant difference. Progoitrin (PRO); Gluconapoleiferin (GF); Gluconapin (GNP); Glucobrassicanapin (GBN); Total (TTL).

[y] Mean separations on main effects were not performed as a result of the presence of significant interactions.

Significance: NS, *Nonsignificant or significant at $P \leq 0.05$.

FW = fresh weight.

Table 4. Glucosinolate concentrations of turnip (*Brassica rapa* subsp. *rapa*) roots in May and August planting dates in 2006–2007 across cultivars, plastic mulch treatments, and years.

	Glucosinolate concn (μmol/100 g FW)													
	PRO		GF		GNP		GBN		1MGB		GNS		TTL	
Source of variation^z	May	August	May	August	May	August	May	August	May	August	May	August	May	August
Cultivar (C)														
Just Right	11 ± 1	6 ± 1	2 ± 0	0 ± 0	63 ± 3	62 ± 2	23 ± 1	21 ± 0	7 ± 2	2 ± 2	47 ± 1	49 ± 0	154 ± 5	141 ± 4
Purple Top	49 ± 3	47 ± 1	25 ± 1	21 ± 1	18 ± 2	23 ± 3	20 ± 1	55 ± 2	5 ± 2	1 ± 3	53 ± 0	42 ± 0	182 ± 5	198 ± 6
Royal Crown	46 ± 2	40 ± 1	24 ± 2	11 ± 1	10 ± 1	21 ± 2	20 ± 1	36 ± 1	6 ± 2	2 ± 3	65 ± 1	47 ± 0	185 ± 6	162 ± 6
Scarlet Queen	49 ± 2	43 ± 3	23 ± 3	22 ± 3	12 ± 1	27 ± 2	43 ± 2	72 ± 1	25 ± 2	7 ± 3	57 ± 1	71 ± 0	217 ± 7	252 ± 9
Significance^y	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Mulch (M)														
Bare soil	41 ± 4 b	34 ± 4	23 ± 3	14 ± 2	24 ± 4	37 ± 4	28 ± 2	48 ± 4	13 ± 2	4 ± 4 a	51 ± 2	55 ± 1	190 ± 8	198 ± 12 a
Blue	48 ± 4 a	34 ± 4	19 ± 3	10 ± 2	30 ± 5	29 ± 4	29 ± 2	45 ± 4	12 ± 2	3 ± 4 ab	58 ± 2	55 ± 0	206 ± 7	180 ± 10 b
Red	34 ± 4 b	34 ± 4	17 ± 3	15 ± 2	30 ± 6	33 ± 4	25 ± 2	45 ± 4	10 ± 3	3 ± 3 ab	47 ± 1	52 ± 0	171 ± 6	188 ± 10 ab
Silver	37 ± 3 b	36 ± 4	22 ± 3	14 ± 3	24 ± 4	33 ± 4	26 ± 2	47 ± 4	10 ± 3	2 ± 3 b	61 ± 1	51 ± 0	189 ± 9	190 ± 10 ab
White	35 ± 4 b	33 ± 4	16 ± 2	15 ± 3	22 ± 4	37 ± 4	25 ± 3	47 ± 4	9 ± 3	2 ± 4 ab	57 ± 2	53 ± 0	171 ± 10	194 ± 13 ab
Yellow	38 ± 4 b	32 ± 4	15 ± 2	14 ± 3	25 ± 4	32 ± 4	26 ± 2	43 ± 4	9 ± 2	3 ± 3 ab	61 ± 1	49 ± 1	181 ± 6	179 ± 11 b
Significance^y	*	NS	*	*	*	*	NS	NS	*	*	*	*	*	*
Year (Y)														
2006	32 ± 2	43 ± 2	25 ± 2	20 ± 2	25 ± 2	45 ± 2	23 ± 1	44 ± 2	13 ± 2	3 ± 2	57 ± 1	67 ± 0	185 ± 5	226 ± 6
2007	46 ± 2	25 ± 2	12 ± 1	8 ± 1	27 ± 3	23 ± 2	30 ± 2	48 ± 2	8 ± 1	2 ± 1	54 ± 1	38 ± 0	184 ± 4	149 ± 4
Significance^y	*	*	*	*	*	*	*	*	*	*	NS	*	NS	*
Interactions^y														
C × M	NS	NS	*	*	*	*	*	NS	NS	NS	NS	NS	NS	NS
C × Y	*	*	*	*	*	*	*	*	*	*	*	*	*	*
M × Y	NS	NS	*	*	*	*	*	NS	*	NS	*	*	*	NS
C × M × Y	NS	NS	*	*	*	*	NS	*	NS	NS	*	NS	*	NS

