Biol Fertil Soils (1994) 18:249–254

ORIGINAL PAPER

G. Cuenca · R. Azcón

Effects of ammonium and nitrate on the growth of vesicular-arbuscular mycorrhizal *Erythrina poeppigiana* O.I. Cook seedlings

Received: 24 June 1993

Abstract *Erythrina poeppigiana*, a woody tropical plant, was inoculated with vesicular-arbuscular mycorrhizal (VAM) fungi *Glomus etunicatum* Becker and Gerdeman, *G. mosseae* Nicol. and Gerd. Gerdeman and Trappe, or *G. intraradices* Schenk and Smith. Growth, N uptake, and nutrition were evaluated in VAM-inoculated plants and controls fertilized with two levels (3 or 6 m*M*) of either NH_4^+-N or NO_3^--N. The response by the mycorrhizal plants to N fertilization, according to N source and/or level differed significantly from that of the control plants. In general, the growth of the mycorrhizal plants was similar to that of the non-mycorrhizal plants when N was provided as NH_4^+. When the N source was NO_3^- the control plants grew significantly less than the VAM plants. Inoculation with VAM fungi gave yield increases of 255 and 268% for *G. etunicatum*-colonized plants, 201 and 164% for *G. mosseae*-colonized plants and 286 and 218% for *G. intraradices*-colonized plants fertilized with 3 and 6 m*M* NO_3^--N, respectively. The increased growth and acquisition of nutrients by plants fertilized with NO_3^--N and inoculated with VAM shows that VAM mycelium has a capacity for NO_3^- absorption. The results also showed that *E. poeppigiana* seedlings preferred NH_4^+ as an N source. *G. etunicatum* was the most effective endophyte, not only increasing N, P, Ca, Mg, and Zn uptake in the presence of NO_3^- fertilizer but also P and Mg in the presence of NH_4^+ applications. From these results we conclude that VAM symbiosis affects N metabolism in *E. poeppigiana* plants and that this species can overcome limitations on the use of NO_3^--N by the mediation of VAM fungi.

G. Cuenca (✉)
Centro de Ecología y Ciencias Ambientales (IVIC),
Apartado 21827, Caracas 1020 A, Venezuela

R. Azcón
Departamento de Microbiología.
Estación Experimental del Zaidín (CSIC),
Apartado 419, E-18008 Granada, Spain

Key words Ammonium · *Erythrina poeppigiana* · Nitrate · VA mycorrhiza · Nutrient uptake · *Glomus* spp.

Introduction

VAM symbiosis is a widespread phenomenon which occurs in 80% of plant species and is an important factor in the uptake of nutrients, especially P, by colonized plants (Powell and Bagyaraj 1984). N uptake by VAM-symbiotic plants has received little attention. In many circumstances N limits plant growth but the significance of VAM fungi in N uptake is still incompletely understood. The ability of mycorrhizal roots to take up N has been attributed in most cases to an indirect effect associated with improved P nutrition. Other studies have demonstrated that VAM fungi can metabolize inorganic N (Ho and Trappe 1975; Ames et al. 1983; Smith et al. 1985). Recently, some authors have reported that mycorrhizal fungi have direct effects on N absorption and assimilation by the symbiotic system (Azcón et al. 1982; Ames et al. 1984; Barea et al. 1987).

The conventional model of increased nutrient uptake by VAM roots attributes the increase to growth of extraradical mycelium into undepleted soil volume. Better exploitation of a large volume of soil is considered an important feature of mycorrhizal systems. Because NO_3^- is at least one order of magnitude more mobile than NH_4^+, the mycorrhizal system might be expected to have more influence on N uptake and translocation if NH_4^+ rather than NO_3^- were the N source. But recent studies (Barea et al. 1989; Azcón et al. 1991; Azcón et al. 1992) have shown an increase in the plant growth response to mycorrhizal infection in the presence of NO_3^--N. In particular, a recent paper by Johansen et al. (1993) showed that mycorrhizal hyphae were able to transport inorganic N as NO_3^-.

The aim of the present study was to compare the effects of NH_4^+-N and NO_3^--N absorption by mycorrhizal roots growing in a sandy substrate. A basal nutrient solution was periodically added to ensure that nutrients did

not limit plant growth. The low adsorbing capacity of this culture medium allowed a fast uptake by VAM fungi of poorly diffusing ions. These conditions of N availability to VAM roots made it possible to assess the importance of fungal metabolism as a physiological element in N acquisition by roots.

