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Acquisition of nitrogen by external hyphae of arbuscular mycorrhizal fungi associated with *Zea mays* L.

BY BEAT FREY* AND HANNES SCHÜEPP

Department of Phytopathology and Soil Microbiology, Swiss Federal Research Station, CH-8820 Wädenswil, Switzerland

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SUMMARY

The objective of this study was to examine the ability of arbuscular mycorrhizal (AM) fungi to take up nitrogen from soil and transport it to the host plant. Maize (*Zea mays* L.) associated with *Glomus intraradices* Schenck and Smith or left uninoculated was grown in containers which were divided by a nylon net into a root compartment and a hyphal compartment. A 40 μm pore size nylon net was used to exclude plant roots while allowing fungal hyphae to grow into soil confined by the net. ^{15}N tracer was supplied either as inorganic N or as organic N to the hyphal compartment at a distance of 5 cm from the net.

Inoculation with the AM fungus increased the ^{15}N content of maize compared to the non-mycorrhizal controls when N was applied as $(^{15}NH_4)_2SO_4$. However, there was no conclusive evidence that AM hyphae could derive N from organic ^{15}N sources. Most of the increased N uptake of mycorrhizal plants occurred by hyphal translocation from the hyphal compartment to the root compartment. Higher N uptake by mycorrhizal plants with access to the hyphal compartment was indicated by depletion of total ^{15}N in the soil of that compartment. Cutting the extraradical hyphae in the hyphal compartment in order to sever their connection with the host roots decreased the ^{15}N uptake of the maize plants. A time-course study with inorganic ^{15}N over 26 d showed that *G. intraradices* transported most of the ^{15}N between 10 and 15 d after ^{15}N application, whereas the non-mycorrhizal control plants had a consistently low concentration of ^{15}N throughout the period of sampling.

Nitrogen transport by external hyphae of three AM fungi, *G. intraradices*, *Acaulospora laevis* Gerdemann and Trappe and *Gigaspora margarita* Becker and Hall associated with maize, was further investigated. The results indicated that different isolates of AM fungi differ in the efficiency of hyphal N transport as a consequence of the different patterns of hyphal spread in the soil or of the different capacity for uptake by unit length of hyphae.

Key words: *Zea mays*, nitrogen, ^{15}N transport, arbuscular mycorrhizal fungi, external hyphae.

INTRODUCTION

Associations between arbuscular mycorrhizal (AM) fungi and plant roots make an important contribution to plant nutrition. Mycorrhizal colonization of roots often promotes increased plant nutrient uptake and growth (Harley & Smith, 1983). Hyphae outside the root can extend several centimetres from the root surface (Li, George & Marschner, 1991) and thereby increase the effective absorptive surface of the root (Read, 1984). This is particularly important when considering the nutrition of plants for immobile nutrients such as phosphorus (Nye & Tinker, 1977), as hyphae are known to absorb P and translocate it to the host plant (Jakobsen, Abbott & Robson, 1992*b*).

Relatively little is known about the effects of the AM symbiosis on the N nutrition of plants. N applications have been reported to both stimulate (Brown, Schultz & Kormanik, 1981; Hepper, 1983) and suppress root colonization (Chambers, Smith & Smith, 1980; Azcon, Gomez-Ortega & Barea, 1982). Sylvia & Neal (1990) suggested that plant N stress, like P stress, promotes root colonization by AM fungi. Johnson, Jarrell & Menge (1984) concluded that the form of N may profoundly affect colonization by AM fungi. In hydroponic sand cultures they found the mycorrhizal colonization to decrease to a higher extent by amending with NH_4^+-N compared to NO_3^--N.

Reviews of the literature suggest that the function of AM fungi in plant N acquisition is variable. Rhodes & Gerdeman (1980) stated that the N contribution by AM to the host plant would probably

* To whom correspondence should be addressed.

be of little significance. Increased N content in arbuscular mycorrhizal plants is occasionally observed in pot experiments (see Cooper, 1984). However, mycorrhizal associations can be important factors influencing the N nutrition of plants, particularly when the relatively immobile NH_4^+-N rather than the mobile NO_3^--N is the major source of plant-available N (Smith, 1980; Bowen & Smith, 1981). The role of AM fungi in N uptake or metabolism in plants is not clear, although Smith *et al.* (1985) concluded that these fungi are able to assimilate ammonium via glutamine synthetase. Uptake and transport of N via AM external hyphae have been reported by Ames *et al.* (1983) and Johansen, Jakobsen & Jensen (1992). Furthermore, a possible role of AM fungi as agents for transferring symbiotically fixed N from a legume to a non-legume in order to reduce N fertilization has been shown by Frey & Schüepp (1992). It is necessary to estimate the qualitative and quantitative importance of AM fungi for the uptake of soil N by plants grown under different conditions.

The objective of the present work was to determine whether the external mycelium is of importance for the host-plant N nutrition using a two-compartment system. Compartmentation of soil volumes containing roots and hyphae and hyphae only allows the hyphae to be fed differentially from the roots (Kothari, Marschner & George, 1990; Faber *et al.*, 1991). ^{15}N as a tracer was placed in the hyphal compartment at a minimum distance of 5 cm from the roots. Three experiments were conducted: (1) to examine the ability of mycorrhizal hyphae to transport N-labelled inorganic N to the host plant, (2) to compare the effectiveness of hyphae of three AM fungi in their N transport to the host, and (3) to examine the ability of AM fungi to utilize two types of organic N.

