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Effect of Nitrogen Species Supply and Mycorrhizal Colonization on Organosulfur and Phenolic Compounds in Onions

HENRIKE PERNER,*,†,‡ SASCHA ROHN,§ GREGOR DRIEMEL,§ NATALIE BATT,§ DIETMAR SCHWARZ,† LOTHAR W. KROH,§ AND ECKHARD GEORGE†,‡

Department of Plant Nutrition, Institute of Vegetable and Ornamental Crops e.V. Grossbeeren, Theodor Echtermeyer Weg 1, D-14979 Grossbeeren, Germany, Plant Nutrition, Institute of Crop Science, Humboldt Universität zu Berlin, Invalidenstrasse 42, D-10115 Berlin, Germany, and Department of Food Analysis, Institute of Food Technology and Food Chemistry, Technische Universität Berlin, Gustav Meyer Allee 25, D-13355 Berlin, Germany

The aim of the present study was to test whether variations in the root environment affect the content of health-related organosulfur compounds, total phenolic compounds, and flavonol glycoside concentrations in onions. For this purpose, greenhouse-grown onions (*Allium cepa* L.) were either inoculated with a commercial arbuscular mycorrhizal inoculum or a sterile inoculum and were provided with two NH_4^+:NO_3^- ratios as a nitrogen source. Onion growth, arbuscular mycorrhizal colonization rate, sugars, and nutrient element concentrations were also quantified. The plant antioxidant activity and quercetin monoglucoside and organosulfur compound concentrations increased with dominant nitrate supply. Furthermore, mycorrhizal colonization increased the antioxidant activity and also concentrations of the major quercetin glucosides. The present study provides clear evidence that antioxidant activity, quercetin glycosides, and organosulfur compounds can be increased in sufficiently supplied onion plants by dominant nitrate supply or application of arbuscular mycorrhizal fungi. This was probably due to increased precursor production and induced defense mechanisms.

KEYWORDS: ***Allium cepa* L.; Alliaceae; arbuscular mycorrhizal fungi; nitrogen species; organosulfur compounds; phenolic compounds; quercetin glucosides; antioxidant activity**

INTRODUCTION

The protective effect of fruit and vegetables in human consumption is attributed to their content of phytochemicals and sometimes also to their antioxidant activity (*1, 2*). The consumption of antioxidants with the diet may be associated with a reduced risk of cardiovascular diseases and cancer, because reactive oxygen species in the body are detoxified. A direct relation between intake of antioxidants in the diet and reduced risk of disease, however, is still discussed controversially (*3*).

In this context, onion (*Allium cepa*) is of central interest. Although food usage of onion is often less conspicuous as compared to commodities like tomatoes or *Brassicas*, with a world production of approximately 57 million tons per year, dry onion is the fourth most important vegetable crop (*4*).

The beneficial role of *Allium* species is often attributed to their sulfur (S)-containing compounds (*5, 6*). These health- and flavor-related organosulfur compounds are suggested to be biologically active as antimicrobiotics, as agents in reducing the risk factors of cardiovascular disease, as potential anticancer agents, and as supporters of the respiratory system (*7*). However, for some *Allium* species—especially for *Allium cepa*—the bioactivity cannot be ascribed solely to the S-containing compounds. Onions are one of the richest sources of flavonoids (*8, 9*), and the antioxidant activity of these compounds is well-known. In spite of the fact that 20 or more flavonols are detectable in onions, quercetin-4′-*O*-monoglucoside and quercetin-3,4′-*O*-diglucoside make up to 80% of the total flavonoid fraction, and quercetin-3,4′-*O*-diglucoside is the major flavonoid (*10*). With a view on human nutrition, it is desirable to test crop production systems that stabilize or even increase the amount of potential health-promoting compounds in onion.

For many years, it has been known that nitrogen (N) supply can affect not only bulb yield but also bulb grade or firmness, maturity, and storability (*11, 12*). Although the flavor of onions is attributed to the organosulfur compounds and therefore primarily influenced by the level of S supply to plants, N supply can also have a considerable effect (*13*). In the present study, we therefore modified the form of N supplied (ammonium vs nitrate), which is a realistic measure to change the quality of plants and harvest products. In horticulture practice, it is

* To whom correspondence should be addressed. Tel: +49 33701 78315. Fax: +49 33701 55391. E-mail: perner@igzev.de.
† Institute of Vegetable and Ornamental Crops e.V. Grossbeeren.
‡ Humboldt Universität zu Berlin.
§ Technische Universität Berlin.

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sometimes recommended to follow a fertilization strategy for onions with dominant ammonium fertilization at an early stage and dominant nitrate fertilization later (*14*).

Many previous studies on onion plant nutrition and quality have not considered that plants usually face a range of microorganisms in their root environment. *Allium* plants grown on low-nutrient soils can be highly dependent on arbuscular mycorrhizal (AM) fungi for satisfactory growth and are thus often colonized to a high degree by these fungi (*15*). In consequence, the yield of *Allium* plants grown on mycorrhizal-free substrates can be increased by mycorrhizal colonization (*12*). On high-nutrient substrates, mycorrhizal colonization often does not lead to increased plant growth but may in some cases affect plant metabolism and composition. We hypothesized that AM fungal colonization increases the antioxidant activity in onions, by increasing phenolic compounds as a consequence of defense mechanisms that are initiated after the contact between roots and mycorrhizal propagules. Several plant genes associated with plant defense are expressed as a result of AM symbiosis in the plant roots (*16*).

