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ARTICLE

Variation of Glucosinolates in Wild Radish (*Raphanus raphanistrum*) Accessions

Mayank S. Malik,[†] Melissa B. Riley,*[,‡] Jason K. Norsworthy,[§] and William Bridges Jr.[∥]

[†]TDRA, Scott Learning Center, Monsanto Company, Scott, Mississippi 38772, United States, [‡]Department of Entomology, Soils, and Plant Sciences, Clemson University, Clemson, South Carolina 29634, United States, [§]Department of Crops, Soils, and Environmental Sciences, University of Arkansas, Fayetteville, Arkansas 72704, United States, and [∥]Department of Applied Economics and Statistics, Clemson University, Clemson, South Carolina 29634, United States

Glucosinolate composition was determined in wild radish accessions from eight states in the northeastern and southern United States to determine the variability of production among accessions. Glucosinolates were evaluated from roots, leaves, flowers, primary, and secondary branches. Seventeen glucosinolates were identified, with glucoerucin, glucoraphenin, glucobrassicin, and gluconasturtiin contributing 90% to 100% of the total glucosinolates. Flowers contained the highest glucosinolate concentrations, 12.07 to 55.36 μmol/g, but flowers contributed only 5.3 to 21.3% to the total glucosinolates. Of the eight accessions, the Mississippi accession produced significantly higher levels of total glucosinolates and glucosinolates which can be degraded to isothiocyanates per plant, totals of 618.97 and 563.53 μmol/plant, respectively. Total plant biomass did not differ between accessions indicating a difference in the ability of the Mississippi accession to produce glucosinolates. Further studies are needed to determine if this accession would consistently produce higher glucosinolate levels under different environmental conditions.

KEYWORDS: Biofumigation; allelopathy; *Raphanus*; wild radish; glucosinolates; isothiocyanates

INTRODUCTION

The biofumigation potential of various Brassicaceae plants for weed management has been investigated (*1*−*5*). Brassicaceae plants incorporated into soil as green manure have led to a reduction of weed emergence in the subsequent crop, but in many cases the green manure incorporation did not provide season-long weed control (*6*−*8*). Incorporation of the Brassicaceae wild radish (*Raphanus raphanistrum* L.), in combination with lower-than-recommended rates of herbicide, resulted in successful weed control in sweet corn (*9*).

Wild radish, a facultative winter annual broadleaf that emerges throughout the year in the southeastern United States (*10*), showed biofumigation potential when aqueous extracts and soil-incorporated air-dried biomass were tested in controlled environments (*11*) Plants tested for sensitivity to the wild radish residues included the crop species cotton (*Gossypium hisutum* L.), corn (*Zea mays* L.), and wheat (*Triticum aestivum* L.); and weed species pitted morningglory (*Ipomoea lacunosa* L.), sicklepod [*Senna obtusifolia* (L.) H. S. Irwin & Barneby], prickly sida (*Sida spinosa* L.), and yellow nutsedge (*Cyperus esculentus* L.). Cotton, sicklepod, and prickly sida were sensitive to wild radish incorporation in soil while yellow nutsedge rhizome production was decreased and morningglory emergence decreased linearly with increases in the percentage of wild radish amendment. In greenhouse studies of the inhibition of yellow nutsedge in wild radish-amended soil (1% w/w), marginal necrosis of leaf margins was observed in both bell pepper (*Capsicum annuum* L.) and tomato (*Lycopersicum esculentum* Mill.) after transplanting into amended soil. Three weeks after transplanting no necrosis was observed with tomato. Bell pepper continued to show a response to the amendments and did not accumulate as much biomass over the 9-week study when compared to those grown in non-amended soil (*12*). The biomass of yellow nutsedge tubers was reduced from 0.32 g/tuber to 0.05 g/tuber in the wild radish-amended soil resulting in a competitive edge for bell pepper over yellow nutsedge, even with the decrease in its biomass (*12*). Subsequent field studies incorporating flowering wild radish biomass into soil along with half the recommended rates of atrazine and *S*-metolachlor resulted in season-long weed control of Florida pusley (*Richardia scabra* L.) and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] in sweet corn (*Zea mays* L.), indicating the potential for using wild radish as a biofumigant (*9*).

The biofumigation potential of Brassicaceae plants, including wild radish, has been linked to the ability of plants to produce glucosinolates. Glucosinolates are sulfur-containing secondary metabolites commonly found in Brassicaceae plants. They are degraded by myrosinase (thioglucoside glucohydrolase) in the presence of water to produce glucose and unstable aglucones, which are broken down into various compounds including isothiocyanates, organic thiocyanates, and nitriles, all of which are active in biofumigation (*13*) (**Figure 1**). In plants, the action of

*Corresponding author. Tel: (864) 656-0580. Fax: (864) 656-0274. E-mail: mbriley@clemson.edu.

