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Effect of the arbuscular mycorrhizal fungus *Glomus fasciculatum* on the uptake of amino nitrogen by *Lolium perenne*

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SUMMARY

The ability of ryegrass (*Lolium perenne* L.) to take up and utilize aspartic acid (Asp) and serine (Ser), and the effect of colonization of the roots by the arbuscular mycorrhizal (AM) fungus *Glomus fasciculatum* (Thax. *sensu* Gerd.) were studied. The seedlings were grown under controlled conditions in a series of micro-lysimeters. All plants were fed with a nutrient solution containing either nitrate, Asp or Ser as the sole N source. After 49 d, they were supplied with ^{15}N labelled nitrate, Asp or Ser for 1 h and harvested.

AM colonization increased the growth and total N content of the plants in all cases. Similarly, the amount of Asp or Ser taken up was higher in AM than in control plants. There were no differences in biomass production between the nitrate and Ser-fed plants. However uptake rates were lower for Ser than for nitrate. Growth of the Asp-fed plants was significantly less than the other two treatments, and uptake of ^{15}N-Asp was lower than uptake of ^{15}N-Ser. Analysis of ^{15}N incorporation into the amino acids extracted from the roots suggests the hydrolysis of Ser followed by re-assimilation of the resulting ammonia via the GS-GOGAT cycle. There were no differences in the patterns of accumulation of amino acids in the root-zone of control and AM-ryegrass. The implication of these results for the pathway of nitrogen transfer between plants is discussed.

Key words: Arbuscular-mycorrhizal fungi, amino acid uptake, ^{15}N, *Lolium perenne* L.

INTRODUCTION

Arbuscular mycorrhizal (AM) colonization is known to have a beneficial effect on plant growth. Many workers have linked this effect with the ability of the symbiosis to improve mobilization from the soil, absorption, and metabolism of phosphate in the colonized roots (Marshner & Dell, 1994). However, there is increasing evidence of a significant role of AM in plant N uptake (Johansen, Jakobsen & Jensen, 1993; Bago *et al.*, 1996; Johansen, Finlay & Olsson, 1996). A capacity for the external hyphae to take up and deliver inorganic N has been demonstrated for both ammonia (Ames *et al.*, 1983) and nitrate (Bago *et al.*, 1996), but the function of AM fungi in organic N acquisition by plants is unclear. Work on *Sorghum bicolor* (Ames *et al.*, 1984) and on *Sorghum vulgare* and *Brachiaria arrecta* (Ibijbijen *et al.*, 1996) has suggested that mycorrhizal roots have access to N sources other than the mineral N, although growth of *Sorghum bicolor* was not modified by AM colonization (Ames *et al.*, 1984).

Nitrogen can be transferred between associated plants by various indirect mechanisms (Ta & Faris, 1987; Bethlenfalvay *et al.*, 1991; Tomm, van Kessel & Slinkard, 1994). Murray & Hatch (1994) showed that N was transferred directly from white clover (*Trifolium repens*) to associated ryegrass (*Lolium perenne* L.) in the presence of insect root herbivores. Further work investigated the effect of the insect on the carbon and N economies of the clover plant and identified the major N containing constituents of root exudates. Two of the major organic compounds in the exudates were the amino acids aspartic acid (Asp) and serine (Ser) (Murray, Hatch & Cliquet, 1996). Recently Jones & Darrah (1993) demonstrated the ability of maize actively to take up amino acids, and Chapin, Moilamen & Kielland (1993) established that amino acids are directly taken up as ions, without hydrolysis, by the arctic sedge *Eriophorum*

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vaginatum. This present work aimed to investigate the ability of ryegrass plants to utilize the compounds released from clover roots, and the effect that AM symbiosis has on their uptake.

MATERIALS AND METHODS

Plant and fungus material

Seeds of perennial ryegrass (*Lolium perenne* L.) were surface sterilized with sodium hypochlorite solution, rinsed three times and germinated in Petri dishes containing demineralized water. The seedlings were transferred to individual microlysimeters after 10 d. The lysimeters were constructed from 60 ml syringe barrels containing sterilized calcined clay (Terra Green, Oil-Dri Company, Chicago, IL, USA) as the growth medium. The lysimeters were kept in controlled conditions with a 16 h photoperiod with a photon fluence rate of 180 μmol m^{-2} s^{-1} at 20 °C.

