Reduced $^{15}$N-nitrogen Transport Through Arbuscular Mycorrhizal Hyphae to *Triticum aestivum* L. Supplied with Ammonium vs. Nitrate Nutrition

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This study compared the influence of NH$_4^+$ or NO$_3^-$ nutrition on the contribution of extraradical hyphae of the arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe (BEG 107) to NH$_4^+$ or NO$_3^-$ uptake by *Triticum aestivum* L. ‘Hano’ (summer wheat) with sufficient or insufficient N supply in semi-hydroponic culture. Roots and root-distant hyphae were spatially separated in compartmentalized pots. Although NH$_4^+$-fed plants supplied with sufficient N had higher N concentrations than their NO$_3^-$-fed counterparts, this did not favourably affect colonization rates, hyphal length densities or $^{15}$N amounts transported via hyphae to the plants. Ammonium supply did not result in higher P or reduced carbohydrate concentrations in the plants, so these factors could not explain the reduced hyphal lengths. It was concluded that the effect of NH$_4^+$ supply on hyphal length may be related to the reduced root growth and/or a direct effect of NH$_4^+$ on hyphal growth. Plant N deficiency reduced the percentage root length colonized, hyphal length, total $^{15}$N uptake by hyphae and dry weight of both NO$_3^-$ and NH$_4^+$-fed mycorrhizal plants. This was more obvious for NO$_3^-$-fed plants because plant biomass and hyphal lengths of NH$_4^+$-fed plants were relatively low in mycorrhizal plants irrespective of the N concentration supplied.


INTRODUCTION

This study compares the effect of NO$_3^-$ or NH$_4^+$ nutrition on arbuscular mycorrhizal (AM) colonization and N uptake through hyphae under controlled nutrient conditions in an inert substrate. Results of Bago et al. (1996) confirm that extraradical hyphae of AM actively take up NO$_3^-$ (probably via an NO$_3^-$:H$^+$ symport), but the actual contribution of this fungal-mediated nitrate uptake is unknown. AM hyphae have also been shown to deplete $^{15}$NH$_4^+$-labelled soil and transport this $^{15}$N to the plant (e.g. Frey and Schiepp, 1993; Johansen et al., 1993).

For practical purposes, most studies of N uptake through AM hyphae have used NH$_4^+$ as the N source (Johansen et al., 1992, 1994; Tobar et al., 1994) although NO$_3^-$ is the predominant N form in most agricultural soils (Marschner, 1995). As a result, very few studies have compared the effect of NH$_4^+$ and NO$_3^-$ ions as sources for mycorrhizal plants while also excluding the effect of these ions on rhizosphere pH. For instance, NH$_4^+$ has been shown to have a deleterious effect on mycorrhizal colonization of plants in soil compared to NO$_3^-$ ions, due to lowered rhizosphere pH with NH$_4^+$ nutrition (Chambers et al., 1980a). Lowered rhizosphere pH can affect spore germination (Green et al., 1976), root colonization and growth of AM fungi (Chambers et al., 1980a). Effects related to the NH$_4^+$ ion but not pH may include a direct salt effect (Chambers et al., 1980a) or a lower carbohydrate status of the root, since NH$_4^+$ uptake requires immediate incorporation of the N into amino acids in the root, which in turn requires C-skeletons from the TCA cycle (Cramer and Lewis, 1993a). Other effects may be increased shoot/root ratio or increased tissue P concentration (Johnson et al., 1984), where the latter may decrease membrane permeability and therefore root exudates that are thought to sustain the germination and growth of the fungus (Graham et al., 1981). Ammonium absorption occurs ‘downhill’ and results in high N contents and allows increased P absorption in some plants. The increased P absorption may be a result of faster synthesis of a nitrogenous P carrier and/or absence of anion competition with NO$_3^-$ for the P uptake site. Therefore, it was expected that NH$_4^+$ nutrition would, due to one or more of the above factors and independently of rhizosphere pH, decrease mycorrhizal colonization and possibly hyphal length, which might affect the amount of N transported through the hyphae to the plant roots.