Soil contains both nitrate and ammonium in varying amounts, depending on soil type, season, and fertilization treatment (*17*). Steward et al. (*18*) concluded from their experiment with N starving *Lycopersicon esculentum* plants that nutrient deficiency can upregulate the flavonoid biosynthetic functions in plant shoots as a protection against further sources of stress such as pathogens. Not only nutrient deficiency but also a predominant uptake of ammonium can be stressful for plants, and ammonium in excess can reduce plant growth (*19*). We therefore hypothesized that a dominant ammonium supply may induce the antioxidant activity in onion plants by increasing flavonol glycoside concentrations.

The objective of the present study was to determine under conditions of sufficient nutrient supply the effect of different ammonium:nitrate ratios and mycorrhizal colonization on onion growth and nutritional characteristics such as shoot dry weight and shoot mineral element concentrations and on shoot quality traits such as sugars and selected phytochemicals. The total pyruvic acid was estimated as a marker for organosulfur compounds. Besides total phenolic concentration, the flavonoid profile of the four major flavonol glycosides was analyzed.

MATERIALS AND METHODS

Onion Cultivation. The commercial substrate Perlite (Knauf Perlite GmbH, Dortmund, Germany) was rinsed with distilled water on a 1 mm sieve to obtain a uniform substrate with a granule size of 1–6 mm and to prevent cation accumulation on the fraction <1 mm. The substrate was autoclaved at 121 °C for 20 min and filled into plastic pots (volume of 10 L). A top layer of gravel on each pot reduced evaporation and algae growth.

Onion sets (*Allium cepa*, cv. Centurion) were suspended in water with 10% H_2O_2 (10 min) for surface sterilization. Subsequently, they were washed with distilled water three times, moistened with $CaSO_4$ solution, and planted directly into the pots.

Twelve onion sets were used per pot. Each treatment level comprised four pots as independent replicates. The pots had small holes at the bottom and were filled with a 5 cm layer of perlite (3.8 L). Then, a 3 cm layer of 10% v/v AM inoculum mixed with perlite was placed in the pot (0.6 L) and covered with perlite (5.6 L). Mycorrhiza inoculum was supplied by Plantworks (TerraVital Hortimix comprising *G. mosseae*, *G. intraradices*, *G. claroideum*, and *G. microaggregatum*, >50 infective units per mL inoculum; Plantworks Ltd., Heeley Close, Sittingbourne, Kent, United Kingdom).

In nonmycorrhizal (NAM) treatments, sterilized (autoclaved at 121 °C for 20 min) Plantworks inoculum was applied. In addition, the drain of nonsterilized Plantworks inoculum was filtered (589/3 blue ribbon paper filter, Schleicher and Schuell Bioscience GmbH, Dassel, Germany) and added to the NAM pots. This procedure was carried out in an attempt to supply similar amounts of nutrients and micro-organisms except AM to all treatments.

The first 2 weeks after planting, twice a day, a fifth-strength modified Hoagland solution at pH 5.6 (*20*) with a MES buffer added was applied to the pots. From the second leaf stage onward, the plants were watered twice a day with a third-strength modified Hoagland solution. A sufficient amount of solution was applied, so that at least one-third of the amount of applied solution drained from the pots. Nitrogen was provided at ammonium:nitrate (NH_4^+:NO_3^-) ratios of 75:25 (dominant NH_4^+ supply) and 5:95 (dominant NO_3^- supply). The nutrient solution consisted of the following macronutrients (mM) (NO_3^-, 1.8, 7.2; and NH_4^+, 5.5, 0.3) depending on the N species treatment: K^+, 2.7; PO_4^{3-}, 0.4; Mg^{2+}, 1.6; SO_4^{2-}, 0.2; Ca^{2+}, 3.6; and micronutrients (μM): Fe^{2+}, 5.5; Mn^{3+}, 2.5; Zn^{2+}, 0.4; BO_3^{3-}, 18; Cu^{2+}, 0.3; MoO_4^{2+}, 0.2; Cl^-, 16133, 5580 depending on the N species treatment. A pH of 5.6 was maintained by adding NaOH and MES buffer at 2 mM. The pots were rinsed with distilled water once a week, to prevent an accumulation of supplied solutes in the substrate. Watering was reduced 2 weeks prior to harvest to let plants dry.

The experiment was carried out from March to June 2005 in a greenhouse facility at Grossbeeren (long. 13°20′E; lat. 51°22′N), Germany. Average air temperatures in the greenhouse during this time were 21 (max 39 °C) day/17 (min 13 °C) night, and the relative humidity was on average during the day 54% and in the night 68%. The daily (15 h) mean light intensity (PAR) was at 16.2 mol m^{-2} (max 662 μmol m^{-2} s^{-1}). The pots were arranged in a completely randomized design.