MATERIAL AND METHODS

Experiment 1 (a) and (b)

The aims of these experiments were to determine whether mycorrhizal hyphae could transport ^{15}N-labelled inorganic N from the soil to the host plant (expt 1*a*) and if so, the time-course of the arrival of the ^{15}N in the host plant's roots and shoots (expt 1*b*).

In expt 1*a* the potential problem associated with physiological differences between mycorrhizal and non-mycorrhizal plants was avoided by the use of mycorrhizal controls in which the mycorrhizal mycelium was physically disrupted repeatedly over the 15 d after ^{15}N application. Thus there were three treatments: I, non-inoculated control; II, mycorrhizal inoculation; III, mycorrhizal inoculation with repeated disturbance of the external hyphae. There were four replicates, giving a total of 12 containers.

Expt 1*b* was conducted to determine the time-course of N transport using treatments I and II only. Again, there were four replicates but these were examined at 6 harvests, giving a total of 48 containers. The maize plants were harvested 2, 6, 10, 15, 20 and 26 d after the addition of ^{15}N.

The experiments were carried out using sweet maize (*Zea mays* L. cv. Honeycomb-F1) as host plant. Seeds were surface sterilized in 5 % sodium hypochlorite for 5 min, rinsed 3 times with sterile water and germinated on moistened filter paper for 3 d. One seedling was transplanted into a two-compartment system (Fig. 1), one for root and adhering mycorrhizal structures ($14{\cdot}5 \times 16 \times 2$ cm) and one for extraradical hyphae in root-free soil ($14{\cdot}5 \times 16 \times 6$ cm). The hyphal compartment was separated from the root compartment using a 40 μm nylon net (Zürich Bolting Cloth Mfg Co. Ltd, 8803 Rüschlikon, Switzerland), which was previously shown to allow penetration by hyphae while preventing penetration by roots (Frey & Schüepp, 1992).

A sandy loam with an organic matter of 3·5 % from a field plot at 'Hessen' (Wädenswil) was used which had not been fertilized for several years. The soil was sieved through a 2 mm mesh and air-dried. After γ-sterilization (10 kGy) the soil was combined 3:1 by volume with washed and autoclaved quartz sand. After sterilization, the soil–sand mixture had the following main characteristics: pH 7·1 (soil:water, 1:2), 8·0 μg $NaHCO_3$-extractable (Olsen) P g^{-1} soil (Olsen & Sommers, 1982), total N 0·24 % with 1 M KCl-extractable 21 μg g^{-1} NH_4^+-N and 30 μg g^{-1} NO_3^--N. The bulk density of the soil–sand mixture was 1·4 g cm^{-3}. The root compartments were filled with 520 g of dry, sterilized soil mix, and the hyphal compartments with 1440 g.

The AM-fungal inoculum used consisted of roots of *Tagetes* sp. colonized by *Glomus intraradices* Schenck and Smith plus associated spores and hyphae. The mycorrhizal fungus was isolated from a grass/clover field near Wädenswil and identified as *Glomus intraradices* by Dr C. Walker (Forestry Commission, Edinburgh). Mycorrhizal treatments (II–III) received 20 g of inoculum mixed uniformly with the soil or the root compartment. The non-inoculated control treatment (I) received 20 g of autoclaved inoculum together with a 2 ml aliquot of a filtrate (< 20 μm) of the AM fungal inoculum to provide a general microbial population, but to avoid indigenous AM fungi.

To measure the effect of hyphal disturbance on N transport to treatment III of expt 1*a*, hyphal connections in the hyphal compartments were severed by completely cross-sectioning the hyphal compartment with a spatula at a distance of 4 cm from the nylon net. The extraradical hyphae were severed immediately before the ^{15}N application was made and then on three subsequent occasions at intervals of 4 d.

The plants were kept in the glasshouse with

supplementary lighting for a 16 h photoperiod from high-pressure sodium vapour lights at an average irradiance of 300 μmol m^{-2} s^{-1} PAR over the waveband 400–700 nm. The temperature regime was 18–22 °C (day) and 16–18 °C (night). Mycorrhizal and non-mycorrhizal plants were given the same amounts of deionized water, and the water consumption of each container was determined daily by weight. Deionized water was supplied only to the root compartment, to keep the soil water content slightly higher in the plant compartment than in the hyphal compartment, but never above field capacity. The aim was to minimize the flow of ions by mass flow and diffusion from the hyphal compartment to the soil almost reached field capacity, but only at the beginning of the experiment. In order to reduce evaporation in the hyphal compartment, the soil surface outside the root compartments was covered with aluminium foil. From the fifth week onwards the plants were fertilized once a week with 20 ml of $\frac{1}{4}$-strengths Hewitt's solution (Hewitt, 1966) by omitting P for mycorrhizal plants. Non-mycorrhizal controls received the same solution containing 20 mg P l^{-1}. The containers were frequently rotated to minimize positional effects.

After the establishment of a hyphal network (48 d after planting), 15 mg of N were applied to the hyphal compartment in the form of 10 atom % $(^{15}NH_4)_2SO_4$ (Isotec Inc.). The labelled N was applied in solution in distilled water and was injected 5 cm from the root compartment. A 5 ml portion of solution was injected at each of 4 positions in order to distribute the label uniformly.