The compartmentalized culture system used in this study (Hawkins and George, 1999) allows the direct comparison of NO$_3^-$ and NH$_4^+$ nutrition of non-mycorrhizal and mycorrhizal plants under pH-buffered and controlled nutrient conditions, thus eliminating any pH effects of the N forms. It was the aim of this study to test (1) the dependence of colonization, hyphal growth and hyphal $^{15}$N transport by *G. mosseae* to *T. aestivum* on N form without the effect of pH, and increase understanding of the mechanism responsible for possible differences in the colonization of NH$_4^+$- and NO$_3^-$-fed AM plants and (2) the dependence...
of colonization, hyphal growth and hyphal $^{15}$N transport by *G. mosseae* to *T. aestivum* on N plant status when plants were supplied with NH$_4^+$ or NO$_3^-$.  

**MATERIALS AND METHODS**

**Experimental culture containers**

Containers with a central root compartment and two outer hyphal compartments were used in a Perlite (2 mm) and drip-irrigation system as described by Hawkins and George (1999).

**Culture and harvest of plants and fungus**

*Triticum aestivum* ‘Hano’ (summer wheat) was cultivated with or without the mycorrhizal fungus *Glomus mosseae* (BEG 107). Two days before harvesting, $^{15}$N was supplied to the hyphal compartments as NO$_3^-$ or NH$_4^+$ (see below). Shoots and roots were harvested into liquid N$_2$, freeze-dried and analysed for plant dry weight, N and P concentrations, $^{15}$N abundance and carbohydrate concentration. Roots were analysed for mycorrhizal colonization and the Perlite in the hyphal compartments was analysed for hyphal length density.

**Nutrient media**

All nutrient media were based on Long Ashton nutrient solution (HANS; Hewitt, 1966), which was modified as described by Hawkins and George (1999) where N was provided as NO$_3^-$ (2 or 0.2 mM) or NH$_4^+$ (2 or 0.2 mM). Three different levels of N and P supply, represented as $^+N + P$ (2 mM N, 0.1 mM P), $^+N - P$ (2 mM N, 10 $\mu$M P) and $^-N - P$ (0.2 mM N, 10 $\mu$M P) were used. The first treatment, containing higher N and P, was used to grow adequately fed control plants. These were compared with $+AM$ and $-AM$ plants that were supplied with either low P, or low N and P. The plants receiving the $+N + P$ treatment are also referred to as controls in tables, figures and text.

Hyphal compartments were irrigated with complete, modified LANS (Hawkins and George, 1999) to allow the hyphae access to a more concentrated nutrient solution than was available to the roots directly. It was calculated that the amounts of N and P supplied to the hyphal compartments were sufficient to ameliorate the expected N or P deficiency, should the total amount of these nutrients be taken up by the hyphae and transported to the plants. Nutrient deficiency and sufficiency were based on expected plant growth rates and adequate levels of N and P in *T. aestivum* (Reuter and Robinson, 1988).

A nitrification inhibitor (e.g. N-serve) was not used since toxic effects below 10 mg kg$^{-1}$ soil or above 15 mg kg$^{-1}$ soil N-serve have been known to occur in soil microorganisms (Laskowski et al., 1975) and AM fungi (Chambers et al., 1980b) respectively.

**Nitrogen, $^{15}$N and P analysis**

Nitrogen concentration was determined from dried, pulverized shoot and root material by dry oxidation (Macro N, Heraeus Holding Ltd, Hanau, Germany) using asparagine as a standard. The percentage atom enrichment of $^{15}$N in dried, pulverized samples was determined by dry oxidation and reduction (Roboprep CN Biological Sample Converter, Europa Scientific Ltd, Crewe, UK) and subsequent mass spectrometry (TraceMass Sample Isotope Analyser, Europa Scientific Ltd) using pulverized pea shoot (2.5% N, 0.45% $^{15}$N) as a standard. Plant P concentration was determined using the molybdenum blue assay (Murphy and Riley, 1962) on dried, pulverized shoot and root material that had been dry ashed at 500°C for 4 h, moistened with H$_2$O and 1:3 (v/v) HNO$_3$, which was evaporated off to split SiO$_2$ from other compounds. Samples were then dissolved in 1:30 (v/v) HCl and boiled for 2 min (to convert metaphosphates and pyrophosphates to orthophosphates) before being made up to volume.