Half of the seedlings were inoculated with the arbuscular mycorrhizal (AM) fungus *Glomus fasciculatum* (Thax. *sensu* Gerd.) taken from colonized roots of leek (*Allium porrum*). The inoculum was disinfected according to the method of Strullu & Romand (1987).

The content of chitin used as a measure of endomycorrhizal colonization was determined according to Bethlenfalvay, Pacovsky & Brown (1981). Three different nutrient solutions containing either 1 mM KNO_3, 1 mM Asp or 1 mM Ser as the N source with 0·137 mM phosphate, pH 6, and other macro- and micro-nutrients as described previously by Lainé *et al.* (1994), were used. For each of these three nutrient solutions 10 replicate plants, either colonized or not (control) with a mycorrhizal fungus, were established. Plants were watered with demineralized water from day 1 to day 7 after sowing, then with 5 ml of nutrient solution at 7-d intervals from day 8 to day 21, and then at 4-d intervals until harvest on day 49. Between days 29 and 49 root-zone solutions were collected every 5 d by flooding the lysimeter with demineralized water and draining immediately before to watering with nutrient solution. The solutions were frozen and freeze-dried for HPLC amino acid analysis.

In the nutrient solutions used on day 49, ^{15}N-labelled compounds were substituted for the corresponding N source (99·9 atom % NO_3, 99·7 atom % Ser and 92 atom % Asp), and fed for 1 h before sampling. The sampled amino acids were analysed by HPLC. At the end of the 1-h labelling period the plants were harvested, by careful washing from the growth medium, and separated into roots and shoots. Half of the replicates were frozen by immersion in liquid nitrogen, and freeze-dried for subsequent amino-acid analysis. The other half were oven-dried at 80 °C for 24 h, ground and analysed for total N and ^{15}N atom %.

Amino acid extraction

Samples (0·5–1 g) were ground in 10 ml of a mixture of a methanol:dichloromethane:water (12:5:3 (v/v/v)). Amino acids were subsequently extracted and purified as described by Murray *et al.* (1996).

Analyses

Total N and ^{15}N atom % were determined in the shoots and roots of the grass plants by use of an automated N analyser (Carlo Erba 1500, Carlo Erba Strumentazione, Rodano, Italy) linked to a mass spectrometer (VG 622 Micromass, VG, Winsford Cheshire, UK) (Preston & Owens, 1983; Marshall & Whiteway, 1985).

The amino acids were quantified by HPLC as *o*-phthaldialdehyde derivatives on a C-18 column using Gold System 8·0 (Beckmann Instruments, San Ramon, CA, USA) as described in Murray *et al.* (1996).

Incorporation of ^{15}N into amino acids in the plants was determined by GC-MS (MD-800, Fisons Scientific Instruments, Crewe, UK) as t.BDMS derivatives. A sample of 0·4 μl was injected in the gas chromatograph fitted with an on-column injector, and a 30 m methylpolysiloxane, 0·25 μm film thickness, fused silica capillary column. Helium was used as a carrier gas at a flow rate of 1 ml min^{-1}, and the oven was temperature-programmed at 60 °C for 1 min, followed by an increase to 120 °C at a rate of 40 °C min^{-1}, and then increased to 260 °C at a rate of 4 °C min^{-1}. Mass spectra were acquired with an electron energy of 70 eV, and the mass range scanned from mass 50 to 600.

RESULTS

In all cases AM colonization significantly increased dry matter yield of both roots and shoots of the ryegrass (Fig. 1). No significant differences were seen between the plants growing on nitrate or Ser as

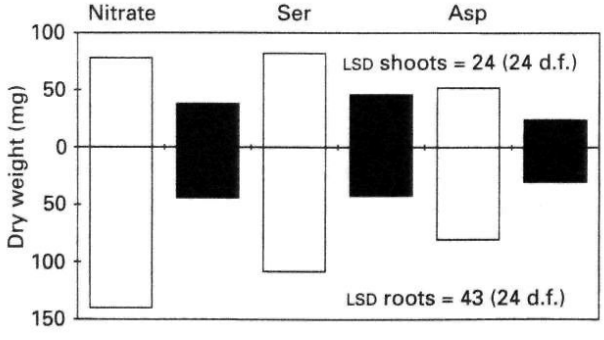

Figure 1. Effect of the arbuscular mycorrhizal (AM) fungus *Glomus fasciculatum* on plant biomass of 49-d-old ryegrass fed with nitrate, Ser or Asp as the nitrogen source. □, AM plants; ■, control plants. Each point is the mean of six independent replicates.