Experiment 2

This experiment was designed to determine whether different AM fungal isolates differ in their efficiency to transport ^{15}N-labelled inorganic N to the host. The experimental design was similar in principle to that in expt 1, except that treatment III (disturbing the external hyphae) was omitted. The three AM fungal isolates studies were: *Gigaspora margarita* Becker and Hall obtained from Dr S. Gianinazzi, INRA, Dijon; *Glomus intraradices* (from the same local source as that used in expt 1); and *Acaulospora laevis* Gerdemann and Trappe obtained from Dr D. Hayman, Rothamsted, Herts. Plants not supplied with a mycorrhizal inoculum were included as controls. The experiment, therefore, contained four inoculation treatments (three fungi plus a control).

The same procedures were used as in expt 1. The root compartments contained, however, two maize seedlings and the test soil was not mixed with quartz sand. There were four replicates in each treatment. The experiment was carried out in a growth chamber with a temperature regime of 25/20 °C (day/night) and a 16 h photoperiod at 400 μmol m^{-2} s^{-1} PAR over the waveband 400–700 nm. After 48 d of growth the ^{15}N-labelled solution was added as described in expt 1: $(^{15}NH_4)_2SO_4$ with 10 % ^{15}N-enrichment dissolved in distilled water was applied at a rate of 25 mg N $container^{-1}$. The plants were harvested 62 d after planting.

Experiment 3

The aim of this experiment was to assess the effect of AM fungal hyphae on the utilization of organic N by the host plant. At the beginning of the experiment the root-free soil compartment of both mycorrhizal and non-mycorrhizal plants received organic N as either ^{15}N-enriched plant material or ^{15}N-enriched fungal tissue.

The experimental design and conditions were the same as for expt 2, but different inoculation treatments were used. Root compartments with maize (two seedlings per compartment) were either inoculated with an AM fungus (+M) or used without inoculation (−M). The fungus used was *Glomus intraradices* (from the same local source as that used in expt 1). The inoculum procedure was as described above, and there were four replicates per treatment. The hyphal compartments in both treatments received an additional microbial population as a 2 ml portion of a filtrate (< 20 μm) of the AM fungal inoculum to ensure a proper degradation of the applied organic material.

Dried and finely ground (< 400 μm) ^{15}N-labelled plant material from previous experiments containing 1·7% N with 1·65 atom % ^{15}N excess was uniformly mixed (1·11 g per replicate) with the growth medium. Figure 1 shows the zone of the hyphal compartment to which the ^{15}N source was applied. The design of the compartment system was the same as that used for expts 1 and 2, but because the ^{15}N was not applied in solution and therefore could not be injected, the zone extending up to 4 cm from the root compartment was filled first with the growth medium, and then the final 2 cm section of the hyphal compartment was filled with a mixture of growth medium and ^{15}N-labelled plant material using the method of Schüepp, Bodmer & Miller (1992). This prevented mixing of ^{15}N-labelled soil with unlabelled soil in the main part of the hyphal compartment adjacent to the root compartment.

^{15}N-labelled fungal material was prepared after a modified method of Schnürer & Rosswall (1987). *Trichoderma harzianum* was grown on malt extract agar (Oxoid) until sporulation (2–3 wk). A mycelium-free spore inoculum was grown in a modified Norkrans-medium (Norkrans, 1963) with 1·13 g $(^{15}NH_4)_2SO_4$ (99 atom % ^{15}N) at 20 °C on a rotary shaker for 50 d. The fungal tissue was then collected, dried at 60 °C and finely ground. The ^{15}N-labelled fungal tissue was uniformly mixed with the corresponding soil (0·35 g each replicate). The filling procedure of the cuvette section at a distance of

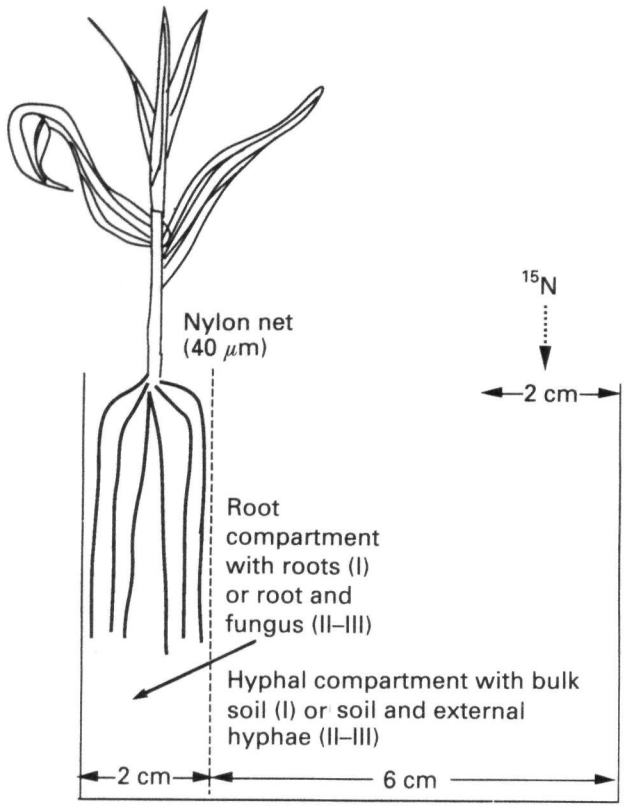

Figure 1. Details of the two-compartment system to separate soil volumes for root and hyphal growth used for studying transport of N by hyphae of arbuscular mycorrhizal fungi associated with *Zea mays*. Arrow with dashed line indicates zone to which ^{15}N was added. Treatments were as follows (expt 1): I, without fungus; II, external mycelium, not restricted and undisturbed; III, external mycelium, not restricted but disturbed by a spatula at a distance of 4 cm from the root compartment.