**Carbohydrate analysis**

Reducing sugars and sucrose were determined according to a modified method of Blakeney and Mutton (1980). About 30 mg of freeze-dried plant material was extracted three times in 2:5 ml 70% (v/v) ethanol (end volume 7.5 ml) by consecutive mixing and centrifugation at 2500 g and 4°C (Minifuge, Hereaus GmbH, Stuttgart, Germany). Chlorophyll in leaf extracts was removed by addition of activated charcoal (about 10 mg ml$^{-1}$), mixing and centrifugation at 1800 g (Mikroliter, Hettich Zentrifugen, Tuttlingen, Germany). The clear supernatant was used for reducing sugar and sucrose determination. Reducing sugars were determined by mixing 0.2 ml of the clear supernatant with 0.8 ml Na-acetate (pH 4.8) and sucrose was determined by mixing 0.1 ml supernatant with 0.1 ml invertase solution (30 U ml$^{-1}$ buffered in 0.2 M Na-acetate) and 0.8 ml 0.1 M Na-acetate (pH 4.8). The mixtures were incubated for 2 h in a 30°C water bath to allow sucrose to be converted to glucose equivalents. After incubation, 5 ml of a colour reagent (0.03 M hydroxybenzoic acid hydrazide, 0.05 M Na-citrate, 0.01 M CaCl$_2$ and 0.5 mM NaOH) was added to the sample solution and boiled for 4 min in a water bath. The cooled yellow solution was measured spectrophotometrically at 415 nm.

Starch was determined in the residual pellet according to Blakeney and Matheson (1984). The pellet was dissolved in 2 ml dimethyl sulfoximine by boiling for 10 min in a water bath. The sample was centrifuged at 2500 g (Minifuge, Hereaus GmbH, Stuttgart, Germany) and washed with 8 ml 0.1 mM Na-acetate (pH 4.8) solution. One millilitre of the sample solution was mixed with 2 ml amyloglucosidase solution (1:2 U ml$^{-1}$ buffered in 0.2 M Na-acetate) and incubated for at least 12 h at 37°C in a water bath. After incubation, 5 ml of the enzyme colour reagent (4000 U glucose oxidase, 1000 U peroxidase, 0.07 M Na$_2$HPO$_4$, 0.4 M NaH$_2$PO$_4$, 0.016 M benzoic acid, 0.5 mM 4-amino antipyrin, 0.01 M p-hydroxy benzoic acid) was added to each 1 ml sample solution and incubated in a 40°C water bath.
bath for 15 min. The cooled pink solution was measured spectrophotometrically at 510 nm.

Mycorrhizal root colonization and hyphal length density

The percentage of root length colonized by mycorrhizal fungi was determined on roots stained in trypan blue (Koske and Gemma, 1989) using the gridline-intersect method (Giovannetti and Mosse, 1980). For the determination of hyphal length density, samples were taken from hyphal compartments using a 10 mm wide cork borer. The Perlite was thoroughly mixed before sampling. These samples were air-dried, weighed and used to determine hyphal length density according to the modified agar film technique described by Li et al. (1991). Hyphal lengths were expressed on a length per unit volume basis to enable comparison with previously reported hyphal lengths from soil extracts.

$^{15}$Nitrogen feeding

Nitrogen (20 ml of 5 mM 95% atom enriched $^{15}$NO$_3$ or $^{15}$NH$_4$; Chemotrade GmbH, Leipzig, Germany) was supplied to each hyphal compartment of both +AM and −AM treatments 48 h before harvesting. The shoots were harvested after 48 h and, at the same time, the hyphal connection between the root and hyphal compartments was severed using a scalpel. Specific uptake rates of $^{15}$N by the plant via hyphae were expressed as μmol $^{15}$N g$^{-1}$ plant d.wt h$^{-1}$ and specific uptake rates per unit hyphae were expressed as nmol $^{15}$N m$^{-1}$ hyphae h$^{-1}$.