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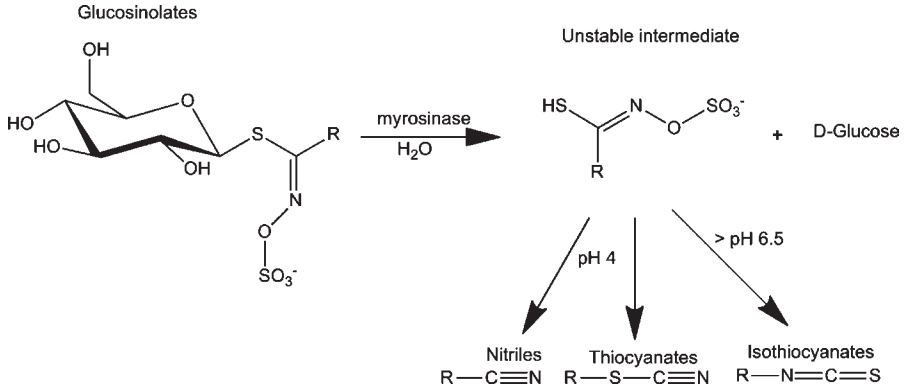
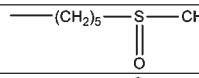
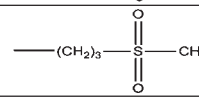
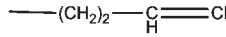
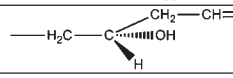
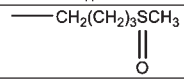
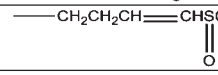
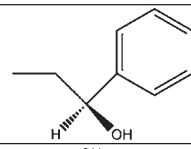
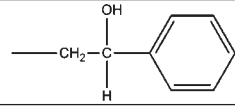
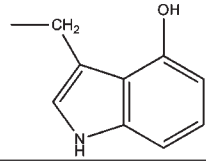
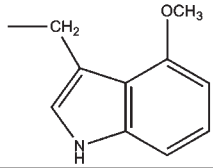
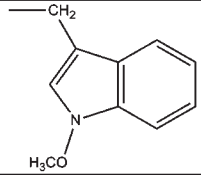


Figure 1. Toxic compounds normally produced during breakdown of glucosinolates by myrosinase (*13*, *15*).

Table 1. Common Names and R-Groups of Glucosinolates Used as Standards during High Performance Liquid Chromatography or Identified in Wild Radish in Addition to Those Previously Identified (*24*), along with Degradation Products Obtained Following Myrosinase Activity[a]

Common Name	R-group	R group structure	Degradation Products
Aliphatic			
Glucoalyssin	5-(Methylsulfinyl)pentyl		isothiocyanates
Glucocheirolin	3-Methylsulfonyl)propyl		Isothiocyanates
Gluconapin	But-3-enyl		Isothiocyanates
Gluconapolieferin	2-Hydroxy-4-pentenyl		Oxazolidine-2-thiones
Glucoraphanin	4-(Methylsulfinyl)butyl		Isothiocyanates, nitriles
Glucoraphenin	4-Methylsulfinyl-3-butenyl		Isothiocyanates
Aromatic			
Glucobarbarin	2-hydroxy-2-phenylethyl		Oxazolidine-2-thione
Glucosibarin	2-hydroxy-2-phenylethyl		Isothiocyanates, 5-phenyl oxazolidine-2-thione
Indole			
4-Hydroxyglucobrassicin	4-Hydroxyindol-3-ylmethyl		Indolyl-3-carbitol Thiocyanate
4-Methoxyglucobrassicin	4-Methoxyindol-3-ylmethyl		Indolyl-3-carbitol Thiocyanate
Neoglucobrassicin	1-Methoxy-3-indolylmethyl		Indolyl-3-carbitol Thiocyanate

[a] Compounds are grouped according to their major chemical class. Chemical classes and degradation products are based on previous reports (*13*, *16*).

Table 2. Comparison of Total Biomass, Total Micromoles of Glucosinolates and Total Micromoles of Isothiocyanates per Plant in Accessions of Wild Radish[a]

accession	total biomass (g)	total μmol of glucosinolates	total μmol of isothiocyanates
Alabama	18.19 ± 1.54 a	391.09 ± 74.92 ab	372.46 ± 68.73 ab
Florida	17.83 ± 1.54 a	237.48 ± 72.37 bc	215.07 ± 66.38 bc
Georgia	17.00 ± 1.26 a	227.81 ± 66.66 bc	204.54 ± 61.00 bc
Maine	15.01 ± 1.26 a	403.26 ± 66.66 ab	383.34 ± 61.00 ab
Mississippi	17.55 ± 1.38 a	618.97 ± 69.33 a	563.53 ± 63.52 a
New York	14.99 ± 1.54 a	331.80 ± 74.92 bc	311.86 ± 68.73 bc
North Carolina	16.70 ± 1.38 a	107.20 ± 69.34 c	105.86 ± 63.52 c
SC-Barnwell	14.60 ± 1.26 a	195.83 ± 66.66 ab	151.40 ± 61.00 c
SC-Colleton	14.96 ± 1.26 a	294.59 ± 66.66 bc	277.38 ± 61.00 bc
SC-Orangeburg	16.02 ± 1.38 a	410.32 ± 69.34 ab	357.67 ± 63.52 b

[a] Values are the average of 3 replicates in each of two experiments followed by standard error. Values followed by the same letter are not significantly different based on Fisher's protected LSD test with a 5% level of significance.

myrosinase on glucosinolates has been suggested to be regulated by the separation of the enzyme from glucosinolates (*14*). Tissue disruption results in the breakdown of this separation (*15*). Products produced by the myrosinase activity on glucosinolates are dependent on the R group (**Figure 1**). Variation of the identified R groups has resulted in the identification of approximately 120 different glucosinolates, belonging to several chemical classes, including aliphatic, aromatic, and heterocyclic indoles containing either straight or branched chain carbons (*16*).

Isothiocyanates from glucosinolate breakdown are toxic to many organisms (*13*). Isothiocyanates are volatile and unstable in soils with maximum levels being observed 30 h after tissue incorporation followed by a drop to approximately 75% within 72 h (*17*). Levels after 72 h (3 nmol/g), however, remained relatively stable through 20 days. With rapeseed meal-amended soils isothiocyanates were rapidly lost, but ionic thiocyanates persisted longer (*18*). The release of isothiocyanates in soil varies according to soil temperature, moisture, and amount of glucosinolates released by plant tissue (*19*).

Isothiocyanates and other degradation products of glucosinolates have been suggested to interact with enzymes responsible for glycolysis and respiration, resulting in inhibition of seed germination (*11*, *20*−*22*). *N*-Butyl and 2-phenethyl isothiocyanates when incorporated into soil inhibited the germination of smooth pigweed (*Amaranthus hybridus* L.) (*22*).