4–6 cm from the roots was the same as described above. The concentrations of N and atom % ^{15}N excess of the fungal material were 6·5 % and over 95 %, respectively.

The plants were maintained in the growth chamber under the same conditions as described for expt 2. The watering regime and fertilization for mycorrhizal and non-mycorrhizal treatments were the same as those in expt 1. The plants were harvested after 84 d of growth.

Harvests and measurements

Soil samples were removed at harvest using a soil corer (1 cm diameter × 6 cm depth) for measurement of hyphal lengths and mineral N analysis in soil. Duplicate soil cores were taken inside root compartments and were pooled. Three replicates of each distance were carried out for sampling from the hyphal compartment representing 1, 3 and 5 cm distance from the nylon net. Cores from each distance were pooled. All the soil samples were air dried. After the soil cores had been taken, the plants were harvested. The roots were carefully washed to remove all soil particles. Before the plant material was dried, a weighed subsample from each root compartment was stained with acid fuchsin in lactic acid (Kormanik, Bryan & Schultz, 1980) to determine AM fungal colonization. The percentage of mycorrhizal colonization (as percentage of total root length) was obtained by the gridline intersect method (Ambler & Young, 1977), observing 200 root-intersections to obtain an error below 3 % (Giovannetti & Mosse, 1980). The rest of the plant material was dried at 70 °C for 48 h and weighed.

The length of hyphae in the soil was measured by the membrane filter technique (Hanssen, Thingstad & Goksoyr, 1974) modified by Frey & Schüepp (1992). The length of mycelium was recorded as m g^{-1} dry soil (Newman, 1966). The hyphal background in hyphal compartments of non-mycorrhizal plants was subtracted from the values found in the hyphal compartment of mycorrhizal plants.

Subsamples from the air-dried soil were used for determination of total N and ^{15}N. The soil was sieved through a 315 μm mesh prior to analysis. Shoots and roots were finely ground using a cyclone mill and analyzed for total content of N and ^{15}N. The N content was measured on duplicate subsamples using an automatic analyzer (Carlo Erba model 1500 N, Milan, Italy) and ^{15}N enrichment was determined using a mass spectrometer (VG Isogas model MM622). To calculate the atom % ^{15}N excess in plant material, the atom % ^{15}N of plants not supplied with ^{15}N served as background.

Statistical analysis

Normality of the data was tested by using the Kolmogorov–Smirnov test. The data were then subjected to analysis of variance for a fully randomized design. The significance of the *F*-ratio was used to indicate statistical significance where there were only two treatments. Where there were more than two treatments the means were compared by calculating least significance difference (LSD) at the 5 % level.

RESULTS

Experiment 1 (a) and 1 (b)

The percentage of root length colonized by *G. intraradices* (Table 1) was 58 % and 64 % in treatments II and III, respectively, while no colonization was found in the non-inoculated control plants (treatment I). The external hyphae proliferated into the hyphal compartments, while no roots were observed in these compartments. Hyphal length in the hyphal compartment of mycorrhizal treatments ranged from 7 to 12 m fungal hyphae g^{-1} dry soil (data not shown). The hyphal background in the bulk soil of the non-mycorrhizal treatment (I) amounted to 0·9 m g^{-1} dry soil.

Shoot and root dry weight of maize was similar in non-mycorrhizal plants and in plants colonized by the AM fungus (Table 1). The N concentration in the shoots and roots of controls was lower than in the

Table 1. *Mycorrhizal colonization and dry weights in maize shoots and roots associated or not (control) with* Glomus intraradices *(expt 1)*

Treatment	Root length colonized (%)	Dry weight (g)	
		Shoot	Root
Control	0 b*	3·6 a	1·2 a
Mycorrhizal	58 a	3·3 a	1·0 a
Mycorrhizal, hyphae disturbed	64 a	3·8 a	1·0 a

* Values are means of four replicates. Within each column, any two means followed by the same letter are not significantly different by least significant difference ($LSD_{x0·05}$).

