JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Phytochemical Changes Induced by Different Nitrogen Supply Forms and Radiation Levels in Two Leafy *Brassica* Species

Carlo Fallovo,[†] Monika Schreiner,[‡] Dietmar Schwarz,[‡] Giuseppe Colla,[†] and Angelika Krumbein*[,‡]

[†]Department of Geology and Mechanical Engineering, Bioengineering and Hydraulics for the Territory, University of Tuscia, Via S. C. De Lellis snc, 01100 Viterbo, Italy

[‡]Leibniz-Institute of Vegetable and Ornamental Crops Grossbeeren and Erfurt e.V., Theodor-Echtermeyer-Weg 1, 14979 Grossbeeren, Germany

ABSTRACT: The effect of three different nitrogen (N) supply forms differing in their ammonium-to-nitrate (NH_4:NO_3) ratio (100% NH_4, 50% NH_4 + 50% NO_3, 100% NO_3) under three different levels of daily photosynthetic active radiation (PAR) (low, 5.0; medium, 6.8; high, 9.0 mol m^{-2} day^{-1}) on a range of desirable health-promoting phytochemicals in *Brassica rapa* subsp. *nipposinica* var. *chinoleifera* and *Brassica juncea* was determined. The 100% NH_4 supply under medium PAR levels led to the highest concentration of glucosinolates based on a low nitrogen/sulfur ratio as well as high levels of carotenoids in the leaves of both *Brassica* species. However, the 100% NH_4 supply under low and medium PAR levels resulted in low concentrations of flavonoids based on high N concentration in the leaves. Thus, the data provided here have strong implications for crop management strategies aimed at optimizing both the concentration and composition of a range of phytochemicals.

KEYWORDS: *Brassica rapa*, *Brassica juncea*, nitrate, ammonium, photosynthetic active radiation, glucosinolates, flavonoids, carotenoids, chlorophylls

INTRODUCTION

In the last years, phytochemical compounds found in vegetables and fruits have aroused great interest due to their highly beneficial properties for human health. Several epidemiological studies, e.g. the meta study of Verhoeven et al.,[1] the Health Professionals' Study,[2] and the bladder cancer study of Tang et al.,[3] have revealed an inverse association between a high consumption of *Brassica* vegetables and a lower risk of cancer incidence. For example, hydrolysis products of certain glucosinolates, such as glucoraphanin, sinigrin, gluconapin, and gluconasturtiin,[4] flavonoids,[5] carotenoids,[6] and chlorophylls,[7,8] are phytochemicals that are associated with anticarcinogenic properties. Thus, it is unsurprising that *Brassica* vegetables in general and leafy Asian *Brassica* vegetables in particular are gaining increasing attention by European consumers since they contain rich sources of glucosinolates as well as high concentrations of flavonoids, carotenoids, and chlorophylls.[9,10] The concentration and composition of phytochemicals vary widely in leafy *Brassica* vegetables, a phenomenon that is influenced by both genetic and environmental factors[4] and exemplified by the two leafy Asian *Brassica* species *Brassica rapa* subsp. *nipposinica* var. *chinoleifera* and *Brassica juncea*, particularly at the level of genetic background. These two leafy Asian *Brassica* species differ widely in their concentration and composition of glucosinolates (aliphatic, indole, and aryl glucosinolates), carotenoids (lutein and β-carotene), and chlorophylls (a and b).[9]

The key environmental factors affecting phytochemical concentration and composition in *Brassicaceae* are the level and the form of nitrogen (N) supply. For example, glucosinolate concentrations were observed to decline with high N supply levels in broccoli (*B. oleracea* var. *italica* L.) and turnip (*B. rapa* subsp. *Rapifera* L.).[11−13] Interestingly, flavonoids were also reported to decrease at high N levels in apple (*Malus domestica* Borkh.) and tomato (*Solanum lycopersicum* L.) leaves,[14,15] whereas lutein, β-carotene, and chlorophylls were observed to increase with increasing N supply in spinach (*Spinacea oleracea* L.) and kale (*B. oleracea* L. var. *sabellica* L.).[16,17]

However, while the influence of the level of N supply is well investigated, studies addressing how the N supply form affects phytochemical concentration and composition are scarce as well as contradictory. To date, it is known that both nitrate and ammonium as inorganic N sources can be utilized by plants and there is preliminary evidence to suggest that, when nitrate and ammonium are used as N supply types, they result in different phytochemical concentration and compositions.[18] However, studies have tended to focus preferentially on growth characteristics,[19] contain only a few *Brassica* species such as kale and rocket salad (*Eruca sativa* Mill) or investigate only a single phytochemical. For example, in rocket salad, the highest glucosinolate concentration occurred at a balanced ratio of ammonium and nitrate in the nutrient solution,[20] whereas in kale, the carotenoid and chlorophyll concentrations increased by partial or total replacement of ammonium by nitrate in the nutrient solution.[17] These findings are in sharp contrast to those which report that ammonium supply increases the chlorophyll content in endive (*Chicorium endivia* L. var. *crispum* Hegi.)[21] and leaves of kohlrabi (*Brassica oleracea* L. var. *gongylodes* L.).[22] In addition, no comprehensive information is available on whether and how the N supply form affects phytochemical concentration and composition in leafy Asian *Brassica* vegetables: a family that shows pronounced differences in its phytochemical profile due to the

Received: December 20, 2010
Accepted: March 12, 2011
Revised: March 11, 2011
Published: March 12, 2011

ACS Publications © 2011 American Chemical Society

dx.doi.org/10.1021/jf1048904 | *J. Agric. Food Chem.* 2011, 59, 4198–4207

different genetic backgrounds of the individual species.[9] Moreover, effects of N supply form on plant growth and metabolism can be modified by plant exposure to different PAR levels.[23,24] For example, the concentrations of flavonoids in broccoli and kale as well as carotenoids and chlorophylls in kale increase with increased photosynthetic active radiation (PAR) levels[25−27] and concentrations of glucosinolates in broccoli show radiation-induced variations.[28]

In this study, we test our hypothesis that partial or total replacement of nitrate by ammonium in the nutrient solution, especially under higher PAR levels, will increase the concentrations of the most important phytochemicals, such as glucosinolates, flavonoids, carotenoids, and chlorophylls, of two leafy Asian *Brassica* species having different phytochemical profiles. While scare information exists how different forms of nitrogen supply can affect phytochemicals the results will be related to the N and sulfur (S) status of the leaves.

MATERIALS AND METHODS

Chemicals. Lutein, β-carotene, chlorophyll a, chlorophyll b and allyl glucosinolate (sinigrin) were purchased from Sigma-Aldrich (Taufkirchen, Germany). Dihydroquercetin, kaempferol and isorhamnetin were obtained from Carl Roth GmbH (Karlsruhe, Germany). Methanol, methylene chloride, acetone and acetic acid were purchased from VWR International (Dresden, Germany). Acetonitrile and aryl sulfatase were obtained from Th. Geyer GmbH (Berlin, Germany). Reagents and solvents were of HPLC or analytical grade quality.

Plant Material. Three hydroponic experiments were carried out in a greenhouse considering three levels of mean daily photosynthetic active radiation (PAR) at the Leibniz-Institute of Vegetable and Ornamental Crops located at Grossbeeren (Germany, latitude 52° N): low (5.0 ± 0.53 mol m^{-2}) from December 6, 2007 to January 22, 2008; medium (6.8 ± 1.32 mol m^{-2}) from January 23, 2008 to February 25, 2008; high (9.0 ± 3.2 mol m^{-2}) from March 6, 2008 to April 9, 2008). The PAR range was chosen to maintain a comparable temperature without cooling, and the maximum PAR is significantly lower compared with field conditions. All three experiments were conducted at the same mean temperature (15 ± 1.6 °C), relative humidity (74%), and CO_2 concentration (400 μmol mol^{-1}) with three replications of each nitrogen treatment. For each experiment, seeds of *Brassicia juncea* (L.) Czern. cv. Red Giant and *Brassica rapa* subsp. *nipposinica* (L.H. Bailey) Hanelt var. *chinoleifera* cv. Mibuna Early were sown in rockwool cubes under greenhouse conditions. The plants at the two true leaf stage, 25 days after sowing, were transferred into 8 m long gullies supplied continuously with nutrient solution. The distance between the 46 plants in each gully was 0.17 m. Nine gullies were randomly arranged in the greenhouse and each corresponded to one replication.