The production of glucosinolates varies considerably within species (*23*). A study of wild radish showed that the greatest glucosinolate levels of wild radish were obtained at the 50% flowering stage (*24*). The objectives of this study were to compare glucosinolate content among different accessions of wild radish at flowering stage, to determine the concentrations of glucosinolates that are converted to isothiocyanates, and to identify accessions that provide better weed management options because of their higher levels of glucosinolates and ultimately isothiocyanate production.

MATERIALS AND METHODS

Seeds of wild radish were collected in 2003 from ten locations across eight states in the northeastern and southern United States including Alabama (Lee County), Florida (Jackson County), Georgia (Tift County), Maine (Penobscot County), Mississippi (Pearl River County), New York (Tompkins County), North Carolina (Sampson County), and South Carolina (Barnwell, Colleton, and Orangeburg Counties). Seeds were dried and stored at 4 °C until used in experiments. Approximately 10 to 15 seeds were planted in 10 cm diameter pots containing a commercial peat moss mix from Farfard Inc. (Anderson, SC), and pots were placed in a growth chamber maintained with a 13 h daylength at 23 °C followed by 8 °C during the dark phase (11 h). When wild radish seedlings reached the 2- to 3-leaf stage, they were thinned to one plant per pot. Three plants were used for determination of glucosinolates for each accession in each of two experiments. Plants were watered three times a week with 0.4% w/v solution of Scotts Miracle Gro fertilizer (Marysville, OH) containing 24% N, 8% P, and 16% K. Plants were harvested at the 50% flowering stage, which corresponds to the maximal level of glucosinolates based on previous research (*24*). Plants were separated into different parts including roots, leaves, flowers, primary branches, and secondary branches. Samples were freeze-dried for 10−14 days. The biomass of each sample component was determined prior to grinding samples to a fine powder that would pass through a 1 mm screen for glucosinolate extraction. Ground samples were stored at 4 °C in sealed plastic bags until analyzed for glucosinolate content.

Glucosinolate Extraction and Analysis. Glucosinolate extraction from plant samples and analysis on high performance liquid chromatography were conducted as described previously (*24*). Glucotropaeolin and sinigrin (1.2 μmol each) were added to one of the three samples, sinigrin (1.2 μmol) was added to a second sample, and the third sample had no surrogates added. Sinigrin was used to correct for recovery of glucosinolates. Glucosinolates were identified by comparison to previously reported HPLC results (*1*, *25*) and by comparison with standards. Glucosinolate standards glucocheirolin, glucoiberin, glucoraphanin, glucoraphenin, glucosibarin, glucobarbarin, and glucoerucin were obtained from KVL (Copenhagen, Denmark). Glucotropaeolin and sinigrin were obtained from Sigma-Aldrich (St. Louis, MO). R-groups, common names, and degradation products of the glucosinolates associated with this study which were not previously reported (*24*) are shown in **Table 1**.

Statistical Analysis. The analysis was based on a randomized complete block design with accession as the treatment factor and the two repeated experiments as blocks. Plant samples were replicated three times within each experiment. Each treatment contained the ground material of wild radish from one accession. Each accession was replicated into three samples with glucotropaeolin and sinigrin added as surrogate to one sample, second sample contained sinigrin as surrogate, and third sample was analyzed alone without any surrogate.

An ANOVA for a randomized complete block design was used to determine the effect of treatment on biomass and total glucosinolates; and the analysis was performed separately on biomass and total glucosinolates for each plant part. If there was a significant treatment effect, Fisher's protected LSD test was used to make comparisons among the treatments and determine specific differences among the treatments. Cluster analysis was also performed to group the treatments on the total array of glucosinolate concentrations. All statistical calculations were performed using SAS and JMP (SAS Institute Inc., Cary, NC), and significance tests were preformed at the 5% level of significance.

RESULTS AND DISCUSSION

Total Glucosinolate Production. The total glucosinolate production varied among the 10 wild radish accessions (**Table 2**). The total concentration of glucosinolates per plant ranged from a low of 107.2 μmol/plant in the North Carolina accession to a high of 618.97 μmol/plant in the Mississippi accession. Total biomass production among the accessions did not differ, indicating that the differences in total glucosinolates are not attributed to differences in total biomass production but are due to differences in production of glucosinolates. The differences are also not due to environmental differences since the accessions were all grown under the same environmental conditions in growth chambers. The accessions from Mississippi, Alabama, Maine, SC-Barnwell and SC-Orangeburg fell into the group with the highest total glucosinolate concentration per plant. This variability in total glucosinolate production would seem to indicate that there are genetic differences between the accessions of wild radish in their ability to produce glucosinolates. These differences do not appear to be based on geographic origin of the accessions since the accessions with the highest levels of glucosinolates were scattered across the eastern United States.

Table 3. Total Amounts of Major and Minor Glucosinolates in Different Plant Tissues of Accessions Collected in Different States in the Northeastern and Southern United States