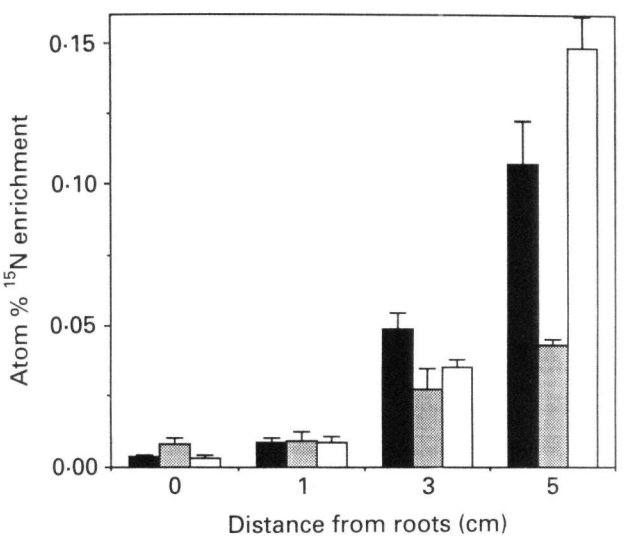

Figure 2. Concentration of ^{15}N in the soil of the root compartment and in the soil of the hyphal compartment with increasing distance from the roots (expt 1). Plants were harvested 15 d after application of $(^{15}NH_4)_2SO_4$. Treatments were as follows (expt 1): ■, without fungus; ▨, external mycelium, not restricted and undisturbed; □, external mycelium, not restricted but disturbed by a spatula at a distance of 4 cm from the root compartment. Lines represent the standard deviation of values.

shoots and roots of mycorrhizal plants (Table 2). The ^{15}N data clearly showed the highest ^{15}N enrichment in maize in the mycorrhizal treatment

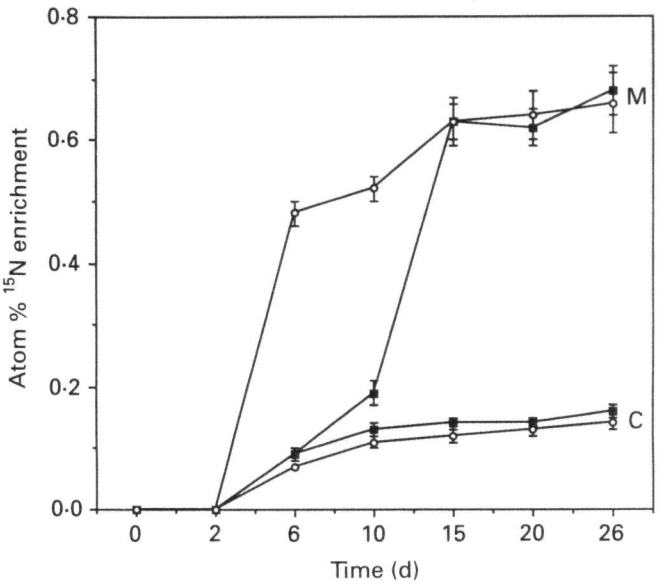

Figure 3. The time-course of appearance of ^{15}N expressed as atom % ^{15}N excess in roots (○) and shoots (■) of mycorrhizal (M) and non-mycorrhizal (C) *Zea mays* after application of $(^{15}NH_4)_2SO_4$ to the root-free soil compartment at a distance of 5 cm from the roots (expt 1). Each sample date represents a mean of four plants (shoot and root). Bars indicate the standard errors of means.

(II) where hyphae were not disturbed (Table 2) compared to the other two treatments (I, III). Non-mycorrhizal maize (I) showed the lowest ^{15}N enrichment, while the ^{15}N enrichment of maize where hyphae were disturbed (III) was slightly increased. The ^{15}N content of mycorrhizal plants in treatment II corresponds to a 39% recovery of applied ^{15}N, whereas the recovery of the other treatments (I, III) was below 10%. The hyphal contribution to plant uptake of ^{15}N from the hyphal compartment accounted for 72%, calculated from the differences of treatments II and III.

Mycorrhizal hyphae significantly depleted ^{15}N in the hyphal compartment at a distance of 4–6 cm from the roots in the undisturbed treatment (II) compared to the other two treatments (I, III, see Fig. 2).

Figure 3 shows the time-course of ^{15}N appearance in maize. The tracer was first detected after 6 d. The

Table 2. *N concentrations and atom % ^{15}N excess in maize shoots and roots associated or not (control) with* Glomus intraradices *(expt 1); $(^{15}NH_4)_2SO_4$ was supplied to the hyphal compartment at a distance of 5 cm from the roots*

Treatment	N concentration (%)		Atom % ^{15}N excess	
	Shoot	Root	Shoot	Root
Control	1·4 b*	1·1 b	0·14 b	0·13 c
Mycorrhizal	2·3 a	1·6 a	0·63 a	0·63 a
Mycorrhizal, hyphae disturbed	2·0 a	1·5 a	0·16 b	0·25 b

* Values are means of four replicates. Significance denoted by ($LSD_{x0·05}$) as in Table 1.

control plants contained only traces of ^{15}N after 6 d. The ^{15}N enrichment of mycorrhizal plants increased with time. The highest uptake of ^{15}N by mycorrhizal plants occurred during the 10–15 d period. In mycorrhizal plants the ^{15}N enrichment in roots was significantly higher than the ^{15}N enrichment in shoots after 6 and 10 d. Non-mycorrhizal plants showed lower levels of atom % ^{15}N excess and remained at a constant low level throughout the study, indicating that mass flow was not completely prevented.

Experiment 2

No mycorrhiza developed in the non-inoculated control pots. Mycorrhizal colonization showed no appreciable difference among the mycorrhizal isolates tested, and 69–84 % of the root length was colonized (Table 3). The length of hyphae (m g^{-1} dry soil) in the hyphal compartment showed different patterns between fungal isolates (Fig. 4). *G. margarita* showed the highest hyphal density close to the roots (1 cm). However, the density decreased at further distances, whereas the hyphal density of *G. intraradices* rose at increasing distances from the roots. The largest hyphal density at the site of ^{15}N application (5 cm distance from the root surface) was recorded with *G. intraradices*. *A. laevis* produced the least amounts of hyphae at all sampling distances.