[z] Means with different letters within each column and source of variation are significantly different at $P \leq 0.05$ as determined by Tukey's honestly significant difference. Progoitrin (PRO); Gluconapoleiferin (GF); Gluconapin (GNP); Glucobrassicanapin (GBN); 1-methoxyglucobrassicin (1MGB); Gluconasturtiin (GNS); Total (TTL).

[y] Mean separations on main effects were not performed due to the presence of significant interactions.

Significance: NS, *Nonsignificant or significant at $P \leq 0.05$.

FW = fresh weight.

than SQ roots (2530 mol TTL/ha), suggesting these cultivar differences in GSL concentrations are not the result of a dilution effect. The fact that SQ, the red cultivar, had higher GSL concentrations than the white cultivar JR, whereas the only partially pigmented (purple crowned) PT and RC roots had intermediate concentrations agrees with previous work in which red- or purple-pigmented brassica varieties had higher GSL concentrations than green or white varieties (Rosen et al., 2005; Volden et al., 2009).

Influence of mulch on glucosinolates. Colored plastic mulches significantly influenced both total and individual GSL concentrations; however, mulch-dependent increases in GSL concentrations were not consistent across tissue types, cultivars, planting dates, and years of the study. In the May planting, M × Y interactions were significant for TTL and all individual shoot GSLs (Table 3). The M × Y interaction resulted from red and blue mulches yielding significantly more TTL (186 and 177 μmol/100 g FW, respectively) than the other mulches in 2006 but not 2007 (Fig. 3). Despite the significance of the M × Y interaction, blue mulch yielded the highest GNP in both years of the study in May plantings. In August plantings, mulch treatments increased TTL in SQ shoots compared with bare soil in both years of the study but did not significantly influence TTL in other cultivars across both years of the study (Fig. 4). Mulch was not a significant factor for any other GSLs in August-planted shoots (Table 3).

Mulches inconsistently influenced root GSLs across planting dates. In May plantings, mulches inconsistently influenced TTL and all individual GSLs except PRO across cultivars and years of the study (Table 4; Fig. 5). Blue mulch consistently yielded on average 30% more PRO in roots than the other mulch treatments across all cultivars and years of the study (Table 4). M × Y interactions were significant for all other individual GSLs in May-planted roots primarily as a result of significant differences between mulches being present for individual GSLs in 2006 but not 2007. In August plantings, bare soil control plots yielded the highest TTL in roots (198 μmol/100 g FW), whereas blue and yellow mulches consistently yielded the lowest TTL across cultivars (179 and 180 μmol/100 g FW, respectively; Table 4). Bare soil control plots also yielded the highest root 1MGB concentration (4 μmol/100 g FW) across cultivars in August plantings.

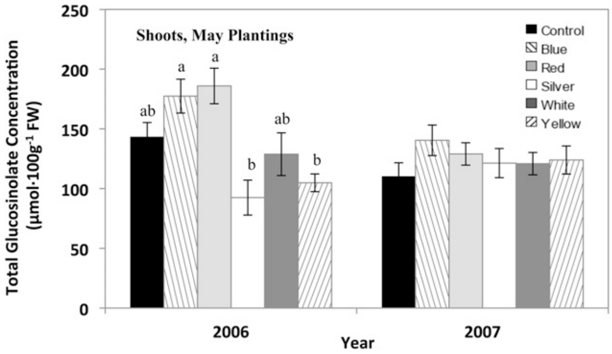

Fig. 3. The interactive effects of colored plastic mulch and year on total glucosinolate concentrations in May-planted shoots. Bars with different letters within each year are significantly different at $P \leq 0.05$ as determined by Tukey's honestly significant difference.