Harvest. Fourteen weeks after they were planted, the plants were harvested. After the roots were separated from the shoots, leaves were cut 3 cm above the bulbs. The fresh weight (fr. wt) of bulbs was recorded. Bulbs of *A. cepa* were cut into cubes to obtain samples (approximately 30 g fr. wt) used immediately for the determination of total pyruvic acid. In addition, material (10 g fr. wt) was frozen at −20 °C and freeze-dried for sugar analysis (sucrose, glucose, and fructose). Two samples of each treatment were dried at 60 °C for 2 days to record the dry weight (dry wt). The remainder of the cut bulbs was frozen at −20 °C and freeze-dried for the estimation of total phenolic concentration, flavonol glycosides, and antioxidant activity. Bulb dry weight samples were ground in a centrifugal grinder with a 0.25 mm sieve and analyzed for total N, P, K, Mg, and S.

Samples were taken randomly from different places in the pot with the help of a sharp knife. In these samples, roots were separated from the substrate by rinsing with cold water and using a set of sieves (smallest sieve size, 1 mm). Roots were then cut in 1 cm pieces and stored in 10% isopropanol for determination of mycorrhizal colonization.

AM Colonization and Mineral Element Concentration. Mycorrhizal root length colonization was determined following the method of Koske and Gemma (*21*) and Giovanetti and Mosse (*22*). Phosphorus, K, N, NO_3^+, and S concentrations were analyzed and measured following standardized procedures. Magnesium formed a red color complex in an alkaline solution containing xylidylblue, which was measured photometrically at 492 nm with an EPOS-analyzer 5060 (Eppendorf, Hamburg, Germany). Details on the methods used for root length colonization and mineral element concentrations are described in Perner et al. (*23*).

Single Sugar Concentration. For sugar analysis, freeze-dried shoot material was ground, mixed with water, and boiled to denature the sugar enzymes. In the filtrate, sucrose, glucose, and fructose were determined with a commercial enzymatic assay (R-Biopharm GmbH, Darmstadt, Germany). The results were recalculated into fresh weight and expressed as g sugar per kg fresh weight.

Pyruvic Acid Concentration. The flavor precursors of *Allium* species are collectively termed S-alk(en)yl cysteine sulfoxides. These S-alk(en)yl cysteine sulfoxides and especially *trans*-(+)-S-(1-propenyl)-L-cysteine sulfoxide have been shown to correspond with pyruvic acid concentration in onions (*24*). S-Alk(en)yl-L-cysteine sulfoxides are activated by the enzyme alliinase to produce pyruvic acid, ammonia, and sulfenic acids, when *Allium* tissue is damaged (*25*). Therefore, in

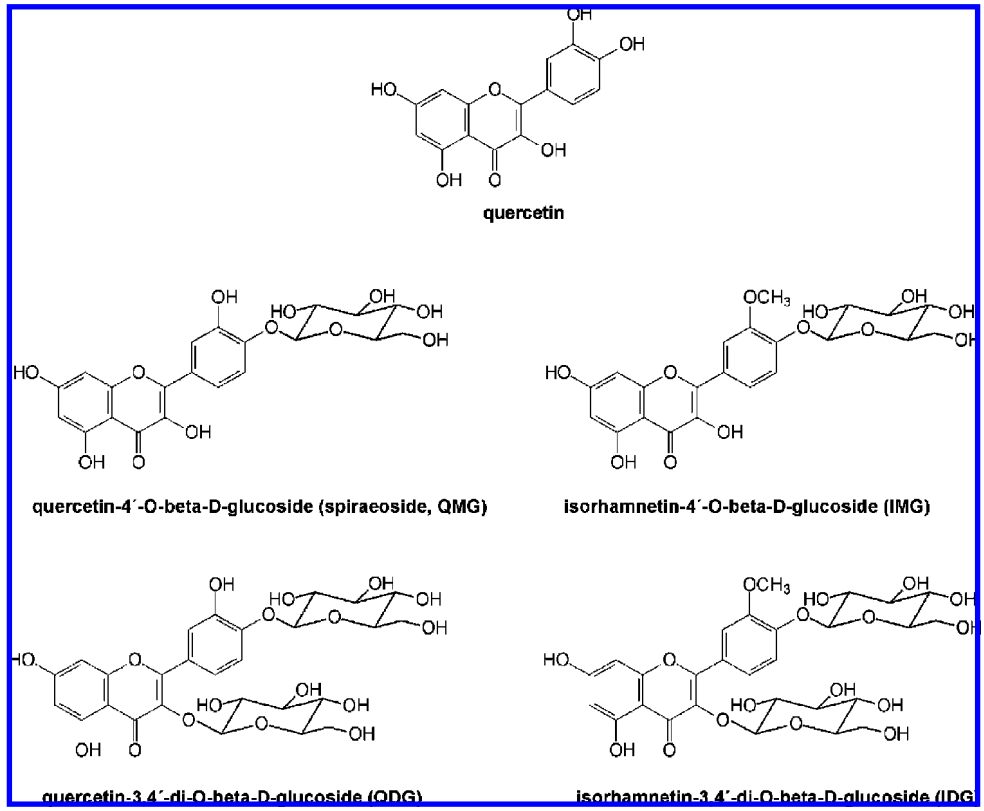

Figure 1. Chemical structures of the four major flavonol glycosides in *Allium cepa*.

the present experiment, pyruvic acid was used as an indicator for organosulfur compounds. Total pyruvic acid was analyzed in 20 g of ruptured tissue of *A. cepa* following the procedure described in Perner et al. (*23*), using the method of Schwimmer and Weston (*26*), modified by Ketter and Randle (*27*). The results are expressed as μM pyruvic acid per g fresh weight.