Statistics

Four replicate containers per treatment with five plants per container were used. For most comparisons, Student's $t$-tests were applied to determine differences due to presence or absence of mycorrhizal fungi within one nutrient treatment or between NO$_3$- and NH$_4$-fed plants with the same N supply (concentration supplied). Root colonization data and other data presented as percent-ages were normalized by arcsine square root transformation before performing Student’s $t$-tests. Differences within all five treatments (+N + P − AM, +N − P − AM, +N − P + AM, −N − P − AM, −N − P + AM) were calculated by mean separation ($P < 0.05$, pair-wise multiple range Tukey test) following a one-way analysis of variance. Results in the tables, text and figures are given as means ± s.e.

RESULTS

Dry weight

Nitrate-fed plants were larger that NH$_4$-fed plants, regardless of mycorrhizal or N treatment ($P < 0.05$, pair-wise multiple range Tukey test, Table 1). The largest differences in dry weight between +AM and −AM plants were evident when the plants were provided with sufficient N (+N − P) and NO$_3$ as the N source. The shoot:root ratio of NH$_4$-fed plants was significantly higher than that of NO$_3$-fed plants due to a relatively smaller root growth ($P < 0.05$, $t$-tests, Table 1).

Nutrient contents and concentrations

With sufficient P (controls), there was, as expected, a higher P concentration in the NH$_4$-fed compared to NO$_3$-fed plants (Fig. 1). However, with insufficient P (+N − P and −N − P treatments), this was not the case and P concentrations were even higher in NO$_3$-fed plants than in NH$_4$-fed plants ($P < 0.05$, Student $t$-tests), regardless of the mycorrhizal or N treatments (Fig. 1). As expected, mycorrhizal colonization resulted in an increased P concentration of the shoots of both NO$_3$- and NH$_4$-fed plants, regardless of the N treatment (Fig. 1). Mycorrhizal colonization significantly increased P concentrations in the root of NO$_3$-fed plants only (Fig. 1).

Nitrate-fed +AM plants had a higher N content compared to −AM plants, irrespective of the level of N supplied while this effect could be seen only for the shoots of NH$_4$-fed, −N − P plants (Fig. 2A). Although the N concentration of shoots of NO$_3$-fed +N − P plants was also greater for mycorrhizal plants (31% increase), as was the case for the roots of NH$_4$-fed −N − P plants (19% increase, Fig. 2B), the effect of mycorrhizal colonization on the N concentration of either NO$_3$- or NH$_4$-fed plants was not large. In general, a greater mycorrhizal effect was visible for NO$_3$-fed as opposed to NH$_4$-fed plants with respect to both N content and N concentration. In +N − P plants, roots of NH$_4$-fed plants had higher N concentrations than those of NO$_3$-fed plants (Fig. 2B, $P < 0.05$, Student’s $t$-test).

Carbohydrate concentrations

The most obvious difference between −AM and +AM plants was seen in the sucrose concentrations of the root and shoot of NO$_3$-fed plants: There was significantly less sucrose in both shoot and root ($P < 0.001$, Student’s $t$-tests) in +AM compared to −AM plants, which resulted in the total sugar concentration being lower ($P < 0.001$, Student’s $t$ tests, Fig. 3). There was no difference between the starch concentration of the shoot while the starch concentration in the root was higher ($P = 0.026$, Student’s $t$-test) in +AM compared to −AM + N − P plants (Fig. 3). There were no significant differences between −AM and +AM plants in the −N − P treatment (Fig. 3).

In contrast to NO$_3$-fed plants, the +AM +N − P NH$_4$-fed plants had higher concentrations of reducing sugars ($P = 0.010$, $P < 0.001$, Student’s $t$-test for shoot and root, respectively) and higher concentrations of sucrose ($P < 0.001$, $P < 0.001$, Student’s $t$-test for shoot and root, respectively) while the starch concentrations were significantly lower ($P = 0.027$, $P < 0.001$, Student’s $t$-test for shoot and root, respectively, Fig. 3) than in the corresponding −AM plants.