Relationship among glucosinolates, mulch, and climatic properties. In our study, mulch treatments altered soil temperatures (Fig. 2) and had significantly different relative light reflectance (Fig. 1). Blue mulch reflected the most blue light and the least red and far-red light. Red mulch reflected the most red and far-red light. Silver, white, and yellow mulches reflected higher amounts of green light than blue and red mulches. Although our mulch treatments varied in terms of their relative reflectance, we found no consistent relationship between the spectral properties of our mulch treatments and GSL concentrations, particularly GSL concentrations in root tissues. For example, $r = 0.02$ and $P > 0.05$ for reflected blue light and TTL. This is in contrast to results reported by Antonious et al. (1996) who attributed the increased GSL concentrations in their mulch treatments to the amount of blue light the mulches reflected. This contradiction between the two studies may be the result of differences in planting density and exposed mulch surface. Our trial used a spacing of 5 cm between plants, whereas Antonious et al. (1996) used a spacing of 30 cm between plants. It is possible that the lower plant density in their study resulted in different reflectance properties by the mulch into the crop canopy.

Antonious et al. (1996) suggested that changes in total GSL concentrations observed in turnip roots grown with colored plastic mulches were mainly the result of changes in reflected light provided by mulches rather than soil temperature because

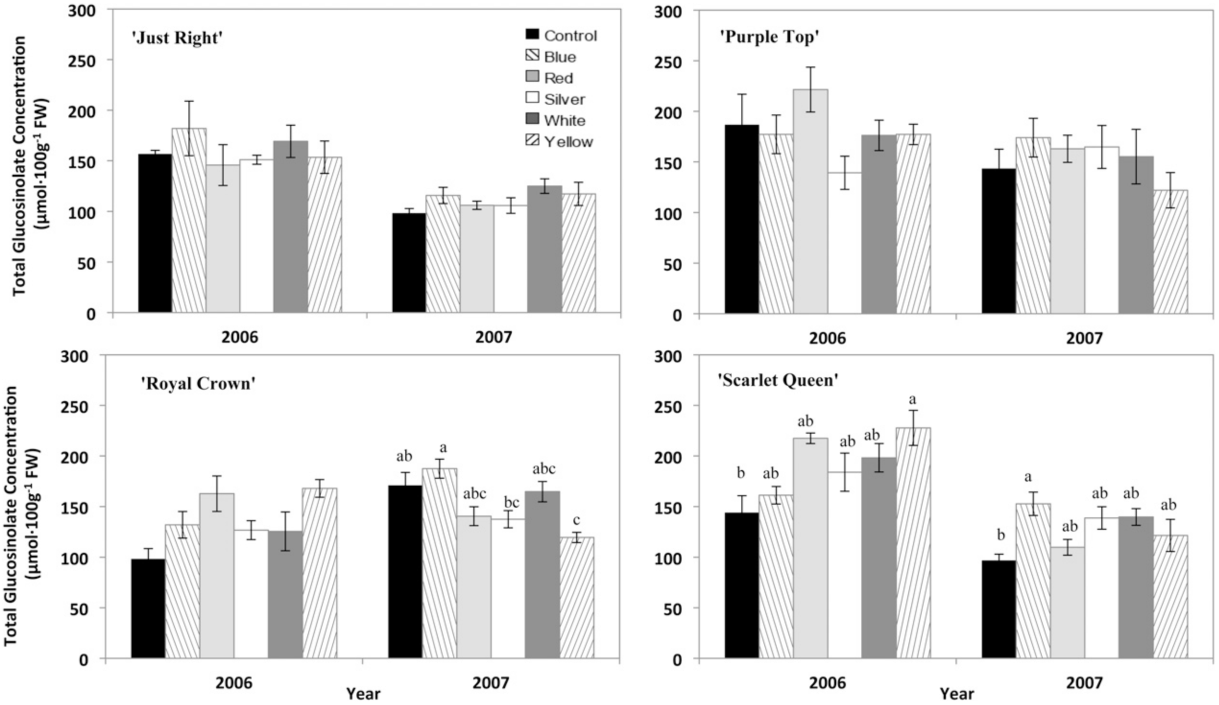

Fig. 4. The interactive effects of colored plastic mulch, cultivar, and year on total glucosinolate concentrations in turnip shoots grown in August plantings. Bars with different letters within each cultivar and year are significantly different at $P \leq 0.05$ as determined by Tukey's honestly significant difference.