Total Phenolic Concentration. The total phenolic concentration was determined using the Folin−Ciocalteu method (*28*) with results expressed as mg gallic acid equivalents (GAE) per g dry weight. Absorbance was measured at 765 nm (LKB-Novaspek II, Pharmacia, Freiburg, Germany). Appropriate dilutions of methanolic extracts were prepared in duplicate with distilled water. The mixture developed a dark blue color and was left to react for 1 h. Duplicate samples and calibration standards were prepared in plastic cuvettes and measured.

Flavonol Glycoside Concentration. Besides total phenolic concentration, the flavonoid profile of the four major flavonol glycosides (quercetin-4′-*O*-monoglucoside, quercetin-3,4′-*O*-diglucoside, isorhamnetin-4′-*O*-monoglucoside, and isorhamnetin-3,4′-*O*-diglucoside) was analyzed. Their structures are given in **Figure 1**.

High-performance liquid chromatography (HPLC) analysis of the flavonols glycosides was performed as described by Buchner et al. (*29*). Reference substances were isolated from onions using multilayer counter current chromatography (*30*). For the analysis of onion bulbs, 2.5 g of lyophilized material was extracted with 50 mL of aqueous methanol (70%) for 30 min under continuous stirring. Following filtration, 4 mL of the filtrate was diluted with the same amount of water and then loaded onto a polyamide (500 mg) solid phase extraction column. The column was washed with 10 mL of water to remove sugars and further water-soluble compounds. The flavonol glycosides were eluted with 10 mL of a methanol/water/acetic acid mixture (90:5:5, v/v). This eluate was applied to the HPLC-diode array detection analysis.

Antioxidant Activity. The antioxidant acticivity of the onion samples was investigated using two different methods. In addition to the trolox equivalent antioxidant capacity assay (TEAC), electron spin resonance (ESR) was used to evaluate the ability of the onion samples to scavenge radicals. In the TEAC assay, this ability is compared to a synthetic antioxidant (trolox), which is a water-soluble vitamin E analogue. In the ESR measurements, the degradation of a synthetic stabilized radical (Fremy's salt) is followed.

The antioxidant activity was determined using the TEAC and measured with a spectrophotometer (Specord 40, analytik jena AG, Jena, Germany) at 734 nm (*31*). Results are expressed as trolox equivalents and have been calculated as mM trolox per g dry weight. To prepare samples, methanolic onion extracts were adequately diluted in duplicate with phosphate-buffered saline (PBS) buffer. A 500 μL aliquot of ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, 500 mM L^{-1}] was added to 100 μL of diluted extract or blanks of PBS buffer, in plastic cuvettes. To initiate the reaction, 200 μL of potassium persulfate was added to each sample. The mixture developed a dark green color. Exactly 6 min after initiation, samples were measured with a spectrophotometer. The procedure was also carried out for the trolox calibration standards.

The degradation of the radical forming compound, Fremy's salt (potassium nitrosodisulfonate), in the presence of antioxidants in the samples was monitored with ESR as described by Roesch et al. (*32*). Appropriate methanolic onion extract dilutions were prepared, and 100 μL aliquots were allowed to react for 20 min with an equal volume of a solution of Fremy's salt (1 mM in phosphate buffer, pH 7.4). The ESR spectra of Fremy's salt radicals were obtained with a Miniscope MS100 spectrometer (Magnettech GmbH, Berlin, Germany) and the software Kinetic Kapillare MS100 Control 2.0. The antioxidant activity, expressed as mM Fremy's salt per g dry weight, was calculated by comparison with a control reaction with 100 μL of Fremy's salt 1 mM and 100 μL of phosphate buffer, pH 7.4.