	μmol					
accession[a]	glucoerucin	glucoraphenin	glucobrassicin	gluconasturtiin	other glucosinolates[b]	total
			Roots			
AL	11.91 ± 2.69 bcd[c]	1.87 ± 0.33 d	0.31 ± 0.06 b	0.91 ± 0.14 ab	0.02 ± 0.01 c	15.03 ± 2.98 def
FL	7.52 ± 0.56 de	2.32 ± 1.31 d	0.86 ± 0.42 b	0.45 ± 0.09 de	0.06 ± 0.02 c	11.20 ± 1.73 f
GA	9.55 ± 1.24 cde	1.53 ± 0.2 d	0.42 ± 0.04 b	1.00 ± 0.09 a	0.13 ± 0.02 c	12.63 ± 1.52 ef
ME	19.79 ± 3.32 ab	1.62 ± 0.29 d	ND[d] b	0.38 ± 0.06 de	0.09 ± 0.02 c	21.87 ± 3.60 cde
MS	22.40 ± 3.86 a	7.65 ± 1.23 a	0.29 ± 0.13 b	0.83 ± 0.10 abc	0.82 ± 0.10 b	31.98 ± 5.26 ab
NY	17.88 ± 2.04 ab	5.25 ± 0.66 bc	0.46 ± 0.06 b	0.60 ± 0.11 bcd	1.08 ± 0.26 ab	25.27 ± 2.58 bc
NC	2.01 ± 0.25 e	5.01 ± 0.67 bc	0.18 ± 0.04 b	0.09 ± 0.02 e	0.11 ± 0.03 c	7.41 ± 0.86 f
SC-BW	17.27 ± 2.77 abc	2.26 ± 0.12 d	0.37 ± 0.08 b	0.99 ± 0.19 a	0.02 ± 0.02 c	20.92 ± 3.06 cde
SC-CL	17.13 ± 4.17 abc	3.12 ± 0.93 cd	1.45 ± 0.47 b	0.51 ± 0.17 cd	0.25 ± 0.08 c	22.46 ± 4.42 bcd
SC-OB	24.82 ± 2.45 a	5.59 ± 0.96 ab	3.95 ± 1.62 a	1.01 ± 0.19 a	1.19 ± 0.18 a	36.56 ± 3.68 a
			Leaves			
AL	49.85 ± 5.01 a	13.86 ± 2.32 cde	5.08 ± 0.38 bc	1.48 ± 0.14 b	1.68 ± 0.53 b	71.95 ± 7.31 a
FL	5.07 ± 1.79 c	10.06 ± 2.58 de	1.92 ± 0.94 def	0.82 ± 0.25 bcd	1.15 ± 0.23 b	19.03 ± 5.19 cde
GA	5.69 ± 0.77 c	6.67 ± 0.65 e	1.03 ± 0.17 ef	0.47 ± 0.03 cd	0.59 ± 0.07 b	14.46 ± 1.44 e
ME	9.46 ± 2.07 c	23.96 ± 5.60 abc	1.66 ± 0.34 ef	0.55 ± 0.15 cd	1.55 ± 0.26 b	37.19 ± 7.78 bcd
MS	13.08 ± 1.77 c	16.95 ± 2.56 bcde	1.62 ± 0.09 ef	1.07 ± 0.09 bc	1.52 ± 0.15 b	34.24 ± 4.28 bcde
NY	7.21 ± 1.27 c	34.01 ± 8.99 a	2.82 ± 0.19 cde	0.98 ± 0.17 bc	4.27 ± 1.55 a	49.29 ± 11.75 b
NC	3.47 ± 0.44 c	11.30 ± 1.41 de	0.19 ± 0.04 f	0.11 ± 0.04 d	1.78 ± 0.30 b	16.86 ± 1.59 de
SC-BW	9.99 ± 2.01 c	20.17 ± 5.34 bcd	5.75 ± 1.25 ab	3.19 ± 0.71 a	0.90 ± 0.19 b	40.01 ± 9.37 bc
SC-CL	26.71 ± 10.22 b	14.92 ± 2.14 cde	4.13 ± 1.24 bcd	0.51 ± 0.16 cd	1.05 ± 0.51 b	47.33 ± 13.82 b
SC-OB	37.19 ± 2.35 b	28.45 ± 2.34 ab	7.64 ± 1.71 a	1.52 ± 0.09 b	4.56 ± 0.49 a	79.36 ± 6.09 a
			Flowers			
AL	9.19 ± 1.97 cde	9.96 ± 1.88 c	0.42 ± 0.08 d	0.06 ± 0.02 cd	0.67 ± 0.13 c	20.31 ± 4.03 cd
FL	8.89 ± 2.39 cde	9.37 ± 1.87 c	2.54 ± 1.05 ab	0.32 ± 0.12 abc	1.57 ± 0.43 bc	22.70 ± 3.60 bcd
GA	14.74 ± 3.77 abc	30.45 ± 7.64 a	2.01 ± 0.50 abc	0.42 ± 0.09 ab	0.99 ± 0.37 c	48.61 ± 12.16 a
ME	12.61 ± 3.48 bcd	29.06 ± 5.52 ab	1.44 ± 0.38 bcd	0.17 ± 0.05 bcd	1.70 ± 0.65 bc	44.98 ± 9.85 a
MS	22.11 ± 3.52 a	19.56 ± 4.69 abc	2.28 ± 0.56 ab	0.58 ± 0.26 a	3.18 ± 0.60 abc	47.71 ± 9.40 a
NY	6.65 ± 1.21 de	31.22 ± 7.07 a	1.22 ± 0.21 bcd	0.11 ± 0.02 cd	4.63 ± 1.29 ab	43.83 ± 8.89 ab
NC	1.68 ± 0.27 e	10.85 ± 1.46 c	0.04 ± 0.01 d	ND d	0.76 ± 0.07 c	13.34 ± 1.72 d
SC-BW	5.77 ± 1.37 de	13.14 ± 2.41 c	2.26 ± 0.52 abc	0.16 ± 0.03 bcd	0.85 ± 0.19 c	22.17 ± 4.28 bcd
SC-CL	10.65 ± 3.09 bcd	17.28 ± 4.32 bc	0.71 ± 0.22 cd	0.20 ± 0.08 bcd	1.08 ± 0.41 c	29.91 ± 7.91 abcd
SC-OB	17.23 ± 2.33 ab	12.60 ± 1.35 c	3.34 ± 0.93 a	0.33 ± 0.09 abc	5.80 ± 3.69 a	39.31 ± 6.62 abc
			Primary Branches			
AL	59.86 ± 14.27 cd	18.75 ± 2.66 d	4.33 ± 0.96 de	1.81 ± 0.42 cd	2.75 ± 0.61 bcd	87.50 ± 18.71 cd
FL	20.55 ± 6.35 e	36.37 ± 9.08 cd	4.54 ± 2.45 de	2.70 ± 0.73 bcd	3.48 ± 1.20 bcd	67.65 ± 15.81 d
GA	23.43 ± 3.52 de	27.87 ± 4.07 cd	8.24 ± 1.29 cd	5.10 ± 0.48 ab	2.72 ± 0.52 bcd	67.37 ± 8.05 d
ME	119.85 ± 29.50 b	34.79 ± 11.34 cd	10.65 ± 3.43 bc	5.23 ± 1.32 a	2.30 ± 0.78 cd	172.81 ± 45.43 b
MS	176.24 ± 15.62 a	70.42 ± 7.44 a	18.10 ± 1.28 a	4.90 ± 0.54 ab	6.51 ± 0.47 b	276.17 ± 13.17 a
NY	22.70 ± 6.53 de	62.03 ± 14.83 ab	5.60 ± 1.41 cde	2.22 ± 0.53 cd	3.41 ± 1.03 bcd	95.96 ± 22.16 cd
NC	14.95 ± 2.14 e	43.22 ± 12.41 bc	0.58 ± 0.19 e	0.33 ± 0.10 d	1.79 ± 0.69 cd	60.88 ± 13.36 d
SC-BW	19.45 ± 6.24 e	20.48 ± 4.06 cd	8.90 ± 0.74 cd	4.13 ± 1.79 abc	15.48 ± 3.44 a	68.43 ± 8.49 d
SC-CL	47.37 ± 15.96 cde	23.32 ± 5.57 cd	4.48 ± 1.49 de	1.59 ± 0.56 d	1.41 ± 0.59 d	78.17 ± 23.66 cd
SC-OB	78.89 ± 5.83 c	29.36 ± 3.20 cd	16.37 ± 3.71 ab	2.00 ± 0.40 cd	5.40 ± 1.06 bc	132.03 ± 12.51 bc
			Secondary Branches			
AL	153.69 ± 29.85 a	24.90 ± 3.68 bc	9.16 ± 1.71 bcd	2.60 ± 0.26 ab	2.82 ± 0.67 bcd	193.18 ± 35.75 ab
FL	20.02 ± 4.67 c	65.64 ± 17.89 a	11.11 ± 4.88 b	1.70 ± 0.45 ab	6.20 ± 1.02 b	104.67 ± 27.04 cd
GA	41.33 ± 4.25 bc	27.22 ± 2.28 bc	9.41 ± 1.40 bc	4.72 ± 0.72 a	2.08 ± 0.26 bcd	84.75 ± 8.45 cd
ME	79.03 ± 12.00 b	42.64 ± 10.97 ab	3.98 ± 1.52 cde	0.22 ± 0.22 b	0.53 ± 0.45 d	126.40 ± 23.39 bc
MS	127.53 ± 32.22 a	62.86 ± 12.08 a	11.55 ± 2.89 b	2.35 ± 0.51 ab	5.46 ± 1.33 bc	209.75 ± 47.79 a
NY	46.61 ± 11.76 bc	63.47 ± 16.08 a	6.57 ± 2.08 bcde	1.81 ± 0.60 ab	5.57 ± 1.00 bc	124.03 ± 28.67 bc
NC	3.89 ± 0.48 c	5.12 ± 0.30 c	0.19 ± 0.03 e	ND b	0.35 ± 0.08 d	9.55 ± 0.75 e
SC-BW	7.77 ± 2.25 c	16.19 ± 4.10 bc	2.90 ± 0.53 de	4.83 ± 3.11 a	12.61 ± 4.02 a	44.30 ± 11.58 de
SC-CL	79.21 ± 30.18 b	27.09 ± 4.34 bc	4.67 ± 1.51 cde	4.13 ± 0.37 a	1.63 ± 0.76 cd	116.72 ± 36.08 cd
SC-OB	70.94 ± 6.51 b	27.98 ± 2.26 bc	19.87 ± 2.11 a	2.51 ± 0.30 ab	4.49 ± 0.57 bcd	125.79 ± 10.96 bc