Total N concentration was the same (11 mM) in all treatments; however, treatments differed in the N supply form: 100% NH_4, 50% NH_4 + 50% NO_3, 100% NO_3). In the 100% NH_4 treatment, NH_4 was supplied using NH_4Cl while NH_4NO_3 was used in the 50% NH_4 + 50% NO_3 treatments. The other macronutrients were supplied in all nutrient solutions at the following concentrations (mM): K 3.6, Ca 4.2, P 0.6, Mg 1.0, S 1.3; micronutrients had the following concentrations (μM): Fe 40.0, M_n 5.0, Zn 4.0, B 30, Cu 0.5, Mo 0.5. During all experiments, the electrical conductivity was kept at 2 dS m^{-1} by adding stock solution or deionized water according to the variations ($\pm$10% of the target value). In all nutrient solutions, a MES buffer was applied at 1.5 mM to keep the pH in the range of 5.6−5.8. Note that phosphoric acid or potassium hydroxide was added when the solution pH drifted above or below the threshold.

Sample Preparation for Compound Measurement. At commercial maturity (45, 32, and 33 days after transplanting for experiments at low, medium, and high PAR, respectively), 20 plants were harvested for each treatment and replication. Commercial maturity is defined for reaching at least 45 and 6−7 fully developed leaves in *B. rapa* and *B. juncea* (before starting the generation growth), respectively, grown at 100% nitrate supply.[19] For each sample of *B. juncea*, the midribs of the leaves were removed by means of a sharp knife. Two mixed subsample of fresh leaves (each 15 g) was used for the double estimation of carotenoids and chlorophylls. To analyze glucosinolate and flavonoid as well as N and S concentrations, another subsample of leaves (300 g) was immediately deep frozen at −40 °C, then freeze-dried, and finely ground.

Glucosinolate Analysis. Glucosinolates were analyzed by HPLC as their desulfo-glucosinolates.[9] Duplicates of freeze-dried sample material (0.5 g) were heated to and incubated at 75 °C for 1 min, extracted with 4 mL of a methanol/water mixture (v/v = 7:3, T = 70 °C) and then centrifuged at 4000 rpm for 10 min. For an internal standard, 200 μL of a 5 mM stock solution of allyl glucosinolate in methanol was added to one of the duplicates just before the first extraction. The residue was extracted twice more with 3 mL of the methanol/water mixture (v/v = 7:3, T = 70 °C). The supernatants were pooled and made up to 10 mL with the methanol/water mixture. From this supernatant, 5 mL were applied to a 250 μL DEAE-Sephadex A-25 ion-exchanger (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, acetic acid-activated) and rinsed with 10 mL of deionized water. Next, 250 μL of a purified solution of aryl sulfatase was applied and left for 12 h. The desulfo-compounds were then flushed with 5 mL of deionized water. The analysis was conducted using a Merck-Hitachi HPLC system (Merck-Hitachi, Darmstadt, Germany) with a Spherisorb ODS2 column (Bischoff, Leonberg, Germany, 5 μm, 250 × 4 mm). A gradient of 0 to 20% acetonitrile in water was selected from minutes 2 to 34, followed by 20% acetonitrile in water until minute 40, and then 100% acetonitrile for 10 min. Glucosinolate determination was conducted at a flow rate of 1.3 mL min^{-1} and a wavelength of 229 nm. Glucosinolate concentration was calculated using allyl glucosinolate as internal and external standard along with the response factor of each compound relative to allyl glucosinolate.[29] The well-known desulfo-glucosinolates were identified according to previous work.[30] The protonated molecular ions $[M + H]^+$ and the fragment ions corresponding to $[M + H - \text{glucose}]^+$ were measured by HPLC−APCI-MS^2 using the Agilent series 1100 MSD (ion trap) (Agilent Technologies, Waldbronn, Germany) in the positive ionization mode.

Flavonoid Analysis. Flavonoids were determined as their aglycons after acid hydrolyses.[31] To 0.5 g of the freeze-dried sample, 40 mL of 62.5% aqueous methanol followed by 10 mL of 8 M HCl was added to this extract. Thus, the extraction solution consisted of 1.6 M HCl in 50% aqueous methanol (v/v). After refluxing at 90 °C for 2 h, the extract was allowed to cool, was adjusted to 100 mL with 50% methanol, and was sonicated for 5 min before being filtered through a 0.45 μm filter for HPLC analyses.

The flavonoid composition and concentration were determined using a series 1100 HPLC (Agilent, Waldbronn, Germany) equipped with a diode array detection system. The extracts were separated on a Prodigy (ODS 3, 150 × 3.0 mm, 5 μm, 100 Å) column (Phenomenex, Aschaffenburg, Germany) with a security guard C18 (ODS 3, 4 × 3.0 mm, 5 μm, 100 Å) at a temperature of 25 °C using a water/acetonitrile gradient.[27] Solvent A consisted of 99.5% water and 0.5% acetic acid, whereas solvent B contained 100% acetonitrile. The following gradient was used: 30−35% B (5 min), 35−39% B (12 min), 39−90% B (5 min), 90% B isocratic (5 min), 90−30% B (5 min), and 30% B isocratic (5 min). The flow was performed using 0.3 mL/min, and the measured detector wavelength was 370 nm. The standards dihydroquercetin, kaempferol and isorhamnetin were used to obtain an external calibration

Table 1. Influence of Nitrogen (N) Supply Form and Photosynthetically Active Radiation (PAR) Level on Glucosinolate (GS) Concentration (mg g^{-1} DM) in *Brassica rapa*[a]

			alkenyl GS			alkyl GS			indole GS					aryl GS
	N form	total GS	total	but-3-enyl	pent-4-enyl	total	4-methylsulfinylbutyl	5-methylsulfinylpentyl	total	indol-3-ylmethyl	4-hydroxyindol-3-ylmethyl	4-methoxyindol-3-ylmethyl	1-methoxyindol-3-ylmethyl	2-phenylethyl
PAR														
low	NH_4	3.58 a	2.11	1.63	0.58 a	0.68 a	0.35 a	0.33 a	0.50 a	0.27 a	0.08	0.08	0.06	0.30
	$NH_4 + NO_3$	2.65 b	1.43	1.05	0.38 b	0.58 b	0.30 b	0.28 b	0.35 b	0.12 b	0.09	0.08	0.06	0.28
	NO_3	2.85 ab	1.63	1.28	0.35 b	0.57 b	0.30 b	0.28 b	0.35 b	0.12 b	0.09	0.08	0.06	0.29
medium	NH_4	4.55 a	3.17 a	2.55 a	0.63 a	0.65 a	0.32 a	0.34 a	0.42 a	0.18 a	0.09	0.09	0.06	0.30 b
	$NH_4 + NO_3$	3.36 b	2.13 b	1.65 b	0.48 b	0.59 b	0.30 b	0.28 b	0.37 b	0.13 b	0.09	0.09	0.06	0.28 b
	NO_3	3.71 b	2.43 b	1.97 b	0.46 b	0.58 b	0.30 b	0.28 b	0.36 b	0.12 b	0.09	0.09	0.06	0.34 a
high	NH_4	4.87 a	3.56 a	2.94 a	0.62 a	0.60	0.31	0.29	0.42 a	0.16 a	0.10 a	0.09	0.08 a	0.29 b
	$NH_4 + NO_3$	3.11 b	1.87 b	1.47 b	0.41 b	0.58	0.30	0.28	0.38 b	0.13 b	0.09 b	0.09	0.07 b	0.28 b
	NO_3	3.11 b	1.82 b	1.41 b	0.42 b	0.58	0.30	0.28	0.37 b	0.13 b	0.09 b	0.09	0.07 b	0.34 a
main effects														
N form	NH_4	4.33 a	2.95 a	2.37 a	0.58 a	0.64 a	0.32 a	0.32 a	0.45 a	0.20 a	0.09 a	0.09	0.07 a	0.30 b
	$NH_4 + NO_3$	3.04 b	1.81 b	1.39 b	0.42 b	0.58 b	0.30 b	0.28 b	0.37 b	0.13 b	0.08 b	0.09	0.07 a	0.28 c
	NO_3	3.22 b	1.96 b	1.55 b	0.41 b	0.58 b	0.30 b	0.28 b	0.36 b	0.12 b	0.08 b	0.09	0.06 b	0.32 a
PAR	low	3.03 b	1.72 b	1.32 b	0.40 b	0.61 a	0.31	0.30 a	0.40 a	0.17 a	0.08 b	0.08 b	0.06 b	0.29 b
	medium	3.87 a	2.58 a	2.06 a	0.52 a	0.61 a	0.31	0.30 a	0.38 b	0.14 b	0.09 a	0.09 a	0.06 b	0.31 a
	high	3.70 a	2.42 a	1.94 a	0.48 a	0.59 b	0.30	0.28 b	0.39 b	0.14 b	0.09 a	0.09 a	0.07 a	0.30 ab
N form		*	*	*	*	*	*	*	*	*	*	ns	*	*
PAR		*	*	*	*	*	ns	*	*	*	*	*	*	*
N form × PAR		ns	ns	ns	ns	*	*	*	*	*	ns	ns	ns	*