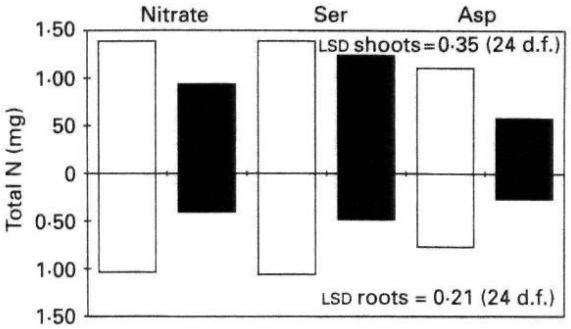

Figure 2. Effect of the arbuscular mycorrhizal (AM) fungus *Glomus fasciculatum* on N content of 49-d-old ryegrass fed with nitrate, Ser or Asp as the nitrogen source. □, AM plants, ■, control plants. Each point is the mean of six independent replicates.

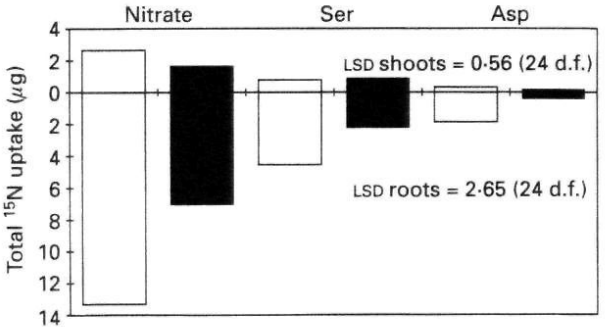

Figure 3. Effect of the arbuscular mycorrhizal (AM) fungus *Glomus fasciculatum* on ^{15}N uptake of 49-d-old ryegrass fed with nitrate, Ser or Asp as the nitrogen source. Plants were fed for 1 h with 4 mM ^{15}N-nitrate, ^{15}N-Ser or ^{15}N-Asp, see 'Materials and Methods'. □, AM plants, ■, control plants. Each point is the mean of six independent replicates.

the sole N source. However Asp-fed plants were significantly smaller than the nitrate or Ser-fed plants. The chitin content in AM plants was 7·4 mg g^{-1} d. wt for nitrate-fed plants (i.e., 21 mg of harvested mycelium g^{-1} f. wt), 7·0 mg g^{-1} d. wt for Ser-fed plants (i.e., 20 mg of harvested mycelium g^{-1} f. wt), and 9·1 mg g^{-1} d. wt for Asp-fed plants (i.e., 26 mg of harvested mycelium g^{-1} f. wt).

In all cases AM colonization significantly increased the total N content of roots. It increased total shoot N content of plants fed with nitrate and Asp, but not that of plants fed with Ser. Asp-fed control plants contained less N than all other plants (Fig. 2).

After labelling for 1 h with ^{15}N-labelled N source, uptake of ^{15}N by AM plants was significantly greater than that of control plants. Uptake of ^{15}N from nitrate was significantly greater than from Ser for both control and AM plants. Similarly uptake of ^{15}N from Ser by both control and AM plants was significantly greater than uptake of ^{15}N from Asp (Fig. 3).

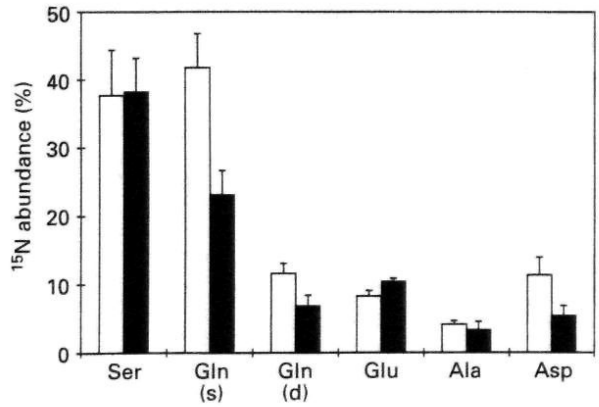

Figure 4. Effect of the arbuscular mycorrhizal (AM) fungus *Glomus fasciculatum* on ^{15}N incorporation into amino acids of root of Ser-fed 49-d-old ryegrass. Plants were fed for 1 h with 4 mM ^{15}N-Ser, see 'Materials and Methods'. □, AM roots, ■, control roots. For GLN s: single ^{15}N-labelling, d: double ^{15}N-labelling. Each point is the mean (±SE) of six independent replicates.