However, in soils or in ecological conditions in which nitrification is inhibited, NH_4^+ is usually the main N source for plants (Rice and Pancholy 1972). Accordingly, plants may prefer either NH_3^--N or NH_4^+-N (Stewart et al. 1992).

In the present work we studied N assimilation in the tropical legume tree *E. poeppigiana* O.I. Cook. This species is widely used in tropical America to shade cacao and coffee plantations. The high N content of *E. poeppigiana* litter represents an important nutrient input to these crops (Aranguren et al. 1982a, b). Abundant root nodules found in *E. poeppigiana* have shown a high potential for N fixation (Escalante et al. 1984). The effects of VAM fungi on *E. poeppigiana* growth and nutrition are not yet known. Whether mycorrhizae improve the use of nutrients by this plant and whether it has a preference for a particular form of N are also important questions.

The purpose of the present study, therefore, was to compare the effects of different levels and sources of N on VAM function, evaluated in terms of growth and nutrient uptake by *E. poeppigiana*. The ability of mycorrhizal *E. poeppigiana* plants to use NH_4^+ or NO_3^- was evaluated in the presence of different VAM fungal species and the results compared with those from non-mycorrhizal plants.

Materials and methods

Experimental design

The experimental treatments consisted of a non-mycorrhizal control and mycorrhizal plants colonized by one of the following endophytes: *G. etunicatum* Becker & Gerdeman, *G. mosseae* Nicol. and Gerd. Gerdeman and Trappe, or *G. intraradices* Schenck and Smith. These treatments were supplied weekly with N at 3 or 6 m*M* as NH_4^+ or NO_3^-. Each treatment was replicated three times for a total of 48 pots.

Plant and soil treatments

Pregerminated *E. poeppigiana* seeds were grown in 2-liter pots filled with an 8:2 (v:v) mixture of quartz sand and steam-sterilized soil (100 °C, 1 h on 3 consecutive days). The soil, colleted from Granada (Spain), had a pH of 7.8, 2.07% organic matter, 0.1% total N, 12 μg P g^{-1} ($NaHCO_3$-extractable P), 311.2 μg K g^{-1} (NH_4Ac-extractable K; Jackson 1976), 35.8% sand, 43.6% silt, and 20.6% clay.

The plants were inoculated with *G. etunicatum*, *G. mosseae*, or *G. intraradices* as spores, mycelium, and mycorrhizal root fragments in a stock culture. The *G. etunicatum* inoculum was obtained from Venezuelan cacao plantations and was cultured in a neutral soil with *Pueraria phaseoloides* as the host plant. The other two VAM inocula were from Granada, Spain. Five grams per pot of inocula were placed directly below the seedling in the planting hole. A soil extract (5 ml pot^{-1} of soil and water in equal volumes filtered through Whatman no. 1 paper) was added to re-introduce microbial populations except for mycorrhizal propagules. Each pot was treated with 5 ml of a *Rhizobium* sp. inoculum that was isolated from field nodules of *E. poeppigiana* an reproduced in M79 medium (Vincent 1975).

Growth conditions

The pots were periodically shifted in a random pattern in a greenhouse maintained under controlled conditions (20–22 °C day time, 12–17 °C night-time, 16/8 h light/dark photoperiod). During the experiment the potting mixture was watered as needed and periodically fertilized with a basal nutrient solution (250 ml pot^{-1} $week^{-1}$; Hepper and O'Shea 1984). This mineral solution was applied at double strength and was also modified to contain N and K in a 1:1 ratio and to provide a total supply of 3 or 6 m*M* N and K per pot, N being added as $Ca(NO_3)_2$ or $(NH_4)_2SO_4$ and K as K_2SO_4.

Measurements

After 10 weeks the plants were harvested. All samples were dried at 80 °C, weighed, and ground in a steel mill before the chemical analyses. Appropriate aliquots were digested in a mixture of $HClO_4$ and H_2SO_4 in the presence of vanadium pentoxide. P was analyzed colorimetrically following the method of Murphy and Riley (1962). For N determination, digestion with H_2SO_4 concentrate in the presence of Se was performed before the micro-Kjeldahl procedure (Jackson 1976). Concentrations of K, Ca, Mg, and Zn were evaluated by atomic absorption spectrophotometry using an acetylene/air flame, and in the presence of La to avoid interference.

Mycorrhizal development was determined in a portion of the root system. The presence of mycorrhizal infection was assessed in subsamples of roots from each plant, previously stained by the method of Phillips and Hayman (1970). To assess VAM colonization (percentage of root length infected) the gridline intersect method of Giovannetti and Mosse (1980) was used. Data were subjected to a random block analysis of variance. Treatment means were compared with Duncan's multiple range test at the 5% probability level.