[a] Different letters indicate significant differences within a treatment; ns, not significant; * significant at $p \leq 0.05$; "total" is the sum of corresponding individual compounds quantified.

dx.doi.org/10.1021/jf1048904 |J. Agric. Food Chem. 2011, 59, 4198–4207

Table 2. Influence of Nitrogen (N) Supply Form and Photosynthetically Active Radiation (PAR) Level on Glucosinolate (GS) Concentration (mg g^{-1} DM) in *Brassica juncea*[a]

			alkenyl GS			indole GS				aryl GS
	N form	total GS	total	allyl	but-3-enyl	total	indol-3-ylmethyl	4-hydroxyindol-3-ylmethyl	4-methoxyindol-3-ylmethyl	2-phenylethyl
PAR										
low	NH_4	4.90	4.09	3.61	0.48	0.38	0.21	0.08	0.10	0.42
	$NH_4 + NO_3$	5.21	4.56	4.12	0.44	0.32	0.14	0.09	0.09	0.32
	NO_3	5.26	4.62	4.17	0.44	0.32	0.14	0.09	0.09	0.32
medium	NH_4	8.40	7.71	7.12	0.59	0.38 a	0.19 a	0.10	0.09	0.32 b
	$NH_4 + NO_3$	6.63	5.99	5.49	0.50	0.34 b	0.15 b	0.10	0.10	0.30 b
	NO_3	8.59	7.84	7.27	0.57	0.36 ab	0.16 b	0.10	0.10	0.39 a
high	NH_4	6.49	5.76	5.22	0.54	0.43 a	0.23 a	0.10	0.09	0.31 b
	$NH_4 + NO_3$	5.15	4.49	4.01	0.48	0.37 b	0.18 b	0.11	0.09	0.29 b
	NO_3	5.45	4.70	4.21	0.49	0.34 b	0.18 b	0.11	0.09	0.40 a
main effects										
N form	NH_4	6.60	5.85	5.32	0.54 a	0.40 a	0.21 a	0.09	0.09	0.35 a
	$NH_4 + NO_3$	5.66	5.01	4.54	0.47 b	0.35 b	0.16 b	0.09	0.09	0.30 b
	NO_3	6.43	5.72	5.22	0.50 b	0.34 b	0.15 b	0.09	0.09	0.37 a
PAR	low	5.12 b	4.42 b	3.97 b	0.46 b	0.34	0.16	0.08 b	0.09 b	0.35
	medium	7.88 a	7.18 a	6.63 a	0.55 a	0.36	0.17	0.10 a	0.10 a	0.33
	high	5.70 b	4.98 b	4.48 b	0.50 ab	0.38	0.19	0.11 a	0.09 b	0.33
N form		ns	ns	ns	*	*	*	ns	ns	*
PAR		*	*	*	*	ns	ns	*	*	ns
N form × PAR		ns	ns	ns	ns	ns	ns	*	ns	*

[a] Different letters indicate significant differences within a treatment; ns, not significant; * significant at $p < 0.05$; "total" is the sum of corresponding individual compounds quantified.

curve. The total concentration of flavonoids was calculated as the sum of the concentration of the individual flavonoid aglycons quercetin, kaempferol and isorhamnetin.

Quercetin, kaempferol and isorhamnetin were identified as deprotonated molecular ions and characteristic mass fragment ions by HPLC−DAD−ESI-MS^2, using an Agilent series 1100 MSD (ion trap) with ESI as an ion source in negative ionization mode. Nitrogen was used as the dry gas (12 L/min, 350 °C) and nebulizer gas (40 psi). Helium was used as the collision gas in the ion trap. The mass optimization was performed for quercetin $[M - H]^-$ m/z 301.

Carotenoid and Chlorophyll Analyses. Carotenoids (β-carotene and lutein) and chlorophylls (chlorophyll a and chlorophyll b) were determined by HPLC.[9] To 15 g of cut material, 1 g of calcium carbonate, 30 g of sodium sulfate, and 30 mL of acetone were added, and the samples were homogenized for 2 min. The extract was then filtered under suction and the solid materials were extracted repeatedly with acetone until the solid materials were colorless. The extract was then filtered through a 0.45 μm filter for HPLC analyses. Carotenoid and chlorophyll concentrations and compositions were determined by HPLC using a C-18 reversed-phase column LiChrospher 100 (5 μm, 250 × 4 mm; VWR International (Dresden, Germany)) with an isocratic eluent of 75% acetonitrile, 15% methanol, and 10% methylene chloride. The analyses were carried out at a flow rate of 1 mL min^{-1}. Wavelengths of 448, 455, 432, and 464 nm were used to determine lutein, β-carotene, chlorophyll a, and chlorophyll b, respectively. Concentrations were quantitatively determined by calibration curves of the related pure standards. Note that determination of phytochemical concentration was performed in duplicate.

N and S Analyses. Total N concentration was determined after dry oxidation by the Dumas method (Elementar Vario EL, Hanau, Germany), and total S concentration was analyzed by an elementary analyzer (high-temperature oxidation) and detected with a nondispersive infrared sensor.

Statistical Analysis. All data were statistically analyzed by two-way ANOVA using SPSS software package (SPSS version 15.0 for Windows) with N supply form and PAR levels as treatment factors. Furthermore, one-way ANOVA was performed separately for each experiment. The means were separated by Tukey's HSD test (significance level 0.05). Significant differences are represented by different letters or asterisks in the tables. Regression analysis was performed to determine the correlation between phytochemical concentrations (total and individual) and leaf N and S concentrations as well as N/S ratio.

RESULTS AND DISCUSSION

Glucosinolates. Ten individual glucosinolates were quantitatively determined in the *Brassica* species investigated: three aliphatic (alkenyl: allyl glucosinolate (sinigrin), but-3-enyl glucosinolate (gluconapin), and pent-4-enyl glucosinolate (glucobrassicanapin); two alkyl (4-methylsulfinylbutyl glucosinolate (gucoraphanin) and 5-methylsulfinylpentyl glucosinolate (glucoalyssin)); four indole (indol-3-ylmethyl glucosinolate (glucobrassicin), 4-hydroxyindol-3-ylmethyl glucosinolate (4-hydroxyglucobrassicin), 4-methoxyindol-3-ylmethyl glucosinolate (4-methoxyglucobrassicin), and 1-methoxyindol-3-ylmethyl glucosinolate (neoglucobrassicin)); and one aryl (2-phenylethyl glucosinolate (gluconasturtiin)). The two cultivars can be differentiated by their glucosinolate composition profiles. For example, in *B. rapa*, the predominant glucosinolate was but-3-enyl glucosinolate followed by pent-4-enyl glucosinolate (Table 1), whereas in *B. juncea*, up to 90% of the total glucosinolate

Table 3. Influence of Nitrogen (N) Supply Form and Photosynthetically Active Radiation (PAR) Level on Leaf N and Sulfur (S) Concentration (mg g^{-1} DM) and N/S Ratio in Leaves of Two *Brassica* Species[a]

		B. rapa			*B. juncea*		
	N form	N	S	N/S ratio	N	S	N/S ratio
PAR							
low	NH_4	72.70	13.48 a	5.41	77.60 a	10.40	7.47
	$NH_4 + NO_3$	74.87	12.51 ab	6.01	74.73 ab	8.80	8.51
	NO_3	63.90	10.69 b	6.11	66.90 b	8.91	7.60
medium	NH_4	69.33 a	13.33 a	5.21 a	73.43 a	12.44 a	5.90
	$NH_4 + NO_3$	60.37 b	13.84 a	4.37 b	67.27 b	10.25 b	6.59
	NO_3	54.53 c	11.19 b	4.88 ab	59.10 c	9.93 b	5.97
high	NH_4	55.80	13.10 a	4.26 b	63.33	10.87 a	5.77 b
	$NH_4 + NO_3$	61.53	10.63 b	5.80 a	67.60	8.09 b	8.37 a
	NO_3	60.13	10.00 c	6.02 a	64.53	7.81 b	8.28 a
main effects							
N form	NH_4	65.94 a	13.30 a	4.96 b	71.12 a	11.24 a	6.38 b
	$NH_4 + NO_3$	65.59 a	12.33 b	5.39 ab	69.87 a	9.05 b	7.82 a
	NO_3	59.52 b	10.62 c	5.67 a	63.51 b	8.88 b	7.29 a
PAR	low	70.49 a	12.23 a	5.84 a	73.08 a	9.37 b	7.86 a
	medium	61.41 b	12.79 a	4.82 b	66.60 b	10.87 a	6.15 b
	high	59.16 b	11.24 b	5.36 ab	64.82 b	8.92 b	7.47 a
N form		*	*	*	*	*	*
PAR		*	*	*	*	*	*
N form × PAR		*	ns	*	*	ns	*

[a] Different letters indicate significant differences within a treatment; ns, not significant; * significant at $p \leq 0.05$.