Dry weight of shoots and roots was unaffected by the different treatments (Table 3). The concentration of N in maize shoots differed significantly between inoculation treatments (Table 3). Plants infected with *G. intraradices* and *G. margarita* had the highest N concentration in the shoots, whereas the non-inoculated control plants had the lowest N concentration. Similarly, atom % ^{15}N excess in the shoots of maize colonized by *G. intraradices* and *G. margarita* was highest. However, atom % ^{15}N excess of maize colonized by *A. laevis* was significantly increased compared to the control plants (Table 3). The ^{15}N enrichments in roots and shoots were similar in non-mycorrhizal plants. However, in mycorrhizal plants the ^{15}N-enrichment in roots was about twice that measured in shoots.

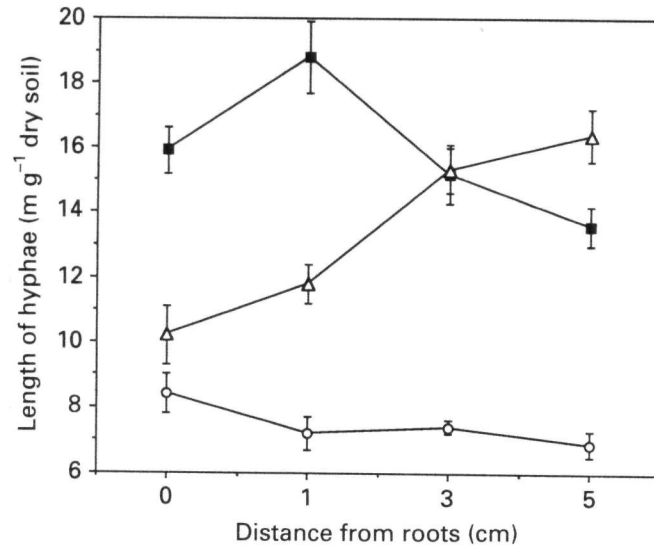

Figure 4. Length of arbuscular mycorrhizal hyphae in soil inside the root compartment (0 cm) and at increasing distances from the roots of *Zea mays* after 62 d of growth (expt 2). ○, *Acaulospora laevis*; △, *Glomus intraradices*; ■, *Gigaspora margarita*. Bars represent the standard errors of the means ($n = 4$).

Experiment 3

Mycorrhizas were present in over 80 % of the total root length of maize after 84 d in the inoculated

Table 3. *Mycorrhizal colonization, plant dry weight, nitrogen concentration and atom % ^{15}N excess in maize associated or not (control) with three AM fungi (expt 2); $(^{15}NH_4)_2SO_4$ was supplied to the hyphal compartment at a distance 5 cm from the roots*

		D. wt (g)	
Fungus	Colonization (%)	Shoot	Root
Control	0 b*	10·1 a	4·8 a
Acaulospora laevis	69 a	10·7 a	4·4 a
Gigaspora margarita	84 a	10·4 a	4·1 a
Glomus intraradices	78 a	10·7 a	4·3 a
	% N	Atom % ^{15}N excess	
Fungus	Shoot / Root	Shoot	Root
Control	0·77 c / 0·72 b	0·14 c	0·14 b
Acaulospora laevis	0·87 b / 0·86 ab	0·46 b	0·86 a
Gigaspora margarita	1·21 a / 1·00 a	0·51 a	0·96 a
Glomus intraradices	1·08 a / 0·87 ab	0·53 a	0·91 a

* Values are means of four replicates. Significance denoted by $LSD_{\alpha 0.05}$ as in Tables 1 and 2.

Table 4. *Mycorrhizal colonization, plant dry weight, nitrogen concentration and atom % ^{15}N excess in maize associated with* Glomus intraradices *or not (control). Organic ^{15}N was supplied to the root-free soil compartment at a distance of 5 cm from the roots as (a) ^{15}N-labelled plant material with 1·65 atom % ^{15}N excess; or (b) ^{15}N-labelled fungal material with 95 atom % ^{15}N excess (expt 3)*

Treatment	Colonization (%)	D. wt (g)		% N		Atom % ^{15}N excess	
		Shoot	Root	Shoot	Root	Shoot	Root
(*a*)							
Control	0*	15·8	5·5	0·62	0·61	0·03	0·03
Mycorrhizal	82	16·4	5·0	0·73	0·69	0·05	0·04
Significance of difference between treatments†		NS	NS	**	**	**	NS
(*b*)							
Control	0	15·4	5·7	0·61	0·63	4·46	3·25
Mycorrhizal	89	17·8	5·4	0·71	0·66	4·30	2·89
Significance of difference between treatments†		NS	NS	**	NS	NS	NS

* Values are means of four replicates. † by analysis of variance; NS, not significant. ** $P < 0·05$.

treatment, while no colonization was observed in the non-inoculated treatment (Table 4). The length of external hyphae ranged between 8 and 14 m g^{-1} dry soil at various distances from the roots.