[a] AL, Alabama; FL, Florida; GA, Georgia; ME, Maine; MS,Mississippi; NY, New York; NC, North Carolina; SC-BW, South Carolina Barnwell County; SC-CL, South Carolina Colleton County; SC-OB, South Carolina Orangeburg County. [b] Other glucosinolates included glucocheirolin, glucoiberin, glucoraphanin, gluconapoleiferin, glucoalyssin, gluconapin, 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin, glucosibarin, glucobarbarin, glucotropaeolin, neoglucobrassicin, and sinigrin. [c] Values are the average of 3 replicates in each of two experiments followed by standard error. Values followed by the same letter are not significantly different based on Fisher's protected LSD test with a 5% level of significance between accessions for each plant part and glucosinolate. [d] ND = none detected.

Table 4. Concentration of Major and Minor Glucosinolates Glucosinolates in Different Plant Tissues of Accessions Collected in Different States in the Northeastern and Southern United States

	μmol/g dry weight					
accession[a]	glucoerucin	glucoraphenin	glucobrassicin	gluconasturtiin	other glucosinolates[b]	total
			Roots			
AL	8.95 ± 3.22 bcd[c]	1.33 ± 0.33 de	0.22 ± 0.06 c	0.66 ± 0.15 ab	0.02 ± 0.01 d	11.17 ± 3.68 cd
FL	4.85 ± 0.63 de	1.36 ± 0.5 de	0.57 ± 0.29 bc	0.31 ± 0.09 bc	0.04 ± 0.01 d	7.12 ± 0.92 d
GA	7.36 ± 1.21 cde	1.19 ± 0.27 e	0.32 ± 0.05 c	0.75 ± 0.04 a	0.10 ± 0.02 cd	9.71 ± 1.52 cd
ME	18.39 ± 3.72 a	1.54 ± 0.34 cde	ND[d] c	0.36 ± 0.08 bc	0.08 ± 0.02 cd	20.36 ± 4.10 ab
MS	16.84 ± 1.79 a	5.84 ± 0.76 a	0.23 ± 0.10 c	0.65 ± 0.10 ab	0.63 ± 0.07 b	24.18 ± 2.61 a
NY	15.88 ± 2.28 a	4.85 ± 1.04 ab	0.39 ± 0.04 c	0.50 ± 0.08 ab	0.89 ± 0.17 a	22.51 ± 3.21 ab
NC	1.40 ± 0.24 e	3.52 ± 0.74 bc	0.12 ± 0.03 c	0.06 ± 0.02 c	0.07 ± 0.01 cd	5.17 ± 0.97 d
SC-BW	13.48 ± 3.05 abc	1.69 ± 0.19 cde	0.29 ± 0.09 c	0.79 ± 0.22 a	0.02 ± 0.02 d	16.27 ± 3.53 bc
SC-CL	16.51 ± 2.89 a	3.33 ± 1.07 bcd	1.45 ± 0.38 ab	0.54 ± 0.19 ab	0.27 ± 0.11 c	22.11 ± 2.38 ab
SC-OB	14.92 ± 1.09 ab	3.44 ± 0.59 bc	2.33 ± 0.97 a	0.61 ± 0.11 ab	0.71 ± 0.09 ab	22.02 ± 1.78 ab
			Leaves			
AL	23.29 ± 2.02 a	6.63 ± 1.19 cd	2.39 ± 0.16 ab	0.70 ± 0.07 b	0.81 ± 0.25 b	33.82 ± 3.34 a
FL	2.37 ± 0.77 c	4.67 ± 1.08 d	0.87 ± 0.38 cd	0.37 ± 0.09 cd	0.54 ± 0.09 b	8.81 ± 2.12 e
GA	2.90 ± 0.30 c	3.60 ± 0.60 d	0.53 ± 0.07 cd	0.25 ± 0.02 de	0.32 ± 0.05 b	7.59 ± 0.88 e
ME	4.84 ± 0.78 c	12.09 ± 1.66 ab	0.87 ± 0.15 cd	0.26 ± 0.06 de	0.81 ± 0.11 b	18.89 ± 2.28 cd
MS	6.04 ± 0.46 c	7.76 ± 0.53 bcd	0.77 ± 0.06 cd	0.50 ± 0.03 bcd	0.72 ± 0.06 b	15.79 ± 0.74 de
NY	3.47 ± 0.47 c	15.72 ± 3.59 a	1.43 ± 0.08 bc	0.48 ± 0.07 bcd	1.96 ± 0.64 a	23.05 ± 4.48 bcd
NC	1.64 ± 0.17 c	5.31 ± 0.50 d	0.09 ± 0.02 d	0.05 ± 0.02 e	0.84 ± 0.12 b	7.93 ± 0.51 e
SC-BW	5.11 ± 0.67 c	10.38 ± 2.20 bc	2.91 ± 0.42 a	1.65 ± 0.28 a	0.46 ± 0.08 b	20.49 ± 3.60 bcd
SC-CL	14.84 ± 5.41 b	7.80 ± 1.30 bcd	2.24 ± 0.68 ab	0.27 ± 0.08 cde	0.55 ± 0.23 b	25.71 ± 7.46 abc