Statistical Analysis. Plants were either inoculated with AM fungi or noninoculated (NAM) and supplied with two different NH_4^+:NO_3^- ratios in supply. Values presented are means of four observations ± standard errors of the mean (SE). Effects of the treatments (mycorrhizal inoculation, myc; NH_4^+:NO_3^- ratios in supply, N) were tested with a two-way analysis of variance (ANOVA) with a significance level of $P < 0.05$. Where appropriate, probability values are reported for the results of the ANOVA. Differences between the levels of AM treatments at each level of N species and differences between the levels of N species at each level of AM treatment were additionally tested by the low

Table 1. Effect of the Treatments N Species (N) and AM Inoculation (myc) on Bulb Fresh Weight, Bulb Dry Weight, Bulb Pyruvic Acid Concentration, and Bulb Total Phenolic Concentration (GAE) of *Allium cepa*[a]

N supply	bulb fresh weight (g pot^{-1})	bulb dry weight (g pot^{-1})	pyruvic acid [μmol (g fr. wt)$^{-1}$]	GAE [mg (g dry wt)$^{-1}$]
dominant NH_4^+				
NAM	476 ± 8 a	67 ± 1 a	1.03 ± 0.02 a	1.08 ± 0.05 a
AM	495 ± 12 A	69 ± 2 A	1.03 ± 0.04 A	1.15 ± 0.01 A
dominant NO_3^-				
NAM	837 ± 37 b	117 ± 6 b	1.37 ± 0.04 b	1.15 ± 0.03 a
AM	797 ± 30 B	114 ± 5 B	1.40 ± 0.07 B	1.29 ± 0.08 A
P (N)	<0.001	<0.001	<0.001	0.058
P (myc)	0.670	0.831	0.744	0.064
P (N × myc)	0.254	0.634	0.733	0.516

[a] Plants were either inoculated with AM fungi or noninoculated (NAM). Effects of the treatments were tested with a two-way ANOVA. Significant differences between means were determined by the Student−Newman−Keuls test ($P < 0.05$). Differences between the two levels of AM treatment at each level of N species were not significant. Differences between the two levels of N species at each level of AM treatment are denoted with small (NAM) or capital (AM) letters. Values are means of four observations ± SE.

Table 2. Effect of the Treatments N Species (N) and AM Treatment (myc) on Bulb N, P, K, Mg, and S Concentrations of *Allium cepa*[a]

	g (kg dry wt)$^{-1}$				
N supply	N	P	K	Mg	S
dominant NH_4^+					
NAM	24.6 ± 0.4 b	3.9 ± 0.1 b	14.2 ± 0.2 a	1.0 ± 0.0 a	2.8 ± 0.0 b
AM	24.8^{ns} ± 0.5 B	3.6^{ns} ± 0.0 B	15.2* ± 0.1 A	0.9 ^{ns} ± 0.0 A	2.7^{ns} ± 0.1 B
dominant NO_3^-					
NAM	21.6 ± 0.4 a	2.8 ± 0.1 a	16.0 ± 0.1 b	1.2 ± 0.0 a	2.3 ± 0.1 a
AM	21.7^{ns} ± 0.6 A	3.0 ^{ns} ± 0.0 A	15.4* ± 0.2 B	1.2 ^{ns} ± 0.0 A	2.3^{ns} ± 0.0 A
P (N)	<0.001	<0.001	0.005	<0.001	<0.001
P (myc)	0.838	0.879	0.617	0.525	0.868
P (N × myc)	0.958	0.022	0.021	0.073	0.390

[a] Plants were either inoculated with AM fungi or noninoculated (NAM). Effects of the treatments were tested with a two-way ANOVA. Significant differences between means were determined by the Student−Newman−Keuls test ($P < 0.05$). Differences between the two levels of AM treatment at each level of N species are denoted as * or ns. Differences between the two levels of N species at each level of AM treatment are denoted with small (NAM) or capital (AM) letters. Values are means of four observations ± SE.

critical value of a Newmann−Keuls test ($P < 0.05$). Data were analyzed using Statistica 6.1 (StatSoft, Tulsa, OK) software.

RESULTS

Mycorrhizal Colonization and Plant Growth. Root length colonization rates of roots from mycorrhizal plants were 55.1 ± 10.7% at dominant NO_3^- supply and 26.1 ± 4.3% at dominant NH_4^+ supply. Roots of noninoculated plants remained free of AM colonization.

Nitrogen species affected bulb fresh and dry weight (**Table 1**). At dominant NH_4^+ supply, bulb growth was significantly decreased as compared to dominant NO_3^- supply. Mycorrhizal inoculation had no significant effect on bulb fresh and dry weight.

Plant Mineral Element Concentrations and Content. Nitrogen species affected bulb N, P, K, Mg, and S concentrations significantly (**Table 2**). Bulb N, P, and S concentrations were significantly increased at dominant NH_4^+ supply as compared to dominant NO_3^- supply. In contrast, bulb K and Mg concentrations were significantly decreased at dominant NH_4^+ supply. Colonization with AM fungi influenced bulb K concentration. At dominant NH_4^+ supply, K concentration was increased in AM plants as compared to NAM plants, whereas at dominant NO_3^- supply, AM colonization decreased the bulb K concentration (**Table 2**). Total bulb N, K, and Mg content was increased significantly at dominant NO_3^- supply as compared to dominant NH_4^+ supply (data not shown). Total bulb P and S contents were not influenced by N species (data not shown). Inoculation with AM fungi had no significant effect on total bulb content of any of the analyzed nutrient elements (data not shown).

Sucrose, Reducing Sugar, Pyruvic Acid, and Total Phenolic Concentration. The concentrations of sucrose and reducing sugars (glucose and fructose) in the bulbs were not significantly affected by AM colonization or N species. Mean sucrose, glucose, and fructose concentrations were determined as 38.47 ± 2.21, 13.22 ± 1.26, and 12.66 ± 1.09 g (kg fr. wt)$^{-1}$, respectively.