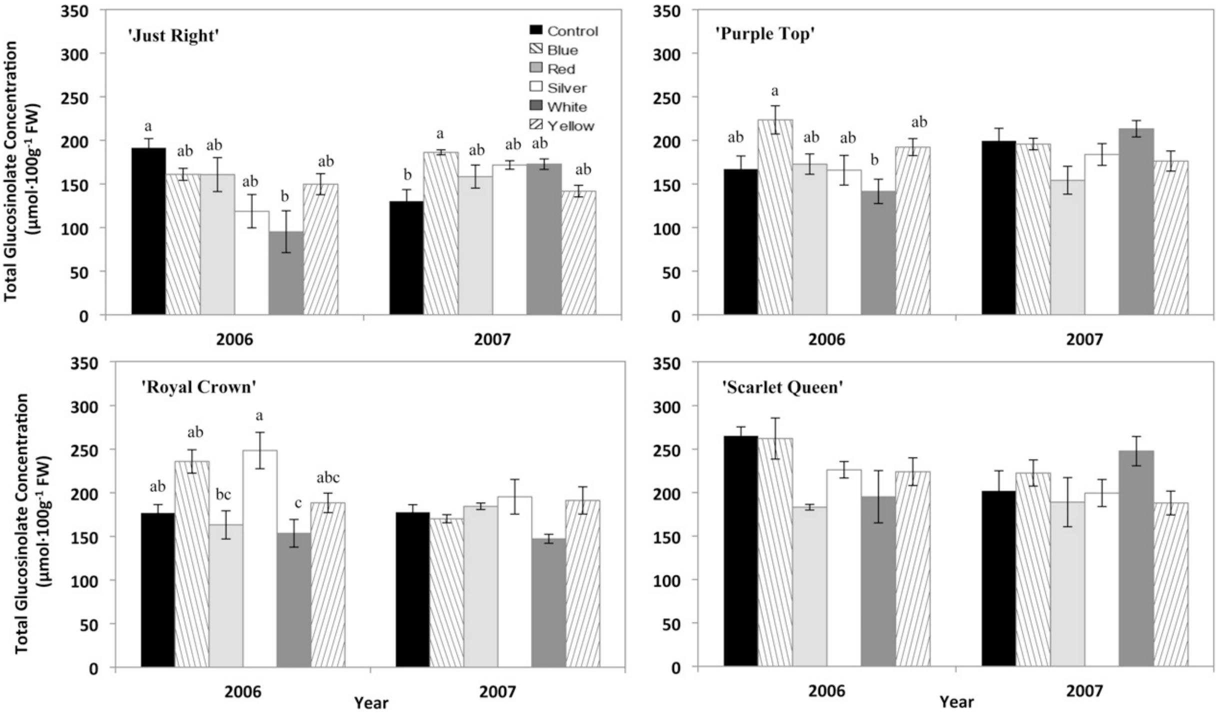

Fig. 5. The interactive effects of colored plastic mulch, cultivar, and year on total glucosinolate concentrations in turnip roots grown in May plantings. Bars with different letters within each cultivar and year are significantly different at $P \leq 0.05$ as determined by Tukey's honestly significant difference.

the range of soil temperatures between the mulch treatments in their study was less than 2 °C. In our study, the range between mean soil temperatures of all mulch treatments within each planting date was ≈8 °C (Fig. 2). Blue and red mulches had the highest mean soil temperatures in most planting dates of the study (Fig. 2). In May plantings, blue and red mulches were 4–6 °C warmer than bare soil treatments. This soil temperature difference was mitigated in August plantings when soil temperatures under blue and red mulch treatments were only 1–4 °C warmer than bare soil treatments. Indeed, there was a slight positive and significant correlation between soil temperature and TTL in roots ($r = 0.17$, $P < 0.001$). The positive relationship between soil temperature and TTL we observed is in agreement with previous research in which elevated temperatures are associated with increases in GSL concentrations in *Brassica oleracea* (Charron et al., 2005; Charron and Sams, 2004).