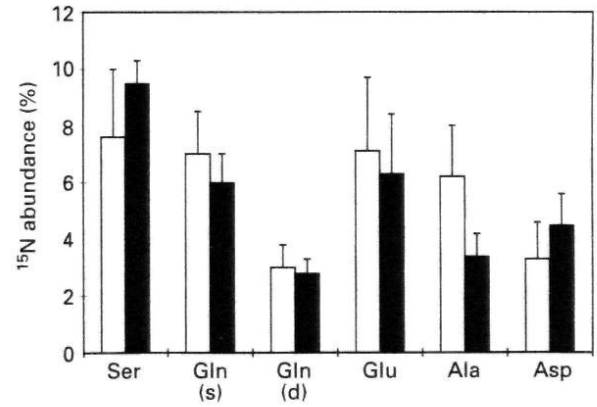

Figure 5. Effect of the arbuscular mycorrhizal (AM) fungus *Glomus fasciculatum* on ^{15}N incorporation into amino acids of root of Asp-fed 49-d-old ryegrass. Plants were fed for 1 h with 4 mM ^{15}N-Asp, see 'Materials and Methods'. □, AM roots, ■, control roots. For GLN s: single ^{15}N-labelling, d: double ^{15}N-labelling. Each point is the mean (±SE) of six independent replicates.

After feeding ^{15}N labelled Ser for 1 h, ^{15}N enrichment of Ser in the roots of Ser-fed plants was *c.* 38%. GLN labelling was also high, particularly in AM plants (Fig. 4). By contrast, in ^{15}N Asp-fed plants, ^{15}N abundance of all amino acids was much lower, peaking at *c.* 10% in Asp (Fig. 5). After feeding ^{15}N-labelled Ser or Asp, ^{15}N enrichment was similar for most of the amino acids in control and AM plants (Figs 4, 5).

Amino acid profiles of the root zone solutions were similar at the different collection dates, and only the total of the five dates is shown in Table I. There were no significant differences in the amino acid composition of root zone solutions from control and AM plants. The amino acid profiles and quantities were equivalent in the root zones of nitrate and Ser-fed plants, where the two most abundant amino acids

Table 1. *Effect of the arbuscular mycorrhizal (AM) fungus* Glomus *on composition of amino acids collected in the root-zone of ryegrass in nmol per plant*

Amino acid	Nitrogen source					
	Asp		Ser		Nitrate	
	−AM	+AM	−AM	+AM	−AM	+AM
Asp	2·04 ± 0·26	1·79 ± 0·23	2·80 ± 0·76	3·02 ± 0·70	3·27 ± 0·61	3·46 ± 0·52
Glu	12·98 ± 1·80	10·55 ± 1·66	7·91 ± 0·86	9·40 ± 1·60	11·24 ± 1·93	7·16 ± 1·03
Ala	2·44 ± 0·45	2·02 ± 0·29	4·22 ± 1·15	4·75 ± 0·99	5·74 ± 1·27	5·16 ± 0·93
Ser	4·69 ± 0·81	4·19 ± 0·68	15·60 ± 4·64	14·38 ± 3·16	17·32 ± 4·20	15·66 ± 2·86
Gln	4·16 ± 0·53	2·96 ± 0·68	3·34 ± 0·61	6·34 ± 0·84	4·53 ± 1·11	4·47 ± 0·66
Gly	2·71 ± 0·82	7·16 ± 1·02	13·28 ± 3·64	11·12 ± 2·31	13·77 ± 3·53	14·13 ± 2·54
Others	5·14 ± 1·21	3·35 ± 0·87	9·50 ± 2·39	9·61 ± 3·01	12·05 ± 3·38	11·72 ± 2·80
Total	34·15 ± 5·90	32·02 ± 5·44	56·65 ± 14·10	58·62 ± 12·60	67·90 ± 16·00	61·76 ± 11·30

Results from five collections were added, see Materials and Methods. Values represent the mean of six independent replicates.

were Ser and Gly. Approximately half the quantity of amino acids was collected from the root zone of Asp-fed plants, where Glu was the most abundant amino acid (Table 1).