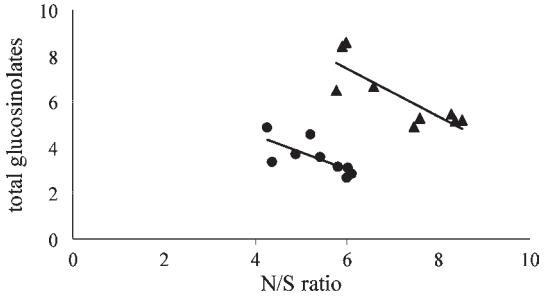

Figure 1. Relationship between total glucosinolate concentration (mg g^{-1} DM) and ratio of total nitrogen (N) to sulfur (S) concentration in leaves of *Brassica rapa* (●) and *Brassica juncea* (▲). Total glucosinolates in *B. rapa* = 7.60 − 0.76(N/S) (r = 0.72*). Total glucosinolates in *B. juncea* = 13.72 − 1.05(N/S) (r = 0.83*). * significant at $p < 0.05$.

concentration was composed of allyl glucosinolate (Table 2). The profile and level of glucosinolates are comparable with data previously reported.[4] In *B. rapa*, the total, alkenyl, alkyl, indole, and aryl glucosinolate concentrations were affected by both N supply form and PAR levels. Interactions were observed for alkyl, indole, and aryl glucosinolates, but not for the predominant alkenyl glucosinolates but-3-enyl glucosinolate and pent-4-enyl glucosinolate. N supplied as 100% NH_4 influenced positively the concentrations of total, alkenyl, alkyl, and indole glucosinolates compared to the supply forms of 50% NH_4 + 50% NO_3 and 100% NO_3. However, the highest concentration of aryl glucosinolate was achieved under 100% NO_3 treatment. The corresponding individual glucosinolates clearly showed a similar trend.

Since N supply form as well as PAR level influence total leaf N and S concentrations, these concentrations were measured and results are summarized in Table 3. In leaves of *B. rapa*, 100% NH_4 resulted in a high S concentration and in a low N/S ratio. The glucosinolate concentration was not correlated to either total N or S concentration, however, a negative linear relationship was found between the total glucosinolate concentration and the N/S ratio (Figure 1).

Similar to *B. rapa*, N supplied as 100% NH_4 influenced positively the concentration of total indole glucosinolates in *B. juncea* due to high levels of indol-3-ylmethyl glucosinolate compared to those of plants supplied with 50% NH_4 + 50% NO_3 or 100% NO_3 (Table 2). A similar trend was also found for buten-3-yl glucosinolate. Surprisingly, the predominant allyl glucosinolate, which determined the concentrations of alkenyl and total glucosinolates, was not affected by N supply form but rather by PAR level. Interestingly, the concentration of the aryl glucosinolate 2-phenylethyl glucosinolate was high at 100% NO_3 as well as 100% NH_4 N supply form as found in *B. rapa*. Similarly to *B. rapa*, 100% NH_4 supply resulted in high S concentrations in leaves (Table 3). A negative linear relationship was found between total glucosinolate concentration and N/S ratio also for in *B. juncea* leaves (Figure 1). However, the N/S ratio combined with the highest total glucosinolate concentration seems to be species specific: N/S ratio amounted to 6:1 for *B. juncea* and 4:1 for *B. rapa*.

In *Brassica* vegetables, it is well know that N and S affect the glucosinolate concentration because glucosinolates are N- and S-containing compounds derived from several amino acids.[11,12,32] In broccoli plants, total glucosinolate concentrations are high at insufficient N, independent of the S level and low at insufficient S in combination with an optimal N supply.[11] In addition, N/S ratios lower than 10:1 are known to have a positive effect on alkyl and indole glucosinolate concentrations.[11] Interactive effects

Table 4. Influence of Nitrogen (N) Supply Form and Photosynthetically Active Radiation (PAR) Level on Flavonoid Concentration (mg g^{-1} DM) in Leaves of Two *Brassica* Species[a]

		B. rapa				*B. juncea*			
	N form	total	kaempferol	quercetin	isorhamnetin	total	kaempferol	quercetin	isorhamnetin
PAR									
low	NH_4	1.72 c	1.05 c	0.10 b	0.56	1.70 b	1.35 b	0.09 b	0.27
	$NH_4 + NO_3$	2.31 b	1.79 b	0.17 a	0.35	3.15 a	2.64 a	0.22 a	0.29
	NO_3	2.69 a	2.07 a	0.20 a	0.42	3.19 a	2.60 a	0.27 a	0.32
medium	NH_4	2.69 b	1.86 b	0.12 b	0.72	2.84 b	2.17 b	0.24 b	0.43
	$NH_4 + NO_3$	3.67 a	2.64 a	0.23 a	0.80	4.05 a	3.23 a	0.34 a	0.48
	NO_3	3.95 a	2.90 a	0.24 a	0.81	4.16 a	3.29 a	0.35 a	0.52
high	NH_4	5.52	3.31	0.39	1.83	4.51 b	3.30 b	0.42	0.80
	$NH_4 + NO_3$	4.74	2.96	0.33	1.45	5.27 a	4.04 a	0.38	0.85
	NO_3	4.88	3.06	0.34	1.48	5.19 a	3.76 ab	0.47	0.96
main effects									
N form	NH_4	3.31	2.07 b	0.20 b	1.04	3.02 b	2.28 b	0.25 b	0.50
	$NH_4 + NO_3$	3.57	2.46 a	0.24 ab	0.87	4.16 a	3.30 a	0.31 a	0.54
	NO_3	3.84	2.68 a	0.26 a	0.90	4.18 a	3.21 a	0.37 a	0.60
PAR	low	2.24 c	1.64 c	0.16 b	0.45 b	2.68 c	2.20 c	0.19 c	0.29 c
	medium	3.44 b	2.47 b	0.20 b	0.78 b	3.68 b	2.90 b	0.31 b	0.48 b
	high	5.05 a	3.11 a	0.35 a	1.58 a	4.99 a	3.70 a	0.42 a	0.87 a
N form		ns	*	*	ns	*	*	*	ns
PAR		*	*	*	*	*	*	*	*
N form × PAR		*	*	*	ns	ns	ns	ns	ns

[a] Different letters indicate significant differences within a treatment; ns, not significant; * significant at $p \leq 0.05$; "total" is the sum of corresponding individual compounds quantified.

between N and S assimilatory pathways are well documented, and *O*-acetylserine is also known to play a key role.[33] Of note is that *O*-acetylserine is a precursor of the N- and S-containing amino acid cysteine that is converted to a number of compounds such as methionine, the precursor of aliphatic glucosinolate synthesis. During conditions of S limitation, *O*-acetylserine accumulates and cysteine decreases,[34] whereas during conditions of N limitation, *O*-acetylserine is reduced.[35] Thus, high N and low S concentrations (high N/S ratio) could lead to an accumulation of *O*-acetylserine and reduced cysteine and methionine syntheses, thereby resulting in a lack of precursors for aliphatic glucosinolate synthesis. Surprisingly, the aliphatic allyl glucosinolate was not affected by the N supply form in *B. juncea*, whereas the aliphatic but-3-enyl glucosinolate and pent-4-enyl glucosinolate showed the highest value at 100% NH_4 supply (high S concentration in the leaves) in both cultivars together with the aliphatic 4-methylsulfinylbutyl glucosinolate and 5-methylsulfinylpentyl glucosinolate in *B. rapa*. Taken together, these results suggest a level of methylthioalkylmalate synthase- (MAM) specific expression as a response to sulfur concentration since several MAMs control the side-chain length of different methionine-derived aliphatic glucosinolates.[36,37]