Although we did not observe consistent differences in GSL concentrations between our mulch treatments, we did observe large differences in GSL concentrations between planting dates and years of our study. Seasonal differences in GSL accumulation have been positively associated with mean air temperatures and solar radiation, particularly within the 2 weeks before harvest (Charron and Sams, 2004). In our study, mean air temperatures, growing degree-days, and solar radiation levels were higher in May plantings than August plantings, particularly during the 10 d before harvest. The highest mean GSL concentrations we observed were in the Aug. 2006 planting (Tables 3 and 4), which had lower mean air temperatures and solar radiation levels than the May planting, particularly near harvest (Table 1). Indeed, a small, negative correlation existed between mean maximum air temperature and shoot TTL ($r = –0.15$ and $r = –0.21$, $P < 0.001$, for harvest and growing season, respectively). Thus, our results do not agree with those of Charron and Sams (2004) who observed a positive relationship between GSL concentration and temperature and GSL concentration and photoperiod, particularly right before harvest. The discrepancy between our data and that of Charron and Sams (2004) may be the result of differences in genotype and environment, because they observed the positive relationship between GSLs and temperature in *B. oleracea* that was grown in a controlled environment.

The presence of significant interactive effects among cultivars, plastic mulch treatments, and years in influencing GSL concentrations across planting dates suggests that mulch treatments alter GSL levels in a cultivar and climate-dependent manner. Thus, using colored plastic mulches to affect any phytochrome or cryptochrome-mediated response associated with increased phytonutrient properties of turnips may not give consistent results. The inconsistency of mulch-dependent GSL enhancement may be the result of interactions among temperature, solar radiation, and mulch properties because these factors individually had a small but significant influence on GSL concentrations. Because of these interactions, we conclude that colored plastic mulches do not provide a sufficient effect to overcome the influence of climatic factors on GSL concentrations in turnip roots and shoots.

Literature Cited

Antonious, G., M. Kasperbauer, and M. Byers. 1996. Light reflected from colored mulches to growing turnip leaves affects glucosinolate and sugar contents of edible roots. Photochem. Photobiol. 64:605–610.

Atkinson, C., P. Dodds, Y. Ford, J. Le Miere, J. Taylor, P. Blake, and N. Paul. 2006. Effects of cultivar fruit number and reflected photosynthetically active radiation on *Fragaria × ananassa* productivity and fruit ellagic acid and ascorbic acid concentrations. Ann. Bot. (Lond.) 97:429–441.

Bones, A.M. and J.T. Rossiter. 1996. The myrosinase-glucosinolate system: Its organisation and biochemistry. Physiol. Plant. 97:194–208.

Carlson, D., M. Daxenbichler, H. Tookey, W. Kwolek, C. Hill, and P. Williams. 1987. Glucosinolates in turnip tops and roots—Cultivars grown for greens and or roots. J. Amer. Soc. Hort. Sci. 112:179–183.

Charron, C., A. Saxton, and C. Sams. 2005. Relationship of climate and genotype to seasonal variation in the glucosinolate–myrosinase system. I. Glucosinolate content in ten cultivars of *Brassica oleracea* grown in fall and spring seasons. J. Sci. Food Agr. 85:671–681.

Charron, C.S. and C.E. Sams. 2004. Glucosinolate content and myrosinase activity in rapid-cycling *Brassica oleracea* grown in a controlled environment. J. Amer. Soc. Hort. Sci. 129:321–330.