DISCUSSION

Previous work has shown that ericoid mycorrhizas can use protein as an N source by excretion of protease into the medium (Leake & Read, 1989). Ames *et al.* (1984) demonstrated that AM-colonized sorghum (*Sorghum bicolor*) can use an organic source of N, although no protease excretion has been found in AM symbiosis. Direct uptake of amino acids has been demonstrated for the ectomycorrhizal fungus *Paxillus involutus* (Chalot *et al.*, 1995) but little is known about endomycorrhiza.

Our work demonstrates that ryegrass plants symbiotic with *G. fasciculatum* are more efficient in their use of Ser and Asp as a source of N. After 1 h of labelling, Ser-fed AM and control plants took up 5·16 and 3·10 μg of Ser-N respectively, whilst Asp-fed AM ryegrass took up 2·20 against 0·60 μg Asp-N by the controls. These differences between AM and control plants could be explained by a greater volume of the growth medium being explored by the colonized roots. Although AM plants tended to take up more organic N than the control, there were no differences in uptake per unit d. wt.

The present study confirms the findings from previous work which demonstrated the importance of amino acids in the overall N nutrition of plants (Chapin *et al.*, 1993; Jones & Darrah, 1994; Shepherd & Davis, 1994). Whereas previous work has relied on quantifying the rate of disappearance of amino acids to determine the extent of their uptake from soil solution (Chapin *et al.*, 1993; Jones & Darrah, 1993; Shepherd & Davis, 1994), in this study we have measured directly the newly absorbed ^{15}N-labelled amino acids in the roots of intact plants. Because we did not measure ^{15}N-labelled amino acids in external mycelium, the form in which the N compounds entered the plant was not determined conclusively. However, the high labelling of Ser in the roots supports the hypothesis that a large proportion of the Ser in the plant was taken up as Ser, regardless of any microbial breakdown in the soil. After 1 h of labelling, from 61 % (AM plants) to 74 % (control plants) of the ^{15}N in the roots was recovered in amino acids, and from 27 % (AM plants) to 35 % (control plants) was recovered in Ser. The data show that GLN is a strong sink for ^{15}N originating from Ser. This could be a result of hydrolysis of Ser in the roots followed by reassimilation of the resulting ammonia via the GS-GOGAT cycle. The higher labelling of Gln in the AM plants could be a product of greater GS activity in mycorrhizal plants (Cliquet & Stewart, 1993). The similar labelling obtained for most of the amino acids in control and AM roots suggests little evidence of a qualitative effect of the mycorrhizal colonization.

The evidence for direct uptake of Asp is less clear. Labelling of amino acids, and uptake of ^{15}N into the roots was relatively lower than with the Ser plants. This could be the result of different carriers for neutral and acidic amino acids as it has been shown in sugar-cane suspension cells (Wyse & Komor, 1984), or of a greater microbial breakdown of Asp in the soil. Both Asp and GLU were the most heavily labelled amino acids in Asp-fed plants. The large proportion of Glu in the root zone of Asp-fed plants could be explained by Asp transformation to Glu, or efflux of Glu as a result of Asp influx to the plant. Our data cannot distinguish between synthesis of Glu from Asp in the roots, by aspartate aminotransferase (Quoreshi *et al.*, 1995), or in the root zone.

Previous work has demonstrated that both ecto- and endomycorrhizas are able to mediate N-transfer between plants (Haystead, Malaczuk & Grove, 1988;

Barea, Azcón & Azcón-Aguilar, 1989; Bethlenfalvay *et al.*, 1991; Arnebrant *et al.*, 1993; Frey & Schuepp, 1994). However, Hamel *et al.* (1991) found that the role of hyphal bridges in interspecific N-transfer was not significant. Although the present work shows that the AM fungus does not alter the equilibrium of amino acids in the root zone, the fungus could play a significant role in utilization of previously released amino acids (Hamel *et al.*, 1991). In grassland systems, where plants are grown in very close association, such mycorrhizal links could be important in the cycling of N between plants, especially between legumes and grasses (Barea *et al.*, 1989).

A previous study found that roots of white clover exuded large quantities of organic N particularly as Ser and Asp, and especially from roots (*Trifolium repens*) which had been damaged by insect larvae (Murray *et al.*, 1996). This work shows that ryegrass plants are able to utilize these exudates directly, that uptake of N from these compounds is greatly enhanced by AM colonization, and that ryegrass plants appear to be able to grow equally well on Ser as on nitrate.

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