A previous study has shown that while genes involved in the methionine biosynthetic pathway are downregulated in *Arabidopsis thaliana* seedlings under S-limited growth conditions, genes of the aromatic amino acids synthesis pathway, such as phenylalanine and tryptophan, are induced.[38] Consistent with this, our results show that the phenylalanine-derived aryl glucosinolate 2-phenylethyl glucosinolate increases in the leaves under 100% NO_3 supply (low S concentration in the leaves), especially in *B. rapa*. Furthermore, the increased production of tryptophan results in increased auxin production under S-limited conditions[34] which could lead to a concomitant decrease in indole glucosinolates since tryptophan is the precursor of indole glucosinolates and auxin.[39] Moreover, in rocket salad, Kim et al.[20] reported highest concentrations of the indole glucosinolates indol-3-ylmethyl glucosinolate and 4-methoxyindol-3-ylmethyl glucosinolate with a 100% NH_4 supply, but highest concentrations of total glucosinolates with a 50% NH_4 + 50% NO_3 supply. This group therefore speculated that these two indole glucosinolates were probably involved in NH_4^+ detoxification.

For *B. rapa*, we found that the highest concentrations of total and alkenyl glucosinolates were induced at medium and high PAR levels and that these were caused by high concentrations of but-3-enyl glucosinolate and pent-4-enyl glucosinolate. For *B. juncea*, medium PAR levels primarily increased the allyl glucosinolate and but-3-enyl glucosinolate and thus the total and alkenyl glucosinolates which did not further increase at high PAR level. Interestingly, the PAR level also influenced the N and S status of the plant. The lowest N/S ratio was found for both species at medium PAR levels resulting in high total and alkenyl glucosinolate concentrations. Furthermore, our study confirms the results found by Wallsgrove and Bennet,[40] who reported that low PAR intensity reduces the glucosinolate content of rape leaves due to decreasing flavin-containing monooxygenases which catalyze the formation of aliphatic aldoxime, a key regulator step in aliphatic glucosinolate biosynthesis. In contrast, we found that the main indole glucosinolate indol-3-ylmethyl glucosinolate was higher at low PAR levels in *B. rapa* which was also found in a study on broccoli.[28] This could not be confirmed for the investigated indole glucosinolates 4-hydroxyindol-3-ylmethyl glucosinolate, 4-methoxyindol-3-ylmethyl glucosinolate, and

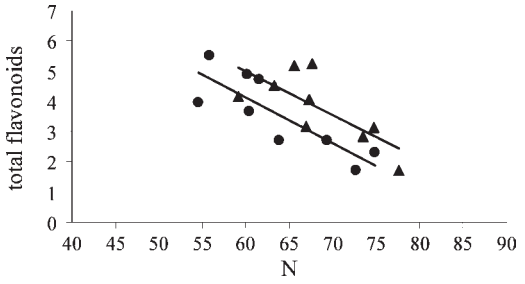

Figure 2. Relationship between total flavonoid concentration (mg g^{-1} DM) and nitrogen (N) concentration in leaves of *Brassica rapa* (●) and *Brassica juncea* (▲). Total flavonoids in *B. rapa* = 13.20 − 0.15(N) (r = 0.84*). Total flavonoids in *B. juncea* = 13.70 − 0.14(N) (r = 0.73*). * significant at $p < 0.05$).

1-methoxyindol-3-ylmethyl glucosinolate which were found in relatively low concentrations in both cultivars. Furthermore, no clear radiation effect was found for the 2-phenylethyl glucosinolate. Taken together, our results are in agreement with previous studies on *B. oleracea* varieties in which the concentrations of total glucosinolates were higher in plants cultivated under high PAR in the spring compared with those grown under low PAR in fall.[41,42]

Thus, our investigation highlights that treatment with 100% NH_4 increases the production of glucosinolates in the leaves concurring with a high S level and a low N/S ratio. Furthermore, medium PAR levels enhance the concentration of alkenyl glucosinolates in both *Brassica* species as well as a high PAR level in *B. rapa.* Of note is that a low PAR level predominantly enhance indole glucosinolates in *B. rapa*.

Flavonoids. Three flavonols were quantified: the predominant flavonol was kaempferol, followed by isorhamnetin and quercetin (Table 4). Recently, kaempferol and isorhamnetin derivatives were detected in *B. juncea*, but no quercetin derivatives were found.[55] The level of flavonols was higher (8.9−27.9 mg g^{-1} DM) than in our study, perhaps because plants were grown in the field in China (high sunlight supply compared with our greenhouse conditions) and different cultivars were used. In both *Brassica* species, kaempferol and quercetin concentrations were affected by both the N supply form and the PAR level, whereas the isorhamnetin concentration was only affected by the PAR level. N supply form and the PAR level interacted in *B. rapa*. Moreover, also in both species, the 100% NH_4 supply led to low kaempferol and quercetin concentrations compared to the 50% NH_4 + 50% NO_3 and 100% NO_3 supplies. Furthermore, the higher the PAR level, the higher the concentrations of flavonols were. At high PAR levels, a significant effect of N supply form was only found for kaempferol in *B. juncea*. Results depict a negative linear relationship between the concentrations of N in leaves and the concentrations of the total flavonols in both species (Figure 2) as well as for the individual flavonols kaempferol and quercetin (data not shown). Furthermore, a high N concentration was found in leaves of both species grown with the 100% NH_4 supply at medium PAR levels, and in *B. juncea*, also at low PAR levels (Table 3). The decrease of total flavonol concentration was correlated to increasing N concentration for both species although the level of flavonols was higher in *B. juncea* (Figure 2).

In onion species (*Allium cepa* L. and *Allium fistulosum* L.), an increased N level in plant tissue of predominantly NH_4 supplied plants in comparison to predominantly NO_3 supplied plants was also found.[43,44] This increased N level led to a lower concentration of quercetin glucosides while isorhamnetin monoglucosides were not affected and isorhamnetin diglucosides increased.[44] However, most studies on N supply and its influence on flavonoid concentration were performed without investigating the N concentration in the plant. Generally, high N supply reduces the concentrations of flavonols in *B. oleracea* such as broccoli and tronchuda cabbage.[45,46] Finally, Steward et al.[47] concluded that reduced N availability in tomato leaf tissue can upregulate the flavonol biosynthetic functions in plants as a protection mechanism against further stress such as pathogen attack or light-induced damage.

Different enzymes of flavonoid biosynthesis seem to be influenced by the nitrogen level. The first step of the phenylpropanoid pathway is the conversion of phenylalanine to cinnamic acid by phenylalanine ammonia-lyase (PAL). To date, most studies have reported that PAL activity decreases with increasing N availability and that this results in a lower flavonoid concentration in e.g. apple leaves.[14] Recently, a study on a medicinal plant (*Echinacea angustifolia*) has shown that PAL activity in the leaves was not influenced by two different NO_3:NH_4 ratios (1:0 and 1:1) in the nutrient solution; however, the PAL activity was reduced in the root tissues of plants grown at a 1:1 NO_3:NH_4 ratio.[48] However, the study does not report whether and how the NO_3:NH_4 ratio affects the N concentration in leaf and root tissues. Moreover, the MYB12 transcription factor, known to regulate flavonol synthesis, is slightly induced by N deficiency in *Arabidopsis thaliana* seedlings, resulting in an accumulation of flavonols.[49] Interestingly, isorhamnetin concentration was not affected under our investigated conditions. A further enzyme, *O*-methyltransferase, catalyzes the conversion of quercetin to isorhamnetin. Thus, this enzyme seems to be not affected by N concentration. Finally, the level of the amino acid phenylalanine in the leaves itself can affect the concentration of flavonoids in vegetable tissue which is dependent on the nutrition status of the plant,[38] a point already discussed for glucosinolate biosynthesis. Therefore, N supplied as NO_3 seems to increase both the phenylalanine-derived aryl glucosinolate 2-phenylethyl glucosinolate and flavonoid concentrations.