SC-OB	13.88 ± 0.42 b	10.55 ± 0.45 bc	2.77 ± 0.55 a	0.57 ± 0.02 bc	1.69 ± 0.12 a	29.46 ± 0.89 ab
			Flowers			
AL	12.39 ± 1.94 bcd	13.45 ± 1.74 de	0.58 ± 0.09 de	0.09 ± 0.02 e	0.90 ± 0.11 b	27.41 ± 3.80 cd
FL	10.56 ± 1.73 cde	11.70 ± 2.38 de	3.12 ± 1.20 a	0.40 ± 0.15 ab	1.93 ± 0.56 b	27.71 ± 2.53 cd
GA	12.08 ± 0.80 cde	25.26 ± 2.71 bc	1.66 ± 0.12 bcd	0.36 ± 0.01 abc	0.74 ± 0.12 b	40.10 ± 2.53 abc
ME	14.46 ± 1.90 bc	37.16 ± 5.38 a	1.62 ± 0.18 bcd	0.19 ± 0.03 cde	1.94 ± 0.44 b	55.36 ± 6.95 a
MS	21.82 ± 1.22 a	18.06 ± 2.06 cde	2.10 ± 0.27 abc	0.50 ± 0.14 a	3.11 ± 0.29 ab	45.59 ± 3.22 ab
NY	7.63 ± 0.95 de	35.48 ± 6.71 ab	1.42 ± 0.20 bcde	0.13 ± 0.02 de	5.08 ± 1.32 a	49.74 ± 7.96 ab
NC	1.50 ± 0.11 f	9.79 ± 0.76 e	0.04 ± 0.01 e	ND e	0.74 ± 0.12 b	12.07 ± 0.87 d
SC-BW	6.53 ± 1.01 ef	15.53 ± 1.95 cde	2.61 ± 0.48 ab	0.18 ± 0.02 cde	1.05 ± 0.23 b	25.90 ± 3.14 cd
SC-CL	13.68 ± 4.38 bc	21.76 ± 6.24 cd	0.87 ± 0.27 cde	0.20 ± 0.07 bcde	1.50 ± 0.60 b	38.00 ± 11.37 bc
SC-OB	17.93 ± 1.66 ab	13.59 ± 1.77 de	3.27 ± 0.64 a	0.32 ± 0.06 abcd	5.02 ± 2.65 a	40.13 ± 3.40 abc
			Primary Branches			
AL	7.07 ± 1.14 de	2.28 ± 0.21 c	0.51 ± 0.06 de	0.21 ± 0.03 d	0.33 ± 0.07 b	10.40 ± 1.46 d
FL	2.73 ± 0.83 ef	5.24 ± 1.50 bc	0.65 ± 0.37 de	0.36 ± 0.09 bcd	0.55 ± 0.23 b	9.53 ± 2.36 d
GA	3.44 ± 0.55 def	4.12 ± 0.61 bc	1.23 ± 0.22 cd	0.76 ± 0.09 a	0.41 ± 0.08 b	9.96 ± 1.34 d
ME	17.86 ± 3.31 b	5.16 ± 1.28 bc	1.57 ± 0.39 bc	0.78 ± 0.14 a	0.34 ± 0.09 b	25.70 ± 5.00 b
MS	23.11 ± 1.71 a	9.30 ± 1.00 a	2.38 ± 0.16 ab	0.64 ± 0.07 abc	0.87 ± 0.09 b	36.30 ± 1.31 a
NY	3.53 ± 0.95 def	9.75 ± 2.16 a	0.90 ± 0.0.24 cde	0.37 ± 0.11 bcd	0.55 ± 0.16 b	15.09 ± 3.24 cd
NC	2.16 ± 0.22 f	5.91 ± 1.44 b	0.09 ± 0.02 e	0.05 ± 0.01 d	0.24 ± 0.08 b	8.43 ± 1.42 d
SC-BW	3.52 ± 1.22 def	3.72 ± 0.91 bc	1.55 ± 0.11 bc	0.72 ± 0.29 ab	2.67 ± 0.56 a	12.18 ± 1.95 d
SC-CL	8.31 ± 2.76 cd	3.96 ± 0.97 bc	0.78 ± 0.25 cde	0.29 ± 0.11 cd	0.25 ± 0.11 b	13.59 ± 4.11 cd
SC-OB	12.06 ± 0.69 c	4.53 ± 0.48 bc	2.50 ± 0.56 a	0.30 ± 0.05 cd	0.81 ± 0.13 b	20.21 ± 1.67 bc
			Secondary Branches			
AL	25.98 ± 3.42 a	4.23 ± 0.39 cd	1.54 ± 0.19 bcd	0.44 ± 0.03 abc	0.48 ± 0.10 bc	32.69 ± 4.01 a
FL	3.67 ± 0.66 ef	12.07 ± 2.68 a	2.00 ± 0.79 b	0.32 ± 0.09 bc	1.20 ± 0.23 b	19.26 ± 3.97 bc
GA	7.49 ± 0.63 def	4.92 ± 0.31 bcd	1.68 ± 0.21 bcd	0.86 ± 0.12 abc	0.38 ± 0.05 bc	15.33 ± 1.22 bc
ME	16.80 ± 2.59 bc	9.07 ± 2.45 ab	0.89 ± 0.37 cde	0.05 ± 0.05 bc	0.13 ± 0.11 c	26.94 ± 5.29 ab
MS	20.94 ± 4.41 ab	10.84 ± 1.36 a	1.92 ± 0.40 bc	0.41 ± 0.06 abc	0.93 ± 0.18 bc	35.04 ± 5.86 a
NY	9.67 ± 2.46 cde	12.10 ± 2.29 a	1.26 ± 0.33 bcd	0.38 ± 0.12 bc	1.14 ± 0.21 bc	24.55 ± 4.62 ab
NC	0.77 ± 0.08 f	1.03 ± 0.09 d	0.04 ± 0.01 e	ND c	0.07 ± 0.02 c	1.92 ± 0.16 d
SC-BW	1.83 ± 0.67 ef	3.45 ± 0.75 cd	0.65 ± 0.12 de	1.27 ± 0.91 a	2.88 ± 1.06 a	10.08 ± 3.12 cd
SC-CL	18.14 ± 7.00 abc	5.89 ± 0.95 bc	1.07 ± 0.38 bcde	0.91 ± 0.13 ab	0.38 ± 0.18 bc	26.39 ± 8.41 ab
SC-OB	15.39 ± 0.89 bcd	6.11 ± 0.35 bc	4.29 ± 0.29 a	0.54 ± 0.05 abc	0.98 ± 0.12 bc	27.31 ± 1.43 ab