Therefore, the pattern and allocation of C into carbohydrates in −AM and +AM plants was similar in NO$_3$- and NH$_4$-fed plants in the −N − P treatment, while the absolute concentrations of starch and sucrose were higher.
in NH\textsubscript{4}\textsuperscript{+}-fed plants \((P < 0.05, \text{Student’s} \ t\text{-tests, Fig. 3})\). For the +N − P treatment, the pattern and allocation of C into carbohydrates in −AM and +AM was significantly different \((P < 0.05, \text{Student’s} \ t\text{-tests})\) and reversed (Fig. 3).

**Mycorrhizal root colonization and hyphal length density**

Plants not inoculated with the fungus were not colonized. There was no significant difference in the colonization rates of NO\textsubscript{3}\textsuperscript{−} and NH\textsubscript{4}\textsuperscript{+}-fed plants, regardless of N treatment (Table 2) while hyphal lengths were significantly reduced in NH\textsubscript{4}\textsuperscript{+}-fed plants, regardless of N treatment (Table 2). In NO\textsubscript{3}\textsuperscript{−}-fed plants, a higher N supply resulted in a significantly higher hyphal length \((P < 0.001, \text{Student’s} \ t\text{-test, Table 2})\) while in NH\textsubscript{4}\textsuperscript{+}-fed plants, hyphal lengths were low, regardless of N supply to the plant.

**15Nitrogen uptake**

There was no significant \(^{15}\text{N}\) enrichment of the NO\textsubscript{3}\textsuperscript{−}-fed −AM plants above the natural abundance level of 0.366 \%, i.e., uptake rates were essentially zero (Table 3) because no mass flow of \(^{15}\text{N}\) occurred from the hyphal to the root compartments in non-mycorrhizal variants. There was some small uptake by the −AM, NH\textsubscript{4}\textsuperscript{+}-fed plants (Table 3). For both the NO\textsubscript{3}\textsuperscript{−} and NH\textsubscript{4}\textsuperscript{+}-fed plants, the

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**Table 1. Dry weight and shoot : root ratio \((S:R)\) of non-mycorrhizal \((-AM)\) and mycorrhizal \((+AM)\) T. aestivum shoots and roots grown with low or high \(P\) supply \((-P; +P)\) and low or high NO\textsubscript{3}\textsuperscript{−} or NH\textsubscript{4}\textsuperscript{+} supply \((-N; +N)\)**

<table>
<thead>
<tr>
<th>Dry weight (g)</th>
<th>+N + P (control)</th>
<th>+N − P</th>
<th>−N − P</th>
<th>−N − P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot</td>
<td>−AM</td>
<td>−AM</td>
<td>+AM</td>
<td>−AM</td>
</tr>
<tr>
<td>Root</td>
<td>36.25 ± 0.64</td>
<td>7.57 ± 0.52\textsuperscript{a}</td>
<td>10.17 ± 0.59\textsuperscript{b}</td>
<td>3.63 ± 0.11\textsuperscript{a}</td>
</tr>
<tr>
<td>S:R</td>
<td>32.59 ± 1.12</td>
<td>5.00 ± 0.64\textsuperscript{a}</td>
<td>13.21 ± 1.15\textsuperscript{b}</td>
<td>3.63 ± 0.20\textsuperscript{a}</td>
</tr>
<tr>
<td>Root</td>
<td>1.12 ± 0.06</td>
<td>1.41 ± 0.18\textsuperscript{a}</td>
<td>0.86 ± 0.17\textsuperscript{a}</td>
<td>1.01 ± 0.04\textsuperscript{a}</td>
</tr>
<tr>
<td>Shoot</td>
<td>14.84 ± 0.50</td>
<td>4.84 ± 0.26\textsuperscript{a}</td>
<td>7.42 ± 0.45\textsuperscript{b}</td>
<td>4.10 ± 0.25\textsuperscript{a}</td>
</tr>
<tr>
<td>Root</td>
<td>2.93 ± 0.30</td>
<td>2.23 ± 0.11\textsuperscript{a}</td>
<td>2.45 ± 0.13\textsuperscript{a}</td>
<td>2.05 ± 0.11\textsuperscript{a}</td>
</tr>
<tr>
<td>S:R</td>
<td>5.17 ± 0.47</td>
<td>2.20 ± 0.21\textsuperscript{a}</td>
<td>3.04 ± 0.14\textsuperscript{b}</td>
<td>2.00 ± 0.03\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Different superscripts indicate a statistically significant difference between −AM and +AM treatments \((P < 0.05, \text{Student’s} \ t\text{-test})\) at the same level of nutrient supply. Values are means ± s.e. of four replicates.
uptake rate of the +AM plants was higher than for the −AM plants, although this was not significant for the NH$_4^+$-fed plants (Table 3). There was no significant difference between the uptake rate of +N − P and −N − P plants but a higher percentage of the total $^{15}$N supplied was taken up into the NO$_3^-$-fed, +N − P plants compared to the −N − P plants ($P = 0.043$, Table 3).