In regard to the effects of different PAR intensities on flavonoid concentration, our results are in agreement with several other studies on *Brassica*. For example, in broccoli, the concentrations of kaempferol and quercetin increased with increasing PAR levels.[26] In addition, exposure of leaves of *B. napus* to supplementary UV radiation resulted in an overall increase in the levels of flavonoids of up to 150% with the level of quercetin being increased by 36-fold.[50] Thus, the PAR level seems to be the determining factor for flavonoid biosynthesis in *Brassica* species.

Numerous studies have largely demonstrated that the accumulation of flavonoids by plants is the main defense mechanism against several environmental stresses including radiation. Flavonoids together with other antioxidant compounds present in green leaves are known to absorb UV and thereby act as a selective filter that protects plant tissue against harmful rays.[51] Interestingly, for both species at high PAR levels, we observed no significant effect of N supply form for quercetin, and in addition, for kaempferol in *B. juncea*. These findings are in agreement with the result that at high PAR intensity N form did not influence N concentration in leaves. Thus, different environmental stress factors, e.g. high PAR intensity, may be able to compensate for each other. Besides the PAR effect on the defense mechanisms of flavonoids our results depict also an effect on the N concentration in

Table 5. Influence of Nitrogen (N) Supply Form and Photosynthetically Active Radiation (PAR) Level on Carotenoid and Chlorophyll Concentration (mg g^{-1} DM) in Leaves of Two *Brassica* Species[a]

		B. rapa						*B. juncea*					
		carotenoids			chlorophylls			carotenoids			chlorophylls		
	N form	total	lutein	β-carotene	total	chlorophyll a	chlorophyll b	total	lutein	β-carotene	total	chlorophyll a	chlorophyll b
PAR													
low	NH_4	1.72	1.04	0.68	14.16	11.30	2.86 b	1.26 b	0.75 b	0.51 c	10.84 b	8.50 b	2.34 b
	$NH_4 + NO_3$	1.81	1.04	0.77	17.71	13.67	4.04 a	1.75 a	0.93 a	0.82 a	17.38 a	13.41 a	3.97 a
	NO_3	1.77	1.01	0.76	17.31	13.28	4.02 a	1.55 a	0.84 ab	0.71 b	15.68 a	12.00 a	3.68 a
medium	NH_4	2.02 a	1.11 a	0.91 a	17.15	13.61 a	3.54	2.11 a	1.06 a	1.05 a	18.99 a	15.16 a	3.83 a
	$NH_4 + NO_3$	1.81 ab	1.01 b	0.80 ab	16.55	12.70 ab	3.85	1.92 a	1.01 a	0.91 b	18.49 a	14.31 a	4.18 a
	NO_3	1.55 b	0.89 ab	0.66 b	14.08	10.78 b	3.30	1.26 b	0.68 b	0.58 c	11.97 b	9.25 b	2.72 b
high	NH_4	1.89	1.03 a	0.85 a	16.19 a	12.71 a	3.48 a	1.52 a	0.78 a	0.74 a	13.87 a	10.78 a	3.09
	$NH_4 + NO_3$	1.56	0.87 b	0.69 b	14.20 b	10.86 b	3.34 ab	1.31 ab	0.69 ab	0.62 ab	12.06 ab	9.26 ab	2.80
	NO_3	1.44	0.81 b	0.63 b	12.85 c	9.79 c	3.06 b	1.16 b	0.64 b	0.52 b	10.43 b	7.98 b	2.45
main effects													
N form	NH_4	1.87 a	1.06 a	0.81 a	15.84	12.54 a	3.30 b	1.63 a	0.86 a	0.76 a	14.56 b	11.48 b	3.09 b
	$NH_4 + NO_3$	1.72 ab	0.97 b	0.75 ab	16.15	12.41 a	3.74 a	1.66 a	0.88 a	0.79 a	15.97 a	12.32 a	3.65 a
	NO_3	1.58 b	0.90 b	0.68 b	14.74	11.28 b	3.46 ab	1.32 b	0.72 b	0.60 b	12.70 c	9.74 c	2.95 b
PAR	low	1.76 ab	1.03 a	0.74	16.39 a	12.75 a	3.64 a	1.52 b	0.84 b	0.68 b	14.63 b	11.30 b	3.33 b
	medium	1.79 a	1.01 a	0.79	15.93 a	12.36 a	3.57 ab	1.76 a	0.91 a	0.85 a	16.48 a	12.91 a	3.58 a
	high	1.62 b	0.90 b	0.72	14.41 b	11.12 b	3.29 b	1.33 c	0.70 c	0.63 c	12.12 c	9.34 c	2.78 c
N form		*	*	*	ns	*	*	*	*	*	*	*	*
PAR		*	*	ns	*	*	*	*	*	*	*	*	*
N form × PAR		*	ns	*	*	*	*	*	*	*	*	*	*

[a] Different letters indicate significant differences within a treatment; ns, not significant;* significant at $p < 0.05$; "total" is the sum of corresponding individual compounds quantified.

the leaves. Low PAR increased the N concentration in the leaves of both species resulting in decreased concentrations of flavonols (Table 3, Figure 2).

Taken together, the results presented here on the influence of N supply form clearly show that the concentration of flavonoids decreases with high N concentration in the leaves as demonstrated by the 100% NH_4 supply. Thus, plants grown using either a 50% NH_4 + 50% NO_3 or 100% NO_3 supply and also at medium or high PAR levels were found to be rich in flavonoids.

Carotenoids and Chlorophylls. In both leafy Asian *Brassica* species, the carotenoids lutein and β-carotene as well as the chlorophylls a and b were quantified. In both *Brassica* species, the lutein concentration was found to be slightly higher than that of β-carotene, while chlorophyll a was the predominant chlorophyll pigment (Table 5). In *B. rapa*, N supply form, changes in the PAR level and their interaction were observed to affect total carotenoid concentration, whereas total chlorophyll concentration responded to PAR levels and the interactive effect of N supply form. Similarly, in *B. juncea*, total carotenoid and chlorophyll concentrations were affected by N supply form, PAR levels, and their interaction. However, in both *Brassica* species, N concentration did not correlate with carotenoid or chlorophyll concentrations (data not shown).

These findings indicate that carotenoids and chlorophylls in both *B. rapa* and *B. juncea* respond differently to the supplied N form and that this is also affected by PAR levels since, e.g., low carotenoid and chlorophyll concentrations occur with the 100% NO_3 supply in combination with medium and high PAR levels and with the 100% NH_4 supply in combination with low PAR levels. Another study has shown that increasing the concentration of NO_3 in a mixed supply of NO_3/NH_4 enhances the carotenoid and chlorophyll concentrations in kale grown in winter/spring under low PAR levels.[17] In *B. rapa* and *B. juncea*, 100% NH_4 supply and in *B. juncea* also the 50% NH_4 + 50% NO_3 treatment resulted in the highest concentrations of total carotenoids, lutein, β-carotene, and chlorophyll a. High concentrations were reached at medium and high PAR levels, whereas low PAR levels showed an opposite trend, and when in combination with the 100% NH_4 supply, the lowest concentrations of total carotenoids and total chlorophylls as well as their individual pigments were found. Moreover, in endive leaves, chlorophyll a and b concentrations were higher in NH_4-supplied plants compared to those supplied solely with NO_3 under high PAR levels.[21] In contrast, carotenoids in carrots (*Daucus carota* L.) were not affected by the N supply form when grown at higher PAR levels.[52] Taken together, these findings suggest a species-specific as well as plant organ-specific response to the form of supplied N under different PAR intensity conditions in respect to both carotenoid and chlorophyll biosyntheses.