[a] AL, Alabama; FL, Florida; GA, Georgia; ME, Maine; MS, Mississippi; NY, New York; NC, North Carolina; SC-BW, South Carolina Barnwell County; SC-CL, South Carolina Colleton County; SC-OB, South Carolina Orangeburg County. [b] Other glucosinolates included glucocheirolin, glucoiberin, glucoraphanin, gluconapoleiferin, glucoalyssin, gluconapin, 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin, glucosibarin, glucobarbarin, glucotropaeolin, neoglucobrassicin, and sinigrin. [c] Values are the average of 3 replicates in each of two experiments followed by standard error. Values followed by the same letter are not significantly different based on Fisher's protected LSD test with a 5% level of significance between accessions for each plant part and glucosinolate. [d] ND = none detected.

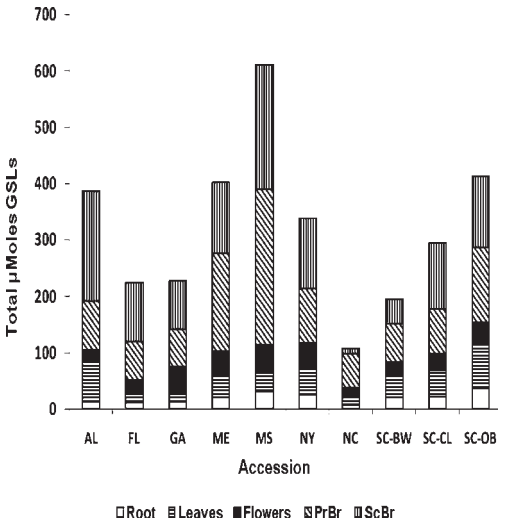


Figure 2. Total μmol of glucosinolates associated with different plant parts of total plant in accessions of wild radish collected from different states in the northeastern and southern United States: AL, Alabama; FL, Florida; GA, Georgia; ME, Maine; MS, Mississippi; NY, New York; NC, North Carolina; SC-BW, South Carolina Barnwell County; SC-CL, South Carolina Colleton County; SC-OB, South Carolina Orangeburg County; PrBr, primary branches; and ScBr, secondary branches. Values represent the average of 3 replicates in each of two experiments.