The bulb concentration of pyruvic acid, an indicator for organosulfur compounds, was significantly increased at dominant NO_3^- supply (**Table 1**), whereas AM colonization had no significant effect. The total phenolic concentration in the onion bulb appeared to be higher at dominant NO_3^- supply and AM colonization, but differences were not significant (**Table 1**).

Profile of Flavonol Glycosides. The bulb quercetin-4′-*O*-monoglucoside concentration was influenced by N species (**Figure 2**). Dominant NO_3^- supply increased its concentration significantly (two factorial ANOVA, $P(N) = 0.001$) as compared to dominant NH_4^+ supply. The bulb concentration of quercetin-3,4′-*O*-diglucoside was not significantly affected by N species but tended also to higher values at dominant NO_3^- supply (**Figure 2**). For both substances, AM colonization significantly increased their concentration [two factorial ANOVA: quercetin-4′-*O*-monoglucoside, $P(myc) = 0.035$; quercetin-4′-*O*-diglucoside, $P(myc) = 0.029$]. Concentrations of the isorhamnetin glucosides were lower [isorhamnetin-3,4′-*O*-diglucoside, 0.03–0.04

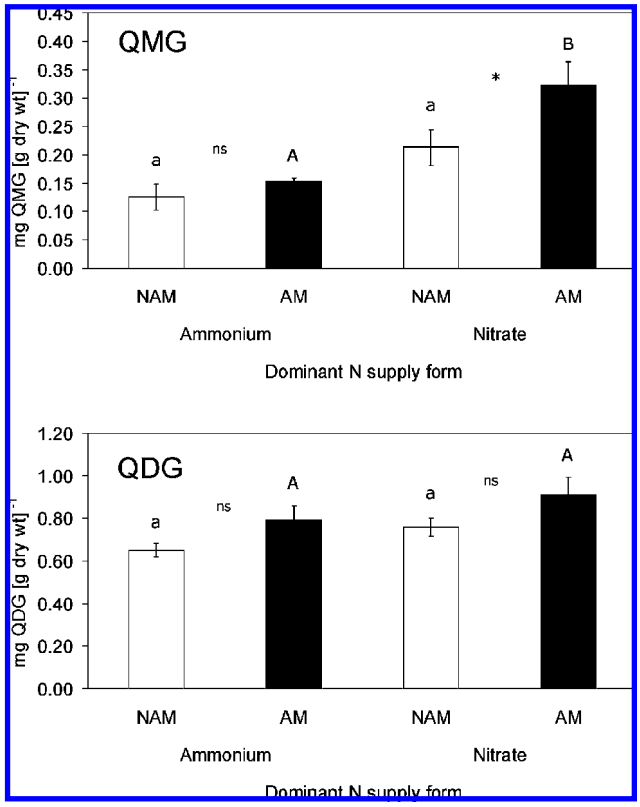

Figure 2. Effects of nitrogen species and AM treatments on quercetin-4′-*O*-monoglucoside (QMG; upper) and quercetin-3,4′-*O*-diglucoside (QDG; lower) concentrations in onion bulbs. Differences between the two levels of AM treatment at each level of N species are denoted as * or ns. Differences between levels of N species at each level of AM treatment are denoted with small (NAM) or capital (AM) letters.

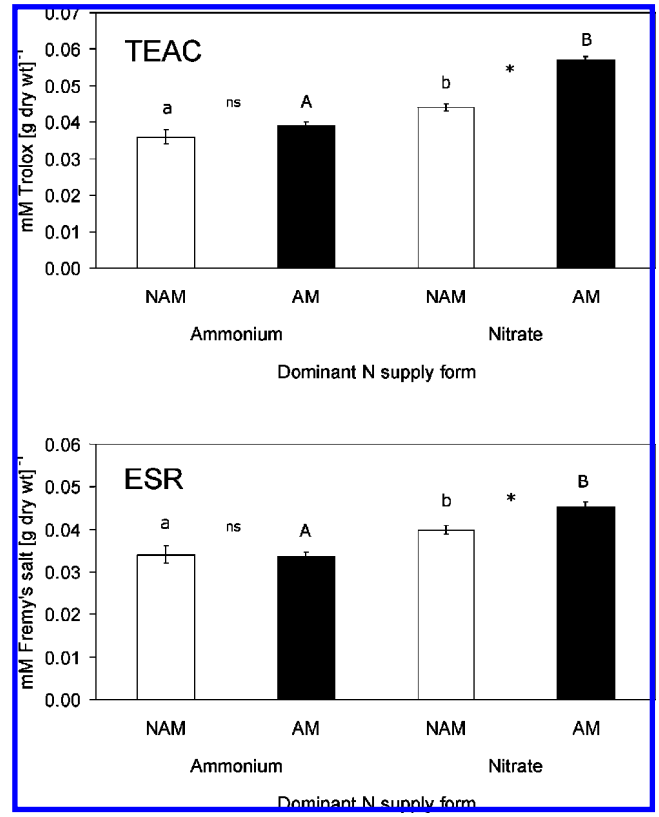

Figure 3. Effects of nitrogen species and AM treatments on antioxidant activity concentration in onion bulbs. The antioxidant activity was determined by TEAC assay (upper) and ESR spectrometry (lower). Differences between the two levels of AM treatment at each level of N species are denoted as * or ns. Differences between levels of N species at each level of AM treatment are denoted with small (NAM) or capital (AM) letters.