NO_3 and NH_4 uptake also influences the uptake of other anions and cations since the uptake of all ions is involved in maintaining electroneutrality within the plant.[53] Increasing the NH_4 supply was also reported to decrease magnesium, calcium, and potassium concentrations in leaf tissues.[23] Magnesium has many functions in photosynthesis since it is present in chlorophyll and is also known to be involved in thylakoid stacking. Thus, a NH_4-induced reduction of magnesium could cause a decrease in chlorophyll concentration possibly promoted under

increasing PAR levels. Decreasing concentrations of chlorophyll observed at increasing PAR levels might also indicate a rising intolerance to NH_4, resulting in a disruption of the chloroplast ultrastructure as was previously demonstrated in citrus leaves receiving a high NH_4 supply.[54]

Interestingly, in broccoli, single treatments with different PAR levels (1.9 to 13.4 mol m^{-2} day^{-1}) did not greatly affect carotenoid and chlorophyll concentrations.[28] In sharp contrast, PAR levels between 7.2 and 35.7 mol m^{-2} day^{-1} were observed to affect carotenoid and chlorophyll concentrations with 11.5−19.3 and 11.5 mol m^{-2} day^{-1} causing the highest concentrations in kale and spinach, respectively.[25] These findings suggest that photodegradation of these phytochemicals takes place at higher PAR levels.[25] Our findings on *B. rapa* and *B. juncea* seem to follow a similar pattern with an increase in carotenoid concentration to a certain PAR level followed by a decrease. However, carotenoid and chlorophylls concentrations were also observed to be affected by the N form supplied. For example, in both *Brassica* species, increasing NH_4 supply resulted in increasing concentrations of carotenoids and chlorophylls at medium and high PAR levels; however, at low PAR levels, the 100% NH_4 supply resulted in the lowest concentrations of carotenoids and chlorophylls.

In conclusion, the results of our study clearly show that the choice of N supply form can lead to increased concentrations of key phytochemicals in *B. rapa* and *B. juncea* and that this can also be further mediated by using an appropriate PAR level. Therefore, the correct supply of N form and PAR intensity is a simple but effective crop management strategy that enables enhanced concentration of health-promoting phytochemical compounds to occur. In the future, molecular and genetic approaches will be used to advance understanding of the phytochemical pathways in *B. rapa* and *B. juncea*, as an additional step to managing both concentration and composition of key phytochemical compounds.

AUTHOR INFORMATION

Corresponding Author

*Tel: +49 (0) 33701-78305. Fax: +49 (0) 33701-55391. E-mail: Krumbein@igzev.de.

ACKNOWLEDGMENT

We sincerely thank Andrea Jankowsky and Ursula Zentner for supporting chemical analyses. We would also like to thank Dr. Ruth Willmott for editorial support and English-language editing.

REFERENCES

(1) Verhoeven, D. T.; Goldbohm, R. A.; van Poppel, G.; Verhagen, H.; van den Brandt, P. A. Epidemiological studies on *Brassica* vegetables and cancer risk. *Cancer Epidemiol., Biomarkers Prev.* **1996**, *5*, 733–748.

(2) Voorrips, L.; Goldbohm, R.; van Poppel, G.; Sturmans, F.; Hermus, R.; van den Brandt, P. Vegetable and fruit consumption and risks of colon and rectal cancer in a prospective cohort study. *Am. J. Epidemiol.* **2000**, *152*, 1081–1092.

(3) Tang, L.; Zirpoli, G. R.; Guru, K.; Moysich, K. B.; Zhang, Y.; Ambrosone, C. B.; McCann, S. Consumption of raw cruciferous vegetables is inversely associated with Bladder cancer risk. *Cancer Epidemiol., Biomarkers Prev.* **2008**, *17*, 938–944.

(4) Verkerk, R.; Schreiner, M.; Krumbein, A.; Ciska, E.; Holst, B.; Rowland, I.; De Schrijver, R.; Hansen, M.; Gerhäuser, C.; Mithen, R.; Dekker, M. Review. Glucosinolates in Brassica vegetables - The influence of the food supply chain on intake, bioavailability and human health. *Mol. Nutr. Food Res.* **2009**, *53*, S219–S265.

(5) Knekt, P.; Kumpulainen, J.; Jarvinen, R.; Rissanen, H.; Heliovaara, M.; Reunanen, A.; Hakulinen, T.; Aromaa, A. Flavonoid intake and risk of chronic diseases. *Am. J. Clin. Nutr.* **2002**, *76*, 560–568.

(6) Stahl, W.; Sies, H. Bioactivity and protective effects of natural carotenoids (review). *Biochim. Biophys. Acta* **2005**, *1740*, 101–107.

(7) Dashwood, R. Chlorophylls as anticarcinigens (review). *Int. J. Oncol.* **1997**, *10*, 721–727.

(8) Ferruzzi, M. G.; Blakeslee, J. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutr. Res.* **2007**, *27*, 1–12.

(9) Krumbein, A.; Schonhof, I.; Schreiner, M. Composition and concentrations of phytochemicals (glucosinolates, carotenoids and chlorophylls) and ascorbic acid in selected *Brassica* species (*B. juncea, B. rapa* subsp. nipposinica var. *chinoleifera*, *B. rapa* subsp. *chinensis* and *B. rapa* subsp. *rapa*). *J. Appl. Bot. Food Qual.* **2005**, *79*, 168–174.

(10) Artemyeva, A. M.; Solovyeva, A. E. Quality evaluation of some cultivar type of leafy *Brassica rapa*. *Acta Hortic.* **2006**, *706*, 121–128.

(11) Schonhof, I.; Blankenburg, D.; Müller, S.; Krumbein, A. Sulphur and nitrogen supply influences growth, product appearance and glucosinolate concentration of broccoli. *J. Plant Nutr. Soil Sci.* **2007**, *170*, 65–72.

(12) Li, S.; Schonhof, I.; Krumbein, A.; Li, L.; Stützel, H.; Schreiner, M. Glucosinolate concentration in turnip (*Brassica rapa* ssp. *Rapifera* L.) roots as affected by nitrogen and sulfur supply. *J. Agric. Food Chem.* **2007**, *55*, 8452–8457.

(13) Omirou, M. D.; Papadopoulou, K. K.; Papastylianou, I.; Constantinou, M.; Karpouzas, D. G.; Asimakopolos, I.; Ethaliotis, C. Impact of nitrogen and sulfur fertilization on the composition of glucosinolates in regulation to sulfur assimilation in different plant organs of broccoli. *J. Agric. Food Chem.* **2009**, *57*, 9408–9417.

(14) Strissel, T.; Halbwirth, H.; Hoyer, U.; Zistler, C.; Stich, K.; Treutter, D. Growth-promoting nitrogen nutrition affects flavonoid biosynthesis in young apple (*Malus domestica* Borkh.) leaves. *Plant Biol.* **2005**, *7*, 677–685.

(15) Bounge-Bartelsman, M.; Phillips, D. A. Nitrogen stress regulates gene expression of enzymes in the flavonoid biosynthetic pathway of tomato. *Plant Physiol. Biochem.* **1995**, *33*, 539–546.

(16) Krumbein, A.; Schonhof, I.; Rühlmann, J. Influence of cultivation methods on carotenoid and chlorophyll concentrations in spinach. *Proc. Ger. Nutr. Soc.* **2003**, *5*, 68.

(17) Kopsell, D. A.; Kopsell, D. E.; Curran-Celentano, J. Carotenoid pigments in kale are influenced by nitrogen concentration and form. *J. Sci. Food Agric.* **2007**, *87*, 900–907.

(18) Marschner, M. In *Mineral nutrition of higher plants*; Academic Press: London, 1995.

(19) Fallovo, C.; Colla, G.; Schreiner, M.; Krumbein, A.; Schwarz, D. Effect of nitrogen forms and radiation on growth and mineral concentration of two *Brassica* species. *Sci. Hortic.* **2009**, *123*, 170–177.

(20) Kim, S. J.; Kawaharada, C.; Ishii, G. Effect of ammonium:nitrate nutrient ratio on nitrate and glucosinolate contents of hydroponically-grown rocket salad (*Eruca sativa* Mill). *Soil Sci. Plant Nutr.* **2006**, *52*, 387–393.

(21) Bonasia, B; Conversa, G.; Gonella, M.; Serio, F.; Santamaria, P. Effects of ammonium and nitrate nutrition on yield and quality in endive. *J. Hortic. Sci. Biotechnol.* **2008**, *83*, 64–70.

(22) Blanke, M. M.; Bacher, W.; Pring, R. J.; Baker, E. A. Ammonium nutrition enhances chlorophyll and glaucousness in Kohlrabi. *Ann. Bot.* **1996**, *78*, 599–604.

(23) Guo, S.; Zhou, Y.; Shen, Q.; Zhang, F. Effect of ammonium and nitrate nutrition on some physiological processes in higher plants - Growth, photosynthesis, photorespiration, and water relations. *Plant Biol.* **2007**, *9*, 21–29.

(24) Tabatabaei, S. J.; Yusefi, M.; Hajiloo, J. Effects of shading and NH_4: NO_3 ratio on the yield, quality and N metabolism in strawberry. *Sci. Hortic.* **2008**, *116*, 264–272.