Engelen-Eigles, G., G. Holden, J.D. Cohen, and G. Gardner. 2006. The effect of temperature photoperiod and light quality on gluconasturtiin concentration in watercress (*Nasturtium officinale* R. Br.). J. Agr. Food Chem. 54:328–334.

EU. 1990. Off. J Eur. Commun. L 170:03.07. p. 27–34.

Hecht, S., S. Carmella, P. Kenney, S. Low, K. Arakawa, and M. Yu. 2004. Effects of cruciferous vegetable consumption on urinary metabolites of the tobacco-specific lung carcinogen 4-(methyinitrosamino)-1-(3-pyridyl)-1-butanone in Singapore Chinese. Cancer Epidemiol. Biomarkers Prev. 13:997–1004.

Hirai, M.Y., K. Sugiyama, Y. Sawada, T. Tohge, T. Obayashi, A. Suzuki, R. Araki, N. Sakurai, H. Suzuki, K. Aoki, H. Goda, O.I. Nishizawa, D. Shibata, and K. Saito. 2007. Omics-based identification of Arabidopsis Myb transcription factors regulating aliphatic glucosinolate biosynthesis. Proc. Natl. Acad. Sci. USA 104:6478–6483.

Kasperbauer, M. 1992. Phytochrome regulation of morphogenesis in green plants - from the Beltsville spectrograph to colored mulch in the field. Photochem. Photobiol. 56:823–832.

Loughrin, J. and M. Kasperbauer. 2001. Light reflected from colored mulches affects aroma and phenol content of sweet basil (*Ocimum basilicum* L.) leaves. J. Agr. Food Chem. 49: 1331–1335.

Mithen, R.F., B.G. Lewis, R.K. Heaney, and G. Fenwick. 1987. Glucosinolates of wild and cultivated Brassica species. Phytochemistry 26:1969–1973.

Neave, A.S., S.M. Sarup, M. Seidelin, F. Duus, and O. Vang. 2005. Characterization of the N-methoxyindole-3-carbinol (NI3C)-induced cell cycle arrest in human colon cancer cell lines. Toxicol. Sci. 83:126–135.

Pham, N., J.W. Jacobberger, A.D. Schimmer, P. Cao, M. Gronda, and D.W. Hedley. 2004. The dietary isothiocyanate sulforaphane targets pathways of apoptosis cell cycle arrest and oxidative stress in human pancreatic cancer cells and inhibits tumor growth in severe combined immunodeficient mice. Mol. Cancer Ther. 3:1239–1248.

Plate, A.Y. and D.D. Gallaher. 2006. Effects of Indole-3-Carbinol and phenethyl isothiocyanate on colon carcinogenesis induced by azoxymethane in rats. Carcinogenesis 27:287–292.

Rosen, C., V. Fritz, G. Gardner, S. Hecht, S. Carmella, and P. Kenney. 2005. Cabbage yield and glucosinolate concentrations as affected by nitrogen and sulfur fertility. HortScience 40: 1493–1498.

Smetanska, I., A. Krumbein, M. Schreiner, and D. Knorr. 2007. Influence of salicylic acid and methyl jasmonate on glucosinolate levels in turnip. J. Hort. Sci. Biotechnol. 82:690–694.

van Dam, N., T. Tytgat, and J. Kirkegaard. 2009. Root and shoot glucosinolates: A comparison of their diversity function and interactions in natural and managed ecosystems. Phytochem. Rev. 8:171–186.

Volden, J., G. Borge, M. Hansen, T. Wicklund, and G. Bengtsson. 2009. Processing (blanching boiling steaming) effects on the content of glucosinolates and antioxidant-related parameters in cauliflower (*Brassica oleracea* L ssp. *botrytis*). LWT-Food. Sci. Tech. (Paris) 42:63–73.

Zhang, H., I. Schonhof, A. Krumbein, B. Gutezeit, L. Li, H. Stützel, and M. Schreiner. 2008. Water supply and growing season influence glucosinolate concentration and composition in turnip root (*Brassica rapa* ssp. *rapifera* L). J. Plant Nutr. Soil Sci. 171:255–265.