There was no significant difference between the specific uptake rates per unit hyphae of +N − P and −N − P variants with the same N form supplied (Fig. 4). This implies that the differences in $^{15}$N uptake into the plants were due to differences in hyphal length and not differences in specific hyphal uptake rates. There was also no significant difference between the specific uptake rates per unit hyphae in the NO$_3^-$ and NH$_4^+$-fed variants with the same N concentration supplied ($P > 0.05$, Student’s t-test), which also implies that the difference in uptake rates and percentage uptake between the NO$_3^-$ and NH$_4^+$-fed variants was due to hyphal length densities and not the specific uptake capacity of the hyphae.

Fig. 2. Nitrogen contents per pot (A) and concentrations (B) of non-mycorrhizal and mycorrhizal T. aestivum shoots and roots supplied with either nitrate or ammonium and grown with low or high P supply (−P; +P) and low or high N supply (−N; +N). Different letters indicate a statistically significant difference between the roots or shoots of −AM and +AM treatments ($P < 0.05$, Student’s t-test) at the same level of nutrient supply. Values are means of four replicates. (□), Shoot; (■), root; (−/+), without/with AM.
DISCUSSION

When N was supplied as NH$_4^+$, plant biomass, hyphal length densities and also $^{15}$N transport via the hyphae to the AM plant were lower than when NO$_3^-$ was supplied to the AM plant, irrespective of plant N status. The N concentrations of plants supplied with NH$_4^+$ were greater than for the corresponding NO$_3^-$-fed plants and this may have been a concentration effect due to the lowered biomass of these plants. Short-term assimilation experiments by Johansen et al. (1996) found that cucumber colonized by G. intraradices assimilated more NH$_4^+$ into amino acids than NO$_3^-$-fed plants.

![Graph](image_url)

**Table 2.** Root colonization and extraradical hyphal length densities of nitrate or ammonium-fed mycorrhizal T. aestivum plants grown with low or high P supply (−P; +P) and low or high N supply (−N; +N)

<table>
<thead>
<tr>
<th>N treatment</th>
<th>+N − P</th>
<th>−N − P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate-fed plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colonization rate (%)</td>
<td>85 ± 2.5$^a$</td>
<td>70 ± 5.6$^a$</td>
</tr>
<tr>
<td>Hyphal length (m cm$^{-3}$)</td>
<td>8.86 ± 0.98$^a$</td>
<td>1.61 ± 0.10$^b$</td>
</tr>
<tr>
<td>Ammonium-fed plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colonization rate (%)</td>
<td>90 ± 0.8$^a$</td>
<td>67 ± 3.5$^b$</td>
</tr>
<tr>
<td>Hyphal length (m cm$^{-3}$)</td>
<td>0.89 ± 0.45$^a$</td>
<td>0.49 ± 0.24$^a$</td>
</tr>
</tbody>
</table>

Different superscripts indicate a statistically significant difference between the +N − P and −N − P treatments (P < 0.05, Student’s $t$-test) at the same N form. Values are means of four replicates ± s.e.