Results

The growth of *E. poeppigiana* was influenced both by the source (NH_4^+ or NO_3^-) and concentration (3 or 6 m*M*) of N applied and also by the VAM fungal species inoculated (Tables 1 and 2). Both levels of N application inhibited nodulation; thus, the results refer to non-nodulated *E. poeppigiana* plants.

With NH_4^+ as the N source the growth of control plants and mycorrhizal plants was similar. Only with the higher NH_4^+ application in the presence of *G. etunicatum* was there a statistically significant difference in shoot growth between control and inoculated plants (Table 1). In contrast, when NO_3^- was applied the differences between control and mycorrhizal plants were evident. In fact, VAM fungi increased the *E. poeppigiana* by 255 and 268% for *G. etunicatum*-colonized plants, 201.5 and 164% for *G. mosseae*-colonized plants, and 286 and 218% for *G. intraradices*-colonized in the presence of 3 and 6 m*M* N applied as NO_3^--N, respectively. With *G. mosseae* and the higher N rate this increase was not statistically significant. Treatment with NO_3^--N was more fa-

Table 1 Effect of N source and concentration on shoot dry weight (g) of *Erythrina poeppigiana* plants colonized with different mycorrhizal fungi compared with control plants. Within rows means followed by the same upper-case letter are not significantly different; within columns means followed by the same lower-case letter are not significantly different (Duncan's multiple range test, $P<0.05$)

	NH_4^+		NO_3^-	
	3 m*M*	6 m*M*	3 m*M*	6 m*M*
Control	5.34b A	4.12b B	2.01a C	1.70a C
Glomus etunicatum	5.07a A	5.96a A	5.12a AB	4.55a B
Glomus mosseae	4.58a A	4.15ab B	4.05ab B	2.79b AC
Glomus intraradices	4.65ab A	4.77ab AB	5.74a A	3.70b AB

vorable to growth at 3 m*M* than at the higher (6 m*M*) rate.

The root growth results (Table 2) were similar to those for shoots, although the higher NH_4^+ rate increased root growth significantly compared with control plants in the presence of *G. etunicatum* or *G. mosseae*. In the presence of NO_3^-, the positive mycorrhizal effect was evident, but with *G. etunicatum* the effect was not as conspicuous as it was without the application of NO_3^-, and there was less root growth with the higher NO_3^- rate than with 3 m*M* NO_3^-. Overall, *G. etunicatum* seemed to be the most efficient inoculum in promoting the growth of *E. poeppigiana* seedlings in this assay (also in NH_4^+ treatments).

N uptake (Table 3) was clearly higher in plants fertilized with NH_4^+-N, particularly at the higher application rate, compared with NO_3^--N. In all cases at the higher rate the difference was statistically significant. In general, the plant N content reflected the same tendencies as the dry weight data; with NO_3^-, the mean increase for the two levels of application was 189% with *G. etunicatum*, 126% with *G. mosseae*, and 175% with *G. intraradices* over the control level.

P uptake was increased by inoculation with mycorrhizal fungi in general in the NO_3^--N treatments (Table 3), but this increase was more noticeable with both NH_4^+ and NO_3^- in the presence of *G. etunicatum*. This fungus increased P uptake by over 400% (3 m*M* NO_3^-).

The K content showed no definite trends except with the addition of 3 m*M* NO_3^--N when the presence of mycorrhiza improved K absorption significantly (Table 3). In non-mycorrhizal plants, uptake of the other nutrients measured (Ca, Mg, and Zn; Table 4) was increased when 3 m*M* NH_4^+-N was added, decreasing at the higher 6 m*M* level. The levels of these nutrients were lower in control plants fertilized with NO_3^--N than with NH_4^+-N. For mycorrhizal plants in general, the effect of N source on nutrient assimilation was irrevlevant except with *G. etunicatum* inoculation. Mg uptake was increased in all treatments regardless of the N source applied when *G. etunicatum* was present. In contrast, the Zn content was influenced by the N source applied in the presence of this fungus, being depressed by NH_4^+-N treatment and increased by NO_3^--N treatment. In the presence of the other two fungi, 3 m*M* NO_3^--N depressed Zn uptake.