mg (g dry wt)$^{-1}$; isorhamnetin-4′-*O*-monoglucoside, 0.09–0.12 mg (g dry wt)$^{-1}$]. The bulb concentration of isorhamnetin-3,4′-*O*-diglucoside was significantly decreased [two factorial ANOVA, $P(N) < 0.001$] at dominant NO_3^- supply (data not shown). Isorhamnetin-4′-*O*-monoglucoside was not significantly influenced by the treatments but tended to higher values at dominant NO_3^- supply (data not shown).

Antioxidant Activity. Both methods of determination showed that the antioxidant activity was significantly [two factorial ANOVA: TEAC, $P(N) < 0.001$; ESR, $P(N) < 0.001$] increased at dominant NO_3^- supply (**Figure 3**). Furthermore, AM colonization increased antioxidant activity significantly in plants at least at dominant NO_3^- supply (**Figure 3**) [two factorial ANOVA: TEAC, $P(myc) = 0.001$; ESR, $P(myc) = 0.183$]. Both methods of measurements resulted in significant interactions between N species treatment and AM treatment [two factorial ANOVA: TEAC, $P(N \times myc) = 0.033$; ESR, $P(N \times myc) = 0.029$].

DISCUSSION

Plant Constitution—Plant Growth, AM Colonization, Element Supply, and Sugar Concentration. At the time of harvest, all onion plants had formed bulbs, irrespective of treatments. Plant growth and AM fungi colonization were both decreased at dominant NH_4^+ supply treatment. Ammonium counter transport with H^+ ions results in intense ATP demand and can cause the acidification of the rhizosphere (*33*). This can lead to growth depression as observed in the present experiment. To prevent pH changes in the rhizosphere, a buffer (MES) was applied with the nutrient solution. However, in perlite culture, the MES buffer capacity may not be sufficiently high to prevent rhizosphere pH decrease (*23*).

AM fungi can stimulate plant growth and increase plant uptake of nutrients, especially P. The effect is based on the characteristics of hyphae to exploit a larger soil volume than roots alone. This way, mycorrhizal plants absorb soil P more efficient than NAM plants and supply significant amounts of P to the plant according to its demand (*34*). In the present experiment, plant growth was not significantly changed by AM colonization. This was expected because all plant nutrients were provided in sufficient amounts to the root surface by regular application of nutrient solution. A comparison of the bulb element concentrations of the present experiment with field grown onions in the literature (*35*) revealed high N concentrations in the present experiment, while K and Mg concentrations were in a lower range. Plant phosphorus concentrations were adequate at dominant NH_4^+ supply, whereas P concentration at dominant NO_3^- supply was low. Nevertheless, no growth depression was observed at dominant NO_3^- supply. Therefore, it appears very likely that in the present experiments plant growth did not rely on the hyphal uptake of P or other nutrients. This allowed us to study the effect of AM colonization on *Allium cepa* plant composition without interfering effects of different plant size between AM treatments.

Total bulb nutrient contents of plants (g per pot) grown at dominant NO_3^- supply were similar or higher as compared to plants supplied with dominant NH_4^+. This indicates that the increased N, P, and S concentrations in the bulb tissue of plants grown with dominant NH_4^+ supply were probably the result of

element accumulation due to slow growth of the plant bulbs. The increased K concentration at dominant NO_3^- supply might be the result of an increased plant K uptake via channels and transporters induced by dominant NO_3^- supply (*36, 37*).

Sugars are the energy source for plant metabolism and are also used to estimate the degree of maturation and quality in fruits and vegetables in practical horticulture. Sucrose concentrations were in a similar range as compared to average sucrose concentrations of 54 cultivars tested by Randle (*38*), whereas glucose and fructose concentrations were slightly lower but still within the variations of the different cultivars. The plant nutrient supply and sugar concentrations indicate that plants were grown under balanced growth conditions regarding nutrient and energy supply.

Organosulfur Compounds. AM fungi had no influence on the broad formation of organosulfur compounds, measured as pyruvic acid, supporting the findings of Guo et al. (*39*) in young onion plants. Guo et al. (*39*) showed that AM colonization increased onion growth, but AM colonization did not affect total pyruvic acid concentration. In contrast, dominant NO_3^- supply increased in the present study the organosulfur compound concentration within the bulb. Previous studies showed that organosulfur compound concentrations in *Allium* species can also be enhanced by increasing S supply in the field (*40*) and increasing N concentration in nutrient solution (*13*) that induced an increased bulb S and N concentration, respectively. In the present study, however, pyruvic acid, as an indicator for organosulfur compound concentrations, did not correspond with the changes in bulb S concentration or bulb N concentration (**Tables 1** and **2**). The main reason for the higher concentrations of organosulfur compounds was probably the optimal metabolic growth conditions for onion plants at dominant NO_3^- supply.