**Table 3.** Uptake rates of $^{15}$N and total percentage of $^{15}$N taken up over the 48 h feeding period in nitrate or ammonium-fed T. aestivum plants grown with low or high P supply (−P; +P) and low or high N supply (−N; +N)

<table>
<thead>
<tr>
<th>N treatment</th>
<th>+N − P</th>
<th>−N − P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate-fed plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+N + P (control)</td>
<td>0.0 ± 0.4</td>
<td>—</td>
</tr>
<tr>
<td>+N − P</td>
<td>0.0 ± 1.4$^a$</td>
<td>56.2 ± 10.1$^b$</td>
</tr>
<tr>
<td>−N − P</td>
<td>0.0 ± 0.6$^b$</td>
<td>66.4 ± 13.5$^a$</td>
</tr>
<tr>
<td>Ammonium-fed plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+N + P (control)</td>
<td>2.9 ± 6.9</td>
<td>—</td>
</tr>
<tr>
<td>+N − P</td>
<td>0.0 ± 5.0$^a$</td>
<td>57.5 ± 26.3$^b$</td>
</tr>
<tr>
<td>−N − P</td>
<td>4.4 ± 5.0$^a$</td>
<td>85.7 ± 49.6$^a$</td>
</tr>
</tbody>
</table>

Percentage of supplied $^{15}$N taken up over 48 h (%)

<table>
<thead>
<tr>
<th>N treatment</th>
<th>−N − P</th>
<th>+N − P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate-fed plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+N + P (control)</td>
<td>0.00 ± 0.1</td>
<td>—</td>
</tr>
<tr>
<td>+N − P</td>
<td>0.00 ± 0.05$^a$</td>
<td>4.8 ± 1.1$^b$</td>
</tr>
<tr>
<td>−N − P</td>
<td>0.00 ± 0.01$^a$</td>
<td>1.8 ± 0.4$^b$</td>
</tr>
<tr>
<td>Ammonium-fed plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+N + P (control)</td>
<td>0.05 ± 0.13</td>
<td>—</td>
</tr>
<tr>
<td>+N − P</td>
<td>0.00 ± 0.07$^a$</td>
<td>1.0 ± 0.5$^a$</td>
</tr>
<tr>
<td>−N − P</td>
<td>0.06 ± 0.07$^a$</td>
<td>1.1 ± 0.6$^a$</td>
</tr>
</tbody>
</table>

Different superscripts indicate a statistically significant difference between the −AM and +AM treatments (P < 0.05, Student’s $t$-test) at the same N supply. Values are means of four replicates ± s.e.
been shown to play a likely role in maintaining biosynthesis. Plants had relatively high N concentrations, this was a consequence of reduced root growth, and the NH$_4^+$ ion is a relatively less favourable N source compared to NO$_3^-$ with respect to hyphal growth and $^{15}$N uptake via hyphae, regardless of N status. In this and a previous study (Hawkins and George, 1999), greater hyphal lengths were generally related to larger amounts of $^{15}$N transported to the plant via hyphae. Various factors (increased P concentration, reduced carbohydrate concentration or a direct NH$_4^+$ ion effect) could be responsible for the reduced hyphal (and root) growth of mycorrhizal NH$_4^+$-fed plants.

Ortas et al. (1996) found that NH$_4^+$ nutrition led to increased P concentration in +AM plants independent of rhizosphere pH. However, the NH$_4^+$-fed plants in this experiment did not have higher P concentrations than the NO$_3^-$-fed plants. Higher P concentration in NH$_4^+$-fed compared to NO$_3^-$-fed plants was therefore not responsible for the reduced hyphal lengths in NH$_4^+$-fed plants.