We conclude that *G. etunicatum* was the most effective endophyte, increasing the N, P, K, Ca, Mg, and Zn con-

Table 2 Effect of N source and concentration on root dry weight (g) of *Erythrina poeppigiana* plants colonized with different mycorrhizal fungus compared with control plants (see Table 1 for explanation of significance)

	NH_4^+		NO_3^-	
	3 m*M*	6 m*M*	3 m*M*	6 m*M*
Control	3.11a A	2.32a B	1.63a C	1.00a A
Glomus etunicatum	2.87a A	5.14b A	3.14ab BC	3.13ab AB
Glomus mosseae	2.22a A	4.65b A	5.12b AB	3.08ab AB
Glomus intraradices	1.53a A	4.50bc AB	6.45c A	3.44ab B

Table 3 Effect of N source and concentration on N, P, and K shoot contents (mg plant^{-1}) of *Erythrina poeppigiana* plants colonized with different mycorrhizal fungi compared with control plants (see Table 1 for explanation of significance)

NH_4^+						NO_3^-					
3 m*M*			6 m*M*			3 m*M*			6 m*M*		
N	P	K	N	P	K	N	P	K	N	P	K
Control											
146.5a A	6.1a A	128.0a A	176.4a A	4.4ab A	60.2b A	63.8b A	1.9b A	39.0b A	56.6b A	1.5b A	42.7b A
Glomus etunicatum											
146.6b A	14.3a B	124.5a A	187.3a A	10.6b B	134.8a B	131.9bc C	9.6b B	145.5a B	96.1c B	5.4c B	113.9a B
Glomus mosseae											
116.7a A	6.3a A	91.7a A	108.9a B	4.4ab A	83.1a AB	88.0ab AB	3.6ab A	109.8a B	64.4b AB	2.8b AB	64.8a AB
Glomus intraradices											
127.5a A	6.8a A	92.4a A	153.3a A	6.2a A	92.9a AB	123.6ab BC	7.0a B	97.3a AB	87.5b AB	4.5a AB	70.3a AB

Table 4 Effect of N source and concentration on Ca (mg plant^{-1}), Mg (mg plant^{-1}), and Zn (μg plant^{-1}) contents of *Erythrina poeppigiana* plants colonized with different mycorrhizal fungi compared with control plants (see Table 1 for explanation of significance)

NH_4^+						NO_3^-					
3 m*M*			6 m*M*			3 m*M*			6 m*M*		
Ca	Mg	Zn	Ca	Mg	Zn	Ca	Mg	Zn	Ca	Mg	Zn
Control											
39.8aA	22.0aA	592aA	19.5bA	14.5bA	362abA	9.2bA	4.3cA	226bA	12.4bA	3.9cA	282bA
Glomus etunicatum											
43.7aA	29.9aB	232aB	50.7aB	31.9aB	225aA	45.3aB	19.5bB	527bB	47.1aB	18.9bB	564bB
Glomus mosseae											
28.3abB	19.5aA	342aB	23.4abC	13.4abA	243aA	33.7aC	13.6abB	184aA	20.4bAC	9.1bAC	196aA
Glomus intraradices											
34.7aAB	20.9aA	267abB	35.8aD	18.9aA	437aA	39.5aBC	19.8aB	202bA	29.4aC	11.3bC	208abA

Table 5 Effect of N source and concentration on percentage vesicular-arbuscular mycorrhizal colonization by *Glomus etunicatum, Glomus mosseae,* and *Glomus intraradices*. Means followed by the same letter are not significantly different using Duncan's multiple range test ($P \leq 0.05$)

	NH_4^+		NO_3^-	
	3 m*M*	6 m*M*	3 m*M*	6 m*M*
Glomus etunicatum	84.1a	74.4ab	86.2a	69.9b
Glomus mosseae	66.3b	67.1b	75.6ab	76.9ab
Glomus intraradices	81.4ab	70.1b	76.3ab	71.2ab

tent not only under NO_3^- applications but also P and Mg under NH_4^+ treatment. The other two endophytes either did not respond significantly with NH_4^+ application or increased some nutrient contents up to the level obtained with the *G. etunicatum* inoculum under NO_3^- (Tables 3, 4).

VAM colonization (Table 5) varied from 66.3% (*G. mosseae*, 3 m*M* NH_4^+) to 86.2% (*G. etunicatum*, 3 mM NO_3^-). No consistent effect of N sources and/or level on this parameter was found. The application of 6 m*M* N, regardless of source, slightly decreased *G. etunicatum* and *G. intraradices* colonization.

Discussion

Even when the biological fixation of N is highly efficient, it does not satisfy all of the N demand by the plant. Indeed, in the presence of *Rhizobium* sp. symbiosis, legumes take up inorganic N from a substrate. The objective of the present work was to study the N nutrition of *E. poeppigiana* under the most common conditions in the field, that