There was no difference in shoot and root dry weight between mycorrhizal and control plants. The N concentration was generally higher in mycorrhizal than in non-mycorrhizal plants (Table 4). The ^{15}N enrichment was higher in all plants than the natural level measured in plants not exposed to ^{15}N (0·3706 atom % ^{15}N). The atom % ^{15}N excess in mycorrhizal plants was increased compared to non-mycorrhizal plants when ^{15}N was applied as ^{15}N-labelled plant material (Table 4*a*). Mycorrhizal plants contained 17 % (SE ± 2), whereas non-mycorrhizal plants contained only 5 % (SE ± 1) of the ^{15}N supplied. However, when the ^{15}N was applied as fungal material, the atom % ^{15}N excess was similar in root and shoot tissues of mycorrhizal plants compared to tissues of non-mycorrhizal plants at harvest (Table 4*b*). Both mycorrhizal and control plants contained 29 % (SE ± 2) of the ^{15}N supplied. The mean recovery of ^{15}N in soil and plant parts was only about 82 %.

DISCUSSION

Inoculation with the AM fungi improved the N nutrition of the host plant in a two-compartment growth system. The experimental compartmentation of roots and hyphae allowed hyphae to be fed independently from the roots, and the contribution of hyphae to the host plant N acquisition could be determined. The increased N uptake of mycorrhizal plants resulted from the hyphal contribution and probably also from increased supply of N to the root surface by mass flow. Higher N uptake by mycorrhizal plants with access to the hyphal compartment was further indicated by depletion of ^{15}N in this compartment. Cutting the extraradical hyphae in the hyphal compartment from their roots adversely affected the ^{15}N uptake by maize. Our results support the view that AM hyphae may improve the capacity of higher plants to acquire inorganic N (Ames *et al.*, 1983; Barea, Azcon-Aguilar & Azcon, 1987; Johansen, Jakobsen & Jensen 1992) but do not fully support the findings of Ames *et al.* (1983) that mycorrhizal plants derived more ^{15}N from an organic source than non-mycorrhizal plants. The present study also shows that different isolates of AM fungi may differ in their efficiency for hyphal N transport.

The increased N acquisition by mycorrhizal plants did not result in improved plant growth, although the fungi may have absorbed nutrients from the hyphal compartment not available to non-mycorrhizal plants. The mycorrhizal contribution accounted for more than 30 % of total N uptake of mycorrhizal plants (expt 1) and is mainly due to the low N fertilization. The absence of growth response to mycorrhizal inoculation was presumably due either to high root densities fully exploring the root compartment or to a suboptimal nutrient supply. The plants did not show any symptoms of P deficiency, but the N supply was suboptimal because typical nitrogen deficiency symptoms such as an enhanced senescence of older leaves (Marschner, 1986) could be seen. Hyphal access to the outer compartments improved N nutrition and may have delayed senescence in mycorrhizal plants. Lack of a mycorrhizal response (shoot dry weight) in small pots with maize has also been documented by Kothari, Marschner & Römheld (1991).

Dissimilarities in root morphology and physiology between mycorrhizal and non-mycorrhizal plants (Koide, 1991) and increased populations of certain microorganisms near the extramatrical hyphae of AM fungi in the soil (Linderman, 1988) can

complicate the study of nutrient transfer by mycorrhizas. In expt 1, such complications were minimized by including a mycorrhizal control treatment in which hyphae were severed. Mycorrhizal roots were equally infected in both mycorrhizal treatments, but with a different access to ^{15}N. This comparison ensured that until the time of ^{15}N application plant roots had all the characteristics of a mycorrhizal plant, including the same nutrient status. It can furthermore be assumed that the microflora other than the external AM mycelium in the hyphal compartment did not differ between the disturbed and undisturbed treatments at the time of ^{15}N application. There was no difference in water use of mycorrhizal plants compared to non-mycorrhizal plants as determined by weighing the containers daily, indicating that increased N uptake in mycorrhizal plants was not due to different mass flow rate between mycorrhizal plants and controls. Since a nitrification inhibitor was not used in the present study, microbial formation of NO_3^--N in the soil cannot be discounted.

Johansen *et al.* (1992) used a nitrification inhibitor to minimize the conversion of applied NH_4^+-N into more mobile NO_3^--N, so that differences in the availability of the added N in mycorrhizal and non-mycorrhizal treatments due to mass flow and diffusion would not obscure differences due to hyphal transport. An additional experimental modification which can be used to provide a barrier to soil water movement between hyphal and root compartments is the introduction of a 2 mm air gap as described by Faber *et al.* (1991) and George *et al.* (1992).

There was a lag in the transfer of N from the fungus to the host plant (Fig. 3), probably owing to the N requirement for growth of the fungus. In mycorrhizal plants, the ^{15}N enrichment in roots was significantly higher than the ^{15}N enrichment in shoots 6 and 10 d after the application of ^{15}N. Similarly, in expt 2 the ^{15}N enrichment in roots was about twice that measured in shoots, whereas ^{15}N enrichments in roots and shoots in non-mycorrhizal plants were similar (Table 3). This may indicate that ^{15}N-labelled N has accumulated in the fungal tissues inside the roots before being translocated into the shoots. Johansen *et al.* (1992) determined the ^{15}N content in the extraradical hyphae in hyphal compartment supplied with ^{15}N, and they showed that a considerable part of the labelled N source was localized in the external hyphae.