It was also hypothesized that under NH$_4^+$ nutrition there would be relatively less carbohydrate available for the fungus (due to the requirement of NH$_4^+$ for immediate assimilation into amino acids with the consumption of C-skeletons) than with NO$_3^-$ nutrition. According to Cramer and Lewis (1993e), the allocation of carbon to the amino-N fraction occurred at the expense of carbohydrate fractions, especially in the root of T. aestivum supplied with NH$_4^+$ as compared to NO$_3^-$ . This was the case for the +AM +N – P treatment. However, the corresponding +AM plants supplied with NH$_4^+$ did not have less carbohydrates in the roots compared to those supplied with NO$_3^-$ . The higher carbohydrate concentrations in +AM NH$_4^+$-fed plants may reflect the relatively smaller sink of the lesser hyphal lengths in NH$_4^+$-supplied plants compared to NO$_3^-$-fed plants.

Therefore, the negative effect of NH$_4^+$ supply on hyphal growth was neither directly related to increased P concentrations nor to decreased carbohydrate concentrations in the root. The reduced colonization, hyphal length and $^{15}$N transport to the plant may be related to the observed reduced root growth and/or NH$_4^+$ may possibly have had a direct inhibitory effect on hyphal growth. The fact that NH$_4^+$ supply to the plant/fungus affected the extraradical hyphal length but not the colonization rate supports the idea that the NH$_4^+$ ion may be deleterious to extraradical fungal growth but has less effect on colonizing structures protected inside the buffered milieu of the root.

In this and a previous study (Hawkins and George, 1999), greater hyphal lengths were generally related to larger amounts of $^{15}$N being transported to the plant. However, in this study, this trend was only significant when NO$_3^-$ was supplied to the plant. In this case the hyphae transported 1.8% (N – P) or 4.8% (+N – P) of the $^{15}$N supplied. For NH$_4^+$-fed plants, the figure was about 1%, regardless of N treatment. The largest mycorrhizal effect with respect to biomass accumulation, hyphal length and $^{15}$N uptake—and even N concentration—was evident for +N – P NO$_3^-$-fed plants. The results of these two studies imply that an adequate N status of the plant is indeed important for mycelial development but that supplying NH$_4^+$ as the sole N source has a detrimental effect on hyphal and root growth, despite an adequate root N status.

**FIG. 4.** Nitrogen uptake into nitrate or ammonium-fed T. aestivum plants expressed per unit hyphal length of G. mosseae in those variants. Plants were grown with low or high N supply [–N ([]); +N (□)] and low P supply. Similar letters indicate that there was no statistically significant difference between the +N – P and −N – P treatments ($P < 0.05$, Student’s $t$-test) with the same N form. Values are means of four replicates + s.e.
This is evidently not the case for the ectomycorrhizal symbiosis which usually grows and preferentially takes up NH$_4^+$ compared to NO$_3^-$ (Finlay et al., 1988, 1989; Martin et al., 1994) although inter- and intraspecific differences occur. This is understandable in evolutionary terms since ectomycorrhizal fungi have largely evolved in environments where NH$_4^+$ is the predominant N form due to reduced nitrification at higher altitudes or latitudes where ectomycorrhiza are prevalent.

Those plants with the largest mycorrhizal effect in terms of biomass accumulation and $^{15}$N transport were also those plants with a reduced sucrose concentration compared to the −AM plants in the +N − P treatment. The reduced sucrose concentration observed in these +AM plants is in agreement with the idea from ectomycorrhizal research that a gradient of sucrose is maintained between plant and fungus by the rapid conversion of plant sugar to fungal sugar by the fungus. In this way, the fungus maintains a strong sink (Wallander, 1992). The actual carbohydrate status of the root (generally low in mycorrhizal plant roots) therefore does not necessarily reflect the degree to which carbohydrate is available to the fungus.

In conclusion, the results indicate that the smaller hyphal lengths of +AM, NH$_4^+$-fed plants were due to a direct ion effect of NH$_4^+$ that reduced root and/or extraradical hyphal biomass. In addition, it was confirmed that sufficient N supply was important for the development of the fungal mycelium and this development was related to the amount of N acquired by the fungus and transported to the plant. Since development of the fungus was generally reduced under NH$_4^+$ nutrition, this latter trend was more obvious for NO$_3^-$-fed plants.

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LITERATURE CITED