(25) Lefsrud, M. G.; Kopsell, D. A.; Kopsell, D. E.; Curran-Celentano, J. Irradiance levels affect growth parameters and carotenoid pigments in kale and spinach grown in a controlled environment. *Physiol. Plant.* **2006**, *127*, 624–631.

(26) Gliszczynska-Swigłoa, A.; Kałuzewicz, A.; Lemanska, K.; Knaflewski, M.; Tyrakowska, B. The effect of solar radiation on the flavonol content in broccoli inflorescence. *Food Chem.* **2007**, *100*, 241–245.

(27) Schmidt, S.; Zietz, M.; Schreiner, M.; Rohn, S.; Kroh, L. W.; Krumbein, A. Genotypic and climatic influences on the concentration and composition of flavonoids in kale (*Brassica oleracea* var. *sabellica*). *Food Chem.* **2010**, *119*, 1293–1299.

(28) Schonhof, I.; Kläring, H.-P.; Krumbein, A.; Claussen, W.; Schreiner, M. Effect of temperature under low radiation conditions on health promoting compounds and ascorbic acid in broccoli. *Agric., Ecosyst., Environ.* **2007**, *119*, 103–111.

(29) Official Journal of the European Communities, 1990, *L 170*, 28−34

(30) Zimmermann, N.; Gerendás, J.; Krumbein, A. Identification of desulpho glucosinolates in *Brassicaceae* by LC/MS/MS: Comparison of electrospray ionisation and atmospheric pressure chemical ionisation mass spectrometry. *Mol. Nutr. Food Res.* **2007**, *51*, 1537–1546.

(31) Krumbein, A.; Saeger-Fink, H.; Schonhof, I. Changes in quercetin and kaempferol concentrations during broccoli head ontogeny in three broccoli cultivars. *J. Appl. Bot. Food Qual.* **2007**, *81*, 136–139.

(32) Yan, X.; Chen, S. Regulation of plant glucosinolate metabolism. *Planta* **2007**, *226*, 1343–1352.

(33) Koprivova, A.; Suter, M.; Den, C. R. O.; Brunold, C.; Kopriva, S. Regulation of sulfate assimilation by nitrogen in *Arabidopsis*. *Plant Physiol.* **2000**, *122*, 737–746.

(34) Nicoforova, V.; Freitag, J.; Kempa, S.; Adamik, M.; Hesse, H.; Hoefgen, R. Transcriptome analysis of sulfur depletion in *Arabidopsis thaliana*: interlacing of biosynthetic pathways provides response specificity. *Plant J.* **2003**, *33*, 633–650.

(35) Leustek, Th.; Martin, M. N.; Bick, J. A.; Davies, J. P. Pathways and regulation of sulfur metabolism revield through molecular and genetic studies. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2000**, *51*, 141–165.

(36) Kroymann, J.; Donnerhacke, S.; Schnabelrauch, D.; Mitchell-olds, T. Evolutionary dynamics of an *Arabidopsis* insect resistance quantitative trait locus. 2003, www.pnas.org/cgi/doi/10.1073/pnas.1734046100.

(37) Textor, S.; de Kraker, J. W.; Hause, B.; Gershenzon, J.; Tokuhisa, J. G. MAM 3 catalyzes the formation of all aliphatic glucosinolate chain lengths in Arabidopsis. *Plant Physiol.* **2007**, *144*, 60–71.

(38) Nicoforova, V.; Bielecka, M.; Gakiere, B.; Krueger, S.; Rinder, J.; Kempa, S.; Morcuende, R.; Scheble, W. R.; Hesse, H.; Hoefgen, R. *Amino Acids* **2006**, *30*, 173–183.

(39) Grubb, C. D.; Abel, S. Glucosinolate metabolism and its control (review). *Trends Plant Sci.* **2006**, *11*, 89–100.

(40) Wallsgrove, R. M.; Bennet, R. N. The biosynthesis of glucosinolates in *Brassicas*. In *Amino Acids and their Derivates in Higher Plants*; Wallsgrove, R. M., Ed.; Society for Experimental Biology Seminar 56; University Press: Cambridge, 1995; pp 243−259.

(41) Charron, C. S.; Saxton, A. M.; Sams, C. E. Relationship of climate and genotype to seasonal variation in the glucosinolate-myrosinase system. I. glucosinolate concentration in ten cultivars of *Brassica oleracea* grown in fall. *J. Sci. Food Agric.* **2005**, *85*, 671–681.

(42) Cartea, M. E.; Velasco, P.; Obregon, S.; Padila, G.; de Haro, A. Seasonal variation in glucosinolate content in *Brassica oleracea* crops grown in northwestern Spain. *Phytochemistry* **2008**, *69*, 403–410.

(43) Perner, H.; Schwarz, D.; Krumbein, A.; Li, X.; George, E. Influence of nitrogen forms and mycorrhizal colonization on growth and composition of Chinese bunching onion. *J. Plant Nutr. Soil Sci.* **2007**, *170*, 762–768.

(44) Perner, H.; Rohn, S.; Driemel, G.; Batt, N.; Schwarz, D.; Kroh, L. W.; George, E. Effect of nitrogen species supply and mycorrhizal colonization on organosulfur and phenolic compounds in onions. *J. Agric. Food Chem.* **2008**, *56*, 3538–3545.

(45) Jones, R. B.; Imsic, M.; Franz, P.; Hale, G.; Tomkins, R. B. High nitrogen during growth reduced glucoraphanin and flavonol content in broccoli (*Brassica oleracea* var. *italica*) heads. *Aust. J. Exp. Agric.* **2007**, *47*, 1498–1505.

(46) Sousa, C.; Pereira, D. M.; Pereira, J. A.; Bento, A.; Rodrigues, M. A.; Dopico-Garca, S.; Valentào, P.; Lopes, G.; Ferreres, F.; Seabra, R. M.; Andrade, P. B. Multivariate analysis of tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC) phenolics: Influence of fertilizers. *J. Agric. Food Chem.* **2008**, *56*, 2231–2239.

(47) Stewart, A. J.; Chapman, W.; Jenkins, G. I.; Graham, I.; Martin, T.; Crozier, A. The effect of nitrogen and phosphorus deficiency on flavonol accumulation in plant tissues. *Plant, Cell Environ.* **2001**, *24*, 1189–1197.

(48) Montanari, M.; Degl'Innocenti, E.; Maggini, R.; Pacifici, S.; Paradossi, A.; Guidi, L. Effect of nitrate fertilization and saline stress on the contents of active constituents of *Echinacea angustifolia* DC. *Food Chem.* **2008**, *107*, 1461–1466.

(49) Lea, U. S.; Slimestad, R.; Smedvig, P.; Lillo, C. Nitrogen deficiency enhances expression of specific MYB and bHLH transcription factors and accumulation of end products in the flavonoid pathway. *Planta* **2007**, *225*, 1245–1253.

(50) Olsson, L. C.; Veit, M.; Weissenbock, G.; Bornman, J. F. Differential flavonoid response to enhanced UV-B radiation in *Brassica* napus. *Phytochemistry* **1998**, *49*, 1021–1028.

(51) Treutter, D. Significance of flavonoids in plant resistance: a review. *Environ. Chem. Lett.* **2006**, *4*, 147–157.

(52) Smolen, S.; Sady, W. The effect of various nitrogen fertilization and foliar nutrition regimes on the concentration of sugars, carotenoids and phenolic compounds in carrot (*Daucus carota* L.). *Sci. Hortic.* **2009**, *120*, 315–324.

(53) Kotsiras, A.; Olympios, C. M.; Drosopoulos, J.; Passam, H. C. Effects of nitrogen form and concentration on the distribution of ions within cucumber fruits. *Sci. Hortic.* **2002**, *95*, 175–183.

(54) Dou, H.; Alva, A. K.; Bondada, B. R. Growth and chloroplast ultrastructure of two *Citrus* rootstock seedling in response to ammonium and nitrate nutrition. *J. Plant Nutr.* **1999**, *22*, 1731–1744.

(55) Harbaum, B.; Hubbermann, E. M.; Zhu, Z.; Schwarz, K. Free and bound phenolic compounds in Leaves of pakchoi (*Brassica campestris* L. ssp. *Chinensis* var. *communis*) and Chinese leaf mustard (*Brassica juncea* Coss). *Food Chem.* **2008**, *110*, 838–846.