Higher ^{15}N uptake by mycorrhizal plants corresponds to a depletion of ^{15}N in the presence of undisturbed hyphae in the hyphal compartment at a distance of 4–6 cm from the roots (Fig. 2, expt 1). This is in agreement with the findings of Johansen *et al.* (1992) and George *et al.* (1992) that mycorrhizal hyphae may efficiently deplete the soil for N. The depletion of soil ^{15}N by the external hyphae may prevent nitrate-leaching and denitrification. In fact, Haines & Best (1976) reported that the presence of AM fungi reduced the amount of nitrate leached from soil cores, which could either be due to enhanced uptake by mycorrhizas or to immobilization by microbes. In the present study, the recovery of ^{15}N in the soil–plant system was between 80 and 90 % in mycorrhizal and non-mycorrhizal treatments, indicating that leaching of N and denitrification losses cannot be ruled out (Barraclough, Greens & Maggs, 1984).

It is not clear in expt 1 (Fig. 2) whether the higher soil ^{15}N level in treatment III (hyphal cutting) compared to treatment I (control) is due to immobilization of ^{15}N by decomposing microorganisms as a consequence of death of truncated hyphae, or to enhanced immobilization of ^{15}N by producing new hyphae. Hyphae might have depleted the soil in the hyphal compartment for ammonium before cutting, as a result of an increase adsorption of the ^{15}N-labelled ammonium in treatment III.

Our study suggests that quantities of N derived from an inorganic ^{15}N source in maize associated with different isolates of AM fungi can vary under the same experimental conditions. *G. intraradices* and *G. margarita* transported about 30 % more ^{15}N to the host plant than *A. laevis*. The hyphal density of *A. laevis* was only half of that observed for the two other fungi (Fig. 3). This may indicate that *A. laevis* was the most efficient of three fungi tested in the hyphal transport of ^{15}N per unit length of hyphae. The hyphal uptake and transport of nutrients from the soil to the host plant may depend on various factors (see Abbott & Robson, 1984). Jakobsen, Abott & Robson (1992*a*) showed that the efficiency of P transport by an AM fungus associated with *Trifolium subterraneum* L. was affected by the spread of the external hyphae, and possibly also by differences in capacity for uptake by unit length of hyphae. Furthermore, hyphae must remain metabolically active and functional with respect to N transport to the host. However, reduction of metabolic activity takes place with time in the extraradical hyphal system (Schubert *et al.*, 1987). The proportion of active hyphal length of *A. laevis* might have been higher compared to the other two fungi. In our study, only a small proportion of the extraradical hyphae is metabolically active during 9 wk of growth, as shown in a previous experiment with *G. intraradices* by Frey & Schüepp (1992).

The results using organic N (expt 3) do not provide conclusive evidence of enhanced availability of organic N to AM hyphae or to the host plant. Data from the experiment with ^{15}N-labelled plant tissue support the findings of Ames *et al.* (1983) that mycorrhizal plants derived more N from the applied ^{15}N-labelled plant tissue than did the control plants. However, results from the fungal material treatment did not show enhanced amounts of ^{15}N becoming available to maize associated with an AM fungus.

This could be a consequence of different C/N ratio (6–7 for fungal material as reported for *T. harzianum* mycelium by Schnürer & Rosswall (1987) and 21–22 for plant tissue (Ames *et al.*, 1983; Schnürer & Rosswall, 1987). The labelled plant material used in this investigation did not decompose readily. This is shown by the low plant availability (17 %) of N in the plant material, whereas the availability to plant of labelled N in the fungal material was comparatively high, with 29 % recovered in the plant on day 84. The high C/N ratio of the plant material may have stimulated N immobilization by micro-organisms, thereby rendering the N unavailable for hyphal uptake. Alternatively, the low C/N ratio of the fungal material might have led to a rapid mineralization and release of N into the soil, with subsequent translocation to plant roots. A large part of this N transported to the roots could be attributed to mass flow during the long period of ^{15}N exposure (84 d).

In the literature there is no evidence of organic breakdown by AM fungi, although Ames *et al.* (1984) observed that mycorrhizal sorghum plants derived N from sources less available to non-mycorrhizal plants. We suggest that organic N has to be mineralized first by other microorganisms before it becomes available to extraradical hyphae of arbuscular mycorrhizas. AM fungi are known to have the ability to assimilate ammonium via glutamine synthetase (Smith *et al.*, 1985). Information on nitrate assimilation by AM fungi is scarce, although some nitrate reductase activity has been shown in spores (Ho & Trappe, 1975) and roots (Oliver *et al.*, 1983). However, there have been no studies of nitrate assimilation by the mycelium. Different responses to inorganic N sources between *G. mosseae* and *G. fasciculatum*-colonized plants were recently found by Azcon, Gomez & Tobar (1992). They suggested that in plants colonized by *G. mosseae* the fungus may play an important role in the acquisition of nitrate by the plants, whereas *G. fasciculatum* showed a preference for ammonium.

The results of this study demonstrate the ability of mycorrhizal hyphae to transport N over considerable distances from the host plant. Disruption of the hyphal network can have implications for N acquisition by the plant. The AM fungi seem to be incapable of utilizing organic sources of nitrogen. The influence of AM fungi on forms of soil N that are generally considered not to be available to plants requires further investigation.

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