Antioxidant Activity—A Result of Total Phenolic Compounds and Flavonol Glucosides? In the present experiment, it was shown that antioxidant activity was enhanced by the application of AM fungi and by dominant NO_3^- supply. Antioxidant activity in onions is mainly attributed to phenolic compounds (*8*).

The measurement of the total phenolic content showed only tendencies toward an increase with AM fungi and dominant NO_3^- supply (**Table 1**). The measurement of the four major flavonol glucosides, however, revealed a significant increase of quercetin-4′-*O*-monoglucoside and quercetin-3,4′-*O*-diglucoside concentrations with AM fungi and/or dominant NO_3^- supply (two-way ANOVA). In contrast to the quercetin glucosides, isorhamnetin-3,4′-*O*-diglucoside was inversely affected, and isorhamnetin-4′-*O*-monoglucoside was not significantly affected. This result and the unknown contribution of all other phenolic compounds may be an explanation for the nonsignificant effect on the total phenolic concentrations. Although the total phenolic concentrations were not significantly modified, the quercetin glucosides may have sufficient activity to be responsible for the increased antioxidant activity due to their increased concentration. Quercetin glucosides are not as effective in scavenging radicals as their aglycone quercetin, but because of their concentrations, they are potent enough to be important for the total antioxidant activity of onions (*8, 41*).

The increased formation of flavonol glycosides in AM colonized plants may be regarded as phytochemicals formation for the defense of plants against stress and exogenous attacks. Transcripts of genes related to plant defense responses were detected in arbuscule-containing root cells, such as major enzymes in phenolic compound biosynthesis (*16*). Chalcone synthase, a key enzyme in flavonoid biosynthesis, and phenylalanine ammonia lyase were induced in mycorrhizal roots (*16, 42, 43*). The latter authors also found a transient accumulation of salicylic acid (a phenolic molecule involved in plant−pathogen interactions) during the early stages of the interaction between rice and *Glomus mosseae*. Until today, most of these observations have only been conducted in roots without considering the effect on the above root plant parts, especially the edible parts. From the present results though, it can be concluded that phenolic compounds, such as quercetin-4′-*O*-monoglucoside and quercetin-3,4′-*O*-diglucoside, may be increased by AM fungi not only in the roots but also in the bulbs of onions.

In the present experiment, the antioxidant activity of the onion bulbs was also increased when plants were fertilized dominantly with NO_3^-. As already mentioned, this may be due to increased quercetin-4′-*O*-monoglucoside and quercetin-3,4′-*O*-diglucoside concentrations. This contradicted our expectation that dominant NH_4^+ supply will induce antioxidant activity by increasing flavonol glycoside.

The biosynthesis of phenolic compounds is in close relationship with plant N supply and N metabolism, because the precursors for all phenolic compounds are the amino acids tyrosine and phenylalanine. In the past, studies on this topic focused on nutrient stress such as N deficiency in tomato plants, leading to an enhanced utilization of the flavonoid biosynthetic pathway (*18, 44*). Quercetin-3-*O*-glucoside content and mRNA levels of chalcone synthase were increased in the N-deficient tomato leaves. A similar study was carried out by Mogren et al. (*45*) under field conditions, examining quercetin concentrations in marketable onion bulbs with reduced N fertilization. The results showed that a reduced N supply in practical horticulture did not cause the required nutrient stress to increase quercetin derivative concentrations.

In the present experiment, the onion plants were not N-deficient but changed their flavonol glycosidic profile and increased the concentrations of the quercetin glucosides and antioxidant activity with a decreased NH_4^+:NO_3^-ratio in the supplied nutrient solution. Stress may be induced by dominant NH_4^+ supply leading to increased isorhamnetin-3,4′-*O*-diglucoside concentration but not to an increased antioxidant activity. Dominant NH_4^+ supply can have a number of physiological consequences for the plant. Viktor and Cramer (*19*) observed, for example, an increased activity of phosphoenolpyruvate carboxylase and carbonic anhydrase activity at dominant NH_4^+ supply as compared to dominant NO_3^- supply in tomato roots.

The AM fungi and dominant NO_3^- supply supplemented each other in their effect on plants antioxidant activity with each other. At dominant NH_4^+ supply, the antioxidant activity was lower in AM plants than at dominant NO_3^- supply. The most obvious reason may be the lower AM colonization rate at dominant NH_4^+ supply, leading to lower induction of defense mechanisms and thus to less production of phenolic compounds and antioxidant activity.

The present study provides clear evidence that antioxidant activity, quercetin glycosides, and organosulfur compounds may not only be increased in nutrient-deficient plants but even in sufficiently supplied plants by changing the NH_4^+:NO_3^- ratios in supply or by application of soil microorganism such as AM fungi. These findings should be followed by more studies on the physiological and molecular level (to understand the principles of formation of important phytochemicals in plants under different environmental conditions) and on

the horticultural level (to define systems for production of onions with specifically high health value).

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