Seventeen different glucosinolates were identified among the various accessions of wild radish. These included glucocheirolin, glucoiberin, glucoraphanin, glucoraphenin, gluconapoleiferin, glucoalyssin, gluconapin, 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin, glucosibarin, glucobarbarin, glucotropaeolin, glucobrassicin, neoglucobrassicin, glucoerucin, sinigrin, and gluconasturtiin. The most prevalent glucosinolates were glucoerucin and glucoraphenin, with glucobrassicin and gluconasturtiin present at levels approximately 10-fold less (**Tables 3**, **4**). The remaining glucosinolates were present at levels less than 0.5 μmol/plant. Of the glucosinolates found in wild radish, all but glucobarbarin, glucobrassicin, 4-hydroglucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin can be degraded with the release of isothiocyanates, with only glucobrassicin being present at greater than 1 μmol/plant (**Table 3**).

In a comparison of the levels of glucosinolates that can be converted to isothiocyanates produced by the different accessions, the Mississippi accession had the highest level of glucosinolates that can be converted to isothiocyanates. The level was significantly greater than all accessions, except those from Alabama and Maine (**Table 2**). The accession from North Carolina had the lowest levels of glucosinolates that can be converted to isothiocyanates, but it was not significantly different from SC-Colleton, New York, Georgia and Florida accessions.

When hierarchical clustering of all accessions was conducted, total glucosinolates in the Mississippi accession grouped separately from other accessions, with the Florida and SC-Orangeburg accessions being the most closely related to it. All other accessions fell into a third group (data not shown).

Glucosinolates in Various Plant Parts. Comparisons were made of the glucosinolates that can convert to isothiocyanates in various plant parts. A majority of the glucosinolates are associated with the primary and secondary branches of plants, with the percentages ranging from 57.6 to 80.8%, an average of 68.2% of the total glucosinolates (**Figure 2**, **Table 3**). Roots supplied the lowest percentage of the total glucosinolates, ranging from 3.3 to 10.7%, an average of 6.6%. The average contribution of branches to the total weight was 73.1%, and roots contributed 8.3% to the total biomass. The average contribution of flowers to the total glucosinolates was 11.1%, which was approximately twice the contribution of flowers to the total weight of plants, which was 5.7%. Flowers and other reproductive organs of plants have previously been shown to contain the highest levels of glucosinolates when compared to other plant parts (*26*, *27*). The overall levels of glucosinolates/g in flowers ranged from 12.07 to 55.36 μmol/g, which were higher than for other plant tissue in each accession (**Table 4**). The highest glucosinolate level in any other tissue was 36.3 μmol/g in the Mississippi primary branch tissue. Even though roots supply low percentages of the glucosinolates, they do provide some glucosinolates that should be considered when looking at cover crops for use in weed suppression.

Individual Glucosinolate Production in Plant Parts. Of the seventeen glucosinolates present in wild radish tissue, glucoerucin and glucoraphenin contributed between 75.4 and 99.7% of the total glucosinolates present in plant parts, with an overall average of 90.8% (**Table 3**). Both of these glucosinolates can be degraded into isothiocyanates, which is important for weed suppression. Minimal differences were noted when looking at the percentage contribution of these two glucosinolates in various plant parts. The contribution of branches can easily be observed, with the total glucosinolates in these plant parts being approximately 3- to 10-fold greater than the levels observed in roots, leaves, or flowers. In assessing the potential of wild radish as a cover crop for weed suppression, it is vital to determine the glucosinolates associated with branch material. In comparing the total glucosinolate contribution of various plant parts among accessions, the Mississippi accession had significantly higher levels of glucosinolates in primary branches and was in the group containing the highest level of glucosinolates along with Alabama, Maine, SC-Orangeburg, New York and SC-Colleton.

Glucosinolate levels present in plant tissue based on per gram dry weight (**Table 4**) were used to compare the values that were previously published for commercial radish (*Raphanus sativus* L.) (*28*−*30*). Both species have generally 2 to 3 major glucosinolates, and the levels in wild radish were similar to those in commercial radish. The most interesting thing is that the major glucosinolates present are different. In wild radish, the major glucosinolates are glucoerucin and glucoraphenin, while the major glucosinolates in commercial radish are 4-methylthio-3-butenyl-glucosinolate (glucoraphasatin) and glucoraphenin. The overall levels are similar between the two species, suggesting that commercial radish may also be an option as a cover crop to increase weed management as has been observed with wild radish (*9*), contingent upon it producing sufficient biomass.

Differences were shown in glucosinolate concentrations among different accessions, indicating that further research is needed to determine if the Mississippi accession can consistently produce more glucosinolates under field conditions, which could result in better weed management. The variation in the accessions is most likely due to differences in the genetics of this accession, but it is also important to remember that wild radish is self-incompatible (*31*) and as a result is cross pollinated, which results in great variability among accessions. Previous studies have also shown that greenhouse tests cannot accurately predict the natural genetic variation (*32*) in wild radish. Further studies are needed for the identification of accessions that produce higher levels of glucosinolates and, more specifically, isothiocyanate-producing glucosinolates along with further field studies to investigate the viability of using wild radish, a weed, for weed management.

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