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Organically Fertilized Onions (*Allium cepa* L.): Effects of the Fertilizer Placement Method on Quercetin Content and Soil Nitrogen Dynamics

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Field-cured onions cv. Hyskin (*Allium cepa* L.) supplied with organic nitrogen fertilizer were studied. The fertilizer was applied by broadcasting and harrowing, broadcasting and rotary cultivation, or placement between rows. Nitrogen dynamics were monitored throughout the growing season by soil sampling. Variation in quercetin content in the onion scales was analyzed by HPLC. The organically fertilized onions were compared with inorganically fertilized onions grown in the same field. Inoculation with arbuscular mycorrhizal fungi (AMF) in the row at sowing or during commercial transplant production was tested but did not significantly affect mycorrhizal root colonization levels in the field. Onions that received no fertilizer at all or that had fertilizer placed between rows had better establishment, probably due to more favorable soil nitrogen concentrations for seedling emergence. Broadcast application led to higher nitrogen concentration in the root zone, resulting in fewer but larger individual onions. Quercetin levels were not significantly altered as a result of nitrogen fertilizer source (inorganic or organic), application method, or mycorrhizal inoculation. However, variation between years was significant, with quercetin levels in 2004 almost twice as high as those in 2005.

KEYWORDS: ***Allium cepa*; onion; quercetin glucosides; organic fertilizer; inorganic fertilizer; nitrogen fertilizer; arbuscular mycorrhizal fungi (AMF); HPLC**

INTRODUCTION

The common yellow onion (*Allium cepa* L.) has a shallow root system; at harvest the final rooting depth is only 0.3 m (*1*). This means that nitrogen fertilization in the upper part of the soil profile is important throughout the whole season. However, excess applications of N fertilizer should be avoided since they have little effect on yields but increase bulb decay (*2*). Using critical nutrient concentrations in onion plant tissue as an indicator of the N nutritional status has been found to be problematic (*3*), but soil N content can be used instead (*4*). The application method may affect the availability of nutrients, and broadcast granular fertilizers are sometimes found to be inefficient at supplying nitrogen to wide-spaced row crops such as onions (*5*).

At present there is considerable debate about the specific components responsible for beneficial health effects from onions, but two main groups of chemical compounds have been proposed: the flavor precursors alk(en)yl sulfoxides (ACSOs) and the flavonoids (*6*). In a recent study, based on a large data set from southern European populations, an inverse association between the frequency of use of onions and the risk of several common cancers was found (*7*).

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Yellow onions contain high concentrations of flavonols, mainly quercetin and its derivatives. In plants, flavonoids occur primarily as glycosides, with different sugar groups linked to one or more of the hydroxyl groups (*8*). The highest concentrations of flavonoids in onion are found in the outer dry scales, so the greatest loss of flavonoids takes place when the onions are peeled (*9*). The formation of flavonol glycosides normally depends on the action of light (*10*) and is induced by UV light (*11*). In onion, the main flavonol glycoside forms have been found to be quercetin 4′-monoglucoside and quercetin 3,4′-diglucoside (*12*).

The factors which motivate people to buy or avoid organic produce are complex (*13*). A slight trend toward lower levels of pesticide residues in vegetables and vegetable products from organic production but no major difference in levels of heavy metals compared with conventionally grown vegetables has been found (*14*). Conventionally cultivated or minerally (inorganically) fertilized vegetables normally have a far higher nitrate content than organically produced or fertilized vegetables (*14*). However, no difference in nitrate content between vegetables from integrated and organic production was found in a Swedish study (*15*).

Differences between cultivars tend to be greater than those found between organic and conventional cultivation systems, and it is a reasonable assumption that increased use of resistant

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Table 1. Soil N_{min} Values (kg of N ha^{-1}) Based on Soil Samples (0–30 cm) Taken in April, June, July, and August and Actual Amount of N (kg ha^{-1}) Added as Fertilizer at Sowing/Planting and in the Beginning of June to the Different Treatments Each Year

year	planting method	N fertilizer	N application method and mycorrhiza treatment	April		June		July	August
				N_{min}	added N	N_{min}	added N	N_{min}	N_{min}
2004	directly sown	no fertilizer		12		30		34	14
	directly sown	Biofer	broadcast, harrowed	12	150	112		149	60
	directly sown	Biofer	broadcast, rotary cultivation	12	150	154		151	64
	directly sown	Biofer	placed	12	150	45		87	44
	directly sown	Biofer	placed + Vaminoc	12	150	35		77	48
	directly sown	NPK, inorganic	broadcast, harrowed	10	30	42	30	96	24
2005	directly sown	no fertilizer		15		46		51	13
	directly sown	Biofer	broadcast, harrowed	15	150	145		112	26
	directly sown	Biofer	placed	15	150	75		69	21
	directly sown	NPK, inorganic	broadcast, harrowed	10	30	32	40	75	12
	transplants	Biofer	placed, no inoculum	21[a]	150[a]	16		55	12
	transplants	Biofer	placed − AMF	21[a]	150[a]	19		55	11
	transplants	Biofer	placed + AMF	21[a]	150[a]	17		55	11

[a] Soil N_{min} analysis and N fertilization were performed before planting in May 2005.

cultivars in organic agriculture will cause higher contents of defense-related secondary metabolites in organic products (*16*). Different food production methods may result in differences in the content of secondary metabolites such as polyphenolic compounds. The food production method has been found to affect the content of the major flavonoid quercetin when diets that reflected a realistic composition from the consumer perspective were studied (*17*). However, the possibility cannot be excluded that the effect originated from the varietal differences between the organically and conventionally produced diets. It has been found that root colonization by arbuscular mycorrhizal fungi (AMF) may influence the composition of flavonoids in plants (*18*). A previous study has shown that limited or high levels of inorganic nitrogen fertilizer had almost no effect on onion quercetin content (*19*).

The aim of the present work was to investigate if the growing system could alter the flavonol concentration and/or composition in the scales of the common yellow onion. The main factors studied were the effect of organic nitrogen fertilizer compared to mineral nitrogen fertilizer, fertilizer placement, planting method, and mycorrhizal inoculation.

MATERIALS AND METHODS

Location of Field Study and Soil Sampling. The field study was performed in 2004 and 2005 at Torslunda Research Station, Öland, in southeastern Sweden (56°38′ N, 16°30′ E). Onion is one of the most important crops grown on Öland. The soil at the site is a sandy loam with a medium organic matter content (3.3%) and a pH of 7.2. In all experiments, water was supplied by irrigation as required on the basis of rain and evaporation data until the middle of July. Soil samples (0–30 cm) were taken 2 days before sowing each year and at 5 cm distance from the onion rows at regular intervals during the growing season of 2004 and 2005. Fresh soil samples were shaken with 2 M KCl for 2 h, and then the extracts were filtered. The content of mineralized nitrogen (N_{min} = ammonium + nitrate) was measured in these extracts by flow injection analysis (FIA) according to the international standard ISO 14 256-2.

Experimental Design and Fertilizer Treatments. *Organically Fertilized Onions, Directly Sown.* In 2004 and 2005, randomized four-block experiments with five and three fertilization treatments, respectively, were conducted. Seeds of onion (*A. cepa* L.) cultivar (cv.) Hyskin F1 without insecticide coating were sown directly in the field at the end of March in 2004 and at the beginning of April in 2005. The rows were placed 0.5 m apart, and seed rate was ≈39 seeds per row meter. No crop protection chemicals were used, and weeds were removed mechanically and by hand.

Nitrogen was supplied before sowing in the form of Biofer (6:3:12) (Gyllebo Gödning AB), a commercial organic pellet fertilizer product consisting of slaughter meat powder and waste products from yeast production. Biofer is approved for usage in certified organic growing in Sweden. Biofer was supplied at a rate equivalent to 150 kg of N ha^{-1}, with an estimated release of 75 kg of N ha^{-1} during the growing season. No extra N was supplied during the growing season (**Table 1**).

In 2004, there were five different treatments. One treatment received no fertilizers at all during the whole season. The second treatment received Biofer broadcast followed by harrowing, while the third treatment received Biofer broadcast followed by rotary cultivation. In the fourth and fifth treatments, Biofer was placed and incorporated between the rows at sowing. The fifth treatment also received Vaminoc (Microbio Ltd.), a granulated mycorrhizal inoculum, at a rate of 10 g per row meter, placed below the seed at a depth of about 5 cm.

In 2005 there were three fertilizer treatments: no fertilizer, Biofer broadcast followed by harrowing, and Biofer placed between the rows at sowing.

Organically Fertilized Onions, Transplants. In 2005, a randomized block experiment with transplanted onions was added. Three different treatments were tested: no inoculum added to the transplant substrate, inoculation of the transplant substrate with soil containing roots of *Sorghum bicolor* (−AMF), and inoculation of the transplant substrate with soil containing roots of *S. bicolor* colonized with the arbuscular mycorrhizal fungus (+AMF) *Glomus intraradices* Schenck & Smith (BEG87). The sorghum plants had been grown for 10 weeks in the greenhouse in pots containing 3:2 soil:sand.

Onion transplants in 5 cm high and 3.7 × 3.7 cm^2 substrate cubes were grown in a greenhouse by a commercial grower until planting in the field. For the two inoculated treatments, the sheared sorghum roots were mixed with a commercial, peat-based substrate to a final concentration of approximately 5% (w/v) before filling of the substrate into trays. Four to six seeds of onion cv. Hyskin F1 without insecticide coating were sown per cube by an automatic sowing device in the beginning of April 2005. The plants were grown in the greenhouse until the middle of May 2005 when they were transplanted into the field at a density of 10 cubes per row meter. The organic fertilizer Biofer was placed between the rows at a rate equivalent to 150 kg of N ha^{-1}, with an estimated release of 75 kg of N ha^{-1} during the growing season. No extra N was supplied during the growing season (**Table 1**). No crop protection chemicals were used, and weeds were removed mechanically and by hand.

Inorganically Fertilized Onions, Directly Sown. The field experiments with directly sown onion seeds and inorganic fertilizers were carried out on separate areas within the same field as the organically fertilized onions in both years. The same seed batch of cv. Hyskin F1 without insecticide coating as in the organically fertilized experiment was used in the inorganically fertilized experiment each year. The seeds were sown directly in the field at the end of March in 2004 and at the beginning of April in 2005. The rows were placed 0.5 m apart, and seed rate was ≈39 seeds per row meter. Pest and weed management was by chemical and mechanical means according to conventional commercial practices in Sweden. The inorganically fertilized onions were part of a larger field experiment carried out in four completely randomized blocks, which is described in ref 20.

On the basis of the soil analyses and using the target value of 40 kg of N ha^{-1} suggested by Gertsson and Björklund (*4*), nitrogen was supplied as NPK-micro to the inorganically fertilized onions at sowing. Additional phosphorus was supplied as PK, and additional potassium was supplied as KMg and KS fertilizers. All fertilizers were broadcast and harrowed down into the soil before sowing. In the beginning of June each year, new soil samples were taken in the same way as at sowing, described above, and the onions were supplied with calcium nitrate on the basis of soil N_{min} analyses up to the target value for June (72 kg of N ha^{-1}), resulting in an additional 30 kg of N ha^{-1} in 2004 and 40 kg of N ha^{-1} in 2005 (**Table 1**).

Harvest. On 17 June 2004 and 15 June 2005, plants from 1 m row length were sampled for determination of plant number, plant weight, and mycorrhizal root colonization. In August the onions were lifted from the ground when 80% of the onion plants had fallen leaves, and they were left on the field to dry and cure in windrows for 10–14 days. After this time the onion bulbs had developed papery outer scales, and the necks were dry and tight. The onions were placed in wooden bins and stored in a ventilated shed at ambient temperature until size grading and extraction of flavonoids in October each year.

Quercetin Extraction. Onions with a diameter of 55–70 mm and a weight of ≈100 g were chosen for extraction. Each onion sample consisted of 10 onions from each experimental plot. The roots, the leaves, and the outer dry skins were removed to mimic domestic peeling. Each onion was divided longitudinally from the top to the base into four wedge-shaped pieces. Two opposite pieces from each onion were chopped and homogenized in a Waring blender. Four portions (2004) or three portions (2005) of each onion sample, each comprising 5.00 g of homogenized onion tissue, were extracted for 2 weeks at −20 °C in 20 mL of acidified (150 mM HCl) ethanol. This long extraction method had been used in previous experiments and was found to be convenient and resulted in good extraction efficiency and repeatability (*19–21*). The extracts were centrifuged at 16500*g* for 10 min at 4 °C, transferred into Eppendorf tubes, and stored for a short period at −20 °C until analysis. Before HPLC analysis, the Eppendorf tubes were thawed and centrifuged at 16500*g* for 5 min at 20 °C.

HPLC Analysis. The analyses of the onion extracts were performed on an Agilent 1100 HPLC system. The column used was a Phenomenex Luna 5u C18(2) (150 × 4.6 mm, 5 μm). The mobile phase consisted of (A) 50 mM acetic acid (HAc) in Millipore ultrapure water with 5% acetonitrile (v/v) and (B) acetonitrile with 5% methanol (v/v). The flow rate was 1.0 mL min^{-1} and the injection volume 10 μL. The binary gradient used was as follows: 0–2 min, 0% eluent B; 2–17 min, 0–45% B; 17–20 min, 45–80% B; 20–21 min, 80% B; 21–23 min, 80–0% B; 23–25 min, 0% B. External standards used for identification and quantification were quercetin (Sigma-Aldrich Chemie Gmbh) and quercetin 4′-glucoside (Extrasynthese). The absorbance was measured at 370 nm using an Agilent 1100 (G1315B) diode array detector (Agilent Technologies). Results are presented as milligrams of quercetin 4′-glucoside equivalent per kilogram fresh weight of onion (mg kg^{-1} fw) for all forms of quercetin.

Mycorrhizal Colonization. Onion roots were separated from the soil by washing and stored in 50% ethanol. The percentage of mycorrhizal root colonization was determined by a gridline-intersection method (*22*) after root clearing and staining (*23*).

Dry Matter and Plant Nutrient Content Analysis. Two portions of ≈10 g of homogenized onion sample from each plot were placed in aluminum cups and dried at 70 °C for 24 h, followed by 105 °C for 1 h. In 2005 plant nutrient contents of the transplanted onions were determined by inductively coupled plasma emission spectroscopy (ICP-ES) after digestion in HNO_3.

Statistical Analyses. The results were statistically analyzed by Minitab release 14.1 (Minitab Inc.) or by SAS 9.1 (SAS Institute Inc.). Results were subjected to ANOVA. Significant differences between means were determined by Tukey's or Duncan's methods of multiple comparisons. Reported results were considered as significantly different at $p < 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Soil Mineral N Contents. In 2004, the soil concentration of ammonium was significantly higher when Biofer was broadcast (BBH and BBR in **Figure 1A**) compared with other fertilizer treatments on the first two soil sampling occasions. The rapid increase in soil ammonium concentrations in the broadcast Biofer plots compared with the placed Biofer plots could be due to more rapid microbial degradation of the pelleted fertilizer because of a much larger contact area between the Biofer and the soil particles in the broadcast application treatments. The soil concentration of nitrate was higher in the broadcast treatments than in the placed or unfertilized treatments on the first two and on the fourth soil sampling occasion (**Figure 1C**). On the second sampling occasion, nitrate content was also significantly higher when Biofer was broadcast and rotary cultivated (BBR in **Figure 1C**) than when Biofer was broadcast and harrowed (BBH in **Figure 1C**). This could be explained by the finding that tillage makes previously protected organic N available (*24*) and that better incorporation of fertilizer into the soil by rotary cultivation leads to earlier mineralization (*25*). The response pattern to fertilizer application method was the same for total mineral N (N_{min}, **Table 1**) as for nitrate (**Figure 1C**). Inoculation with Vaminoc did not significantly affect soil nitrate or ammonium concentration.

In 2005 the soil concentration of ammonium was significantly higher when Biofer was broadcast (BBH in **Figure 1B**) compared to placed and no fertilizer (BP and NON in **Figure 1B**) at the second and third soil sampling occasions. As in 2004 the soil concentration of nitrate was higher in the broadcast treatment in the critical period in June and July when the bulb growth is initiated (**Figure 1D**).

Plant Growth and Yield. *Organically Fertilized Onions, Directly Sown.* In 2004, the number of plants was significantly higher ($p < 0.001$) when no fertilizer was added or when Biofer was placed in combination with Vaminoc compared with broadcast fertilizer (**Figure 2A**). Onion seed germination is negatively affected by high nutrient concentrations (*26*), so this could be an effect of better establishment due to lower nitrogen fertilizer concentrations in the immediate vicinity of the onion seeds.

At final harvest, the total number of onions (**Figure 2C**) and number of first class onions (data not shown) were higher in the unfertilized plots. The number of onions was higher when the Biofer was placed in combination with Vaminoc compared with broadcast fertilizer application. However, there was no difference between harrowing or rotary cultivation for the broadcast treatments and no significant effect of Vaminoc for the placed treatments. The tendency toward a positive effect of mycorrhizal inoculation on seedling establishment may be explained by higher mycorrhizal colonization (**Figure 2B**), leading to better nutrient uptake when Vaminoc is added in combination with placed Biofer.

At final harvest, the weight of individual onions (**Figure 2D**) was inversely related to the number of onions ha^{-1} at harvest (**Figure 2C**). It has been found that onions utilize soil N relatively poorly from nonfertilized soil (*27*). In our experiment the unfertilized onions had significantly lower mean bulb diameter, shown as a high proportion of onions sized 40–55 mm and a low proportion with a diameter 55–70 mm (**Table 2**). Onions fertilized with broadcast Biofer produced a significantly higher proportion of large bulbs, diameter >70 mm, compared with all other treatments. No differences in marketable yield between the treatments were found in 2004 (**Table 2**). In conclusion, the unfertilized treatment as expected resulted in

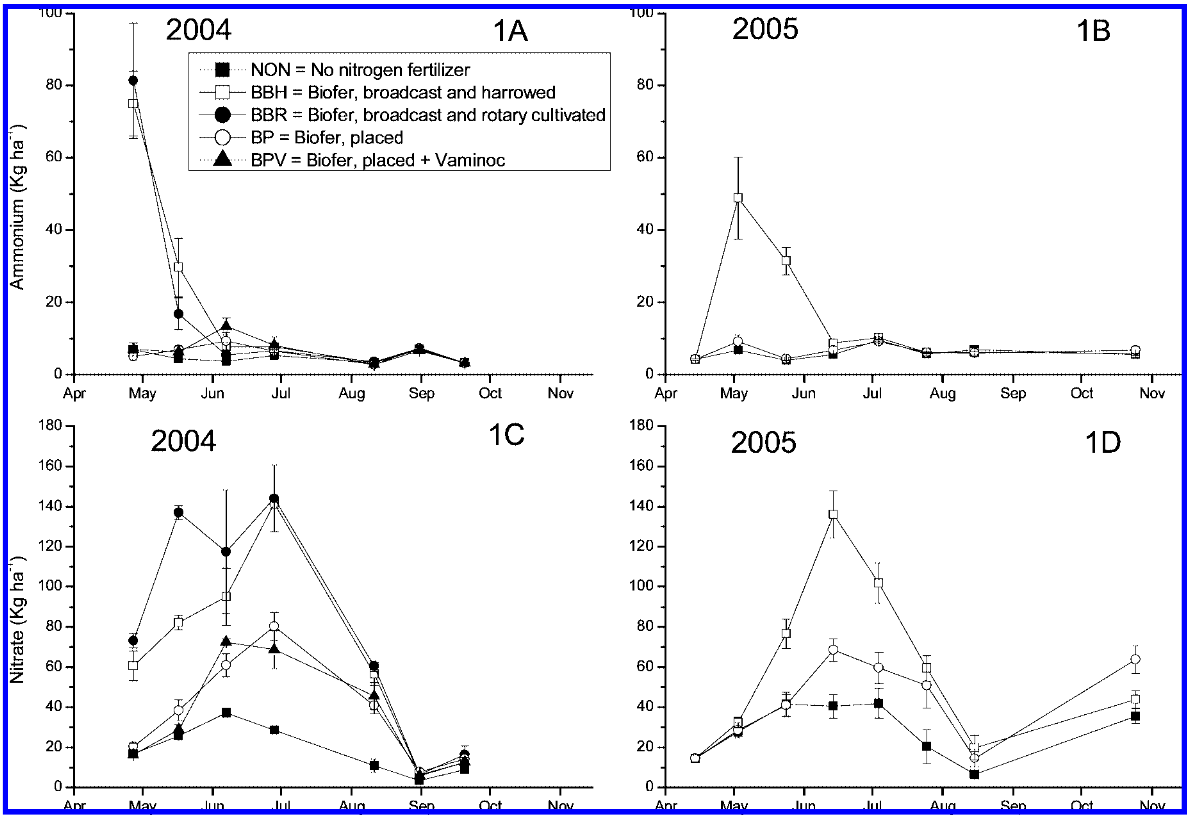

Figure 1. Soil ammonium content in 2004 (**A**) and in 2005 (**B**). Soil nitrate content in 2004 (**C**) and in 2005 (**D**). Error bars denote standard error of the mean ($n = 4$).

good establishment, but with many small-sized onions. Broadcast application resulted in a lower number of established onions in June (**Figure 2A**), but those present had good availability of nitrogen fertilizer (**Table 1**) and space during the subsequent bulbing phase, resulting in a higher proportion of large-sized onions (**Table 2**) and a higher individual mean onion weight (**Figure 2D**).

A lower percentage of onion root colonization at high application rates of organic fertilizers was observed by Linderman and Davis (*28*). In this experiment, the mycorrhizal root colonization was not significantly affected by the fertilizer treatments or by addition of Vaminoc (**Figure 2B**). However, when Biofer was placed (BP in **Figure 2B**), mycorrhizal colonization tended to be lower. An explanation for this could be that local increases in soil P concentrations might be expected when Biofer is placed in contrast to broadcast. The large variation in mycorrhizal colonization in 2004 can be explained by a significant block effect ($p < 0.05$). Root colonization was higher in one block (22%) than in the three other blocks (12–14%).

In 2005, no significant difference in yield or size between the treatments was found, and no differences in dry weight ratio were found between the different organically fertilized treatments in either of the years (**Table 2**).

Organically Fertilized Onions, Transplanted. Preinoculated onion plants have been shown to have higher biomass than noninoculated control plants (*29*), and interactions between inoculation and soil treatment had a significant effect on dry biomass and final bulb diameter (*30*). Vosatka (*31*) generally found increased root P concentration in mycorrhiza-colonized onion roots. In our case, on 16 June 2005, plant (root + shoot) phosphorus (P) concentration was slightly higher (2.36 ± 0.06 mg g^{-1} dw) when *G. intraradices* had been added (+AMF) compared with the uninoculated control (2.14 ± 0.06 mg g^{-1} dw). In the present experiment, plant K concentration was markedly higher for the control inoculated with nonmycorrhizal roots (−AMF) (40.5 ± 1.6 mg g^{-1} dw) compared with the two other treatments (32–35 mg g^{-1} dw). No differences in yield, in size, or in fresh weight to dry weight ratio were found between the three transplant treatments in 2005 (**Table 2**). However, inoculation resulted in a higher proportion of marketable onions compared to no inoculation (**Table 2**). Mycorrhizal colonization was 25 ± 2% and was not affected by the inoculation treatments.

Inorganically Fertilized Onions, Directly Sown. Generally, no difference in dry matter content is found in conventionally or organically grown root and tuber vegetables, but higher dry matter is often found in organically grown leaf vegetables such as spinach, lettuce, and white cabbage (*14*). Fjelkner-Modig et al. (*15*) found no difference in dry matter content of integrated produced and organically produced onions. The inorganically fertilized treatment in the present study received NPK broadcast and harrowed, and the application method is almost identical to, and can be directly compared with, the treatment that received organic fertilizer broadcast and harrowed. In 2004, the inorganically fertilized onions had significantly higher fresh weight to dry weight ratio (11.7%) than the organically fertilized broadcast and harrowed treatment (10.9%) (**Table 2**). However, in 2005 no difference was found.

In 2004 there was a tendency (not significant) for higher yield in the inorganically fertilized treatment. However, in 2005 this difference was significant (**Table 2**). No difference in size was found between onions that received inorganic or

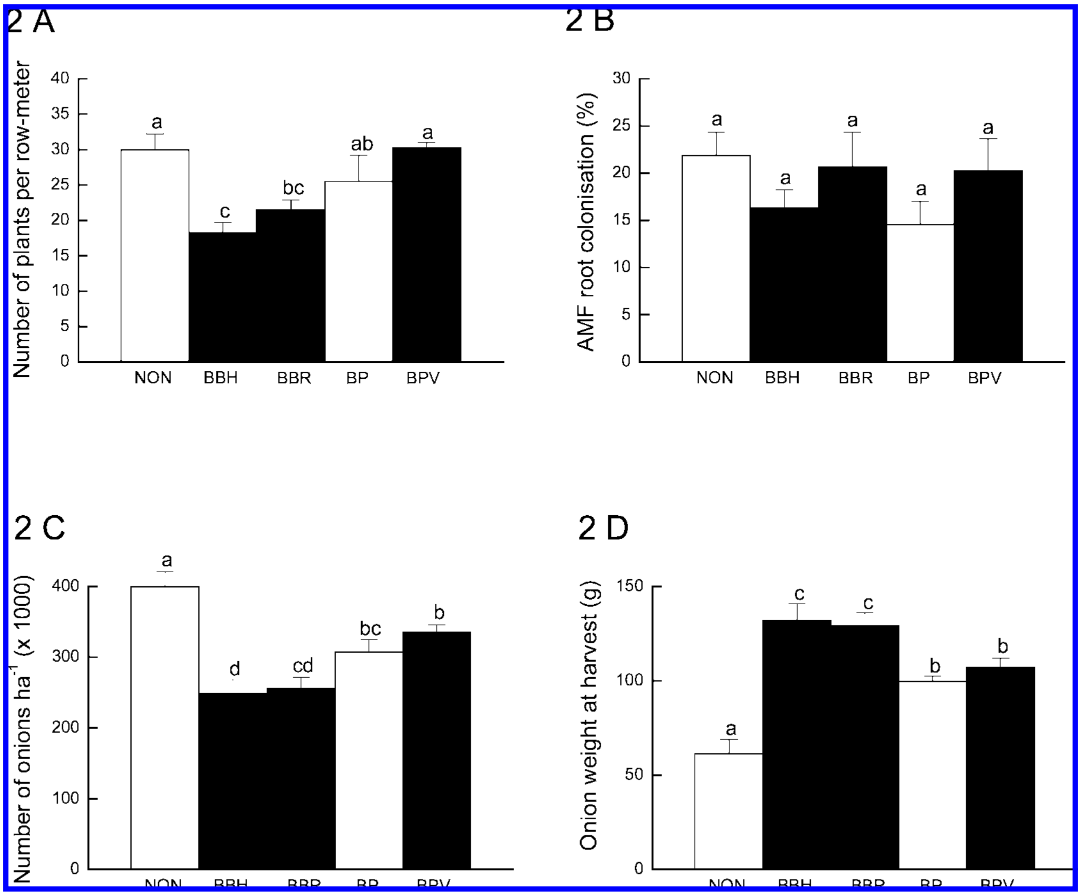

Figure 2. (**A**) Estimation of successful onion seedling establishment measured as the number of organically fertilized onion plants per row meter, 17 June 2004. (**B**) Mycorrhizal root colonization (% AMF) of the organically fertilized onion roots on 17 June 2004. (**C**) Number of organic onions per hectare at harvest 2004. (**D**) Mean fresh weight of individual organic onions at harvest 2004. NON = no nitrogen fertilizer; BBH = Biofer, broadcast and harrowed; BBR = Biofer, broadcast and rotary cultivated; BP = Biofer, placed; BPV = Biofer, placed plus Vaminoc. Means are separated by Duncan's multiple range test ($p < 0.05$). Error bars denote standard error of the mean ($n = 4$), and bars marked with the same letter are not significantly different ($p < 0.05$).

Table 2. Effect of N Fertilizer Treatment on Quercetin Content (mg kg^{-1} fw of Onions with a Diameter of 55–70 mm), Dry Weight to Fresh Weight Ratio (%), Yield (Tons ha^{-1}), Onion Diameter (mm), and Proportion of Marketable Onions (wt % of Total Yield) in October of Each Year ($n = 4$)[a]

year	planting method	N application method	Q_{DG}[b,c]	Q_{MG}[b,c]	total[b]	dw/fw	yield[b]	wt % of marketable yield <40	40–55	55–70	>70	marketable yield
2004	directly sown	no fertilizer	197 ± 25 a	349 ± 29 a	546 ± 53 a	10.8 a	24 ± 5 a	2 a	61 a	35 a	2 a	96.4 a
	directly sown	broadcast, harrowed	187 ± 12 a	351 ± 43 a	538 ± 53 a	10.9 a	33 ± 8 a	<1 a	13 b	68 b	19 b	91.1 a
	directly sown	broadcast, rotary cultivation	166 ± 4 a	318 ± 13 a	484 ± 12 a	10.9 a	33 ± 7 a	<1 a	13 b	71 b	16 b	88.7 a
	directly sown	placed	174 ± 6 a	327 ± 15 a	501 ± 20 a	11.0 a	31 ± 2 a	1 a	23 b	69 b	7 a	91.3 a
	directly sown	placed + Vaminoc	166 ± 16 a	322 ± 44 a	488 ± 60 a	11.1 a	36 ± 4 a	<1 a	23 b	70 b	7 a	90.0 a
	directly sown	broadcast, harrowed, inorganic NPK	179 ± 27	328 ± 36	507 ± 63	11.7	43 ± 13	2	34	61	4	95.2
2005	directly sown	no fertilizer	78 ± 9 a	130 ± 15 a	208 ± 21 a	11.3 a	32 ± 5 a	2 a	48 a	47 a	4 a	98.9 a
	directly sown	broadcast, harrowed	82 ± 7 a	135 ± 16 a	217 ± 21 a	11.4 a	40 ± 3 b	<1 b	19 b	67 b	13 b	96.4 a
	directly sown	placed	83 ± 12 a	129 ± 20 a	212 ± 31 a	11.4 a	36 ± 2 ab	1 ab	37 a	57 ab	5 ab	97.8 a
	directly sown	broadcast, harrowed inorganic NPK	93 ± 11	171 ± 24	264 ± 34	11.6	52 ± 2	<1	27	67	5	97.7
	transplants	placed, no inoculum	73 ± 9 a	115 ± 13 a	188 ± 22 a	11.4 a	46 ± 1 a	2 a	63 a	35 a	1 a	96.3 a
	transplants	placed − AMF	72 ± 8 a	114 ± 14 a	186 ±21 a	11.4 a	44 ± 2 a	2 a	67 a	31 a	<1 a	98.1 ab
	transplants	placed + AMF	74 ± 11 a	116 ± 15 a	190 ± 25 a	11.7 a	46 ± 1 a	1 a	62 a	36 a	<1 a	98.2 b

[a] The organic fertilizer Biofer was used unless otherwise stated. [b] Values are means ± standard deviation. Values followed by the same letter within each column, year, and planting method are not significantly different ($p < 0.05$). Means are separated by Tukey's test. [c] Q_{DG} = quercetin 3,4′-diglucoside; Q_{MG} = quercetin 4′-monoglucoside.

organic fertilizer broadcast and harrowed, apart from a significantly higher proportion of large (>70 mm) onions in the organic fertilizer broadcast and harrowed treatment in 2004.

Quercetin Content. No significant differences in quercetin glucoside content between the directly sown organically fertilized onion treatments could be found in either 2004 or 2005 (**Table 2**). There were differences in soil nitrogen fertilizer levels (**Table 1**) between the treatments in both years, so nitrogen fertilizer level does not seem to have any significant effect on onion quercetin content.

In the transplanted organically fertilized onion treatments in 2005, no difference in quercetin content was found between onions from the three transplant treatments (**Table 2**). Ponce et al. (*18*) reported that the flavonoids quercetin, acacetin, and rhamnetin accumulated in the roots of white clover plants inoculated with the mycorrhizal symbiont *G. intraradices*, whereas they were not detected in noninoculated plants. In the present experiments with directly sown (2004) or transplanted (2005) onions where mycorrhizal inoculum was added, no clear effect on plant root colonization compared with the background colonization by native AM fungi was observed. Hence, in the present study, no differences in flavonoid contents due to mycorrhizal inoculation could be expected.

In 2004, no difference in quercetin content was found between organically fertilized and inorganically fertilized onions that had their fertilizer broadcast and harrowed (**Table 2**). However, in 2005 the content of quercetin monoglucoside was significantly higher in the inorganically fertilized onions.

Concluding Remarks. Differences were observed in the effect of organic and inorganic fertilizers on the yield and size of onions, and these were probably caused by differences in time and amount of release during the season. The method of nitrogen application, broadcast or placement in the row, also affected the size of the onions. Onion seed and seedling emergence were confirmed to be sensitive to soil nitrogen concentrations. Variation in mycorrhizal root colonization between blocks was large, and no significant effect of inoculation with AM fungi in the row at sowing was found. The content and composition of quercetin tended to be at similar levels, irrespective of the source of nitrogen fertilizer or fertilizer application method, but differed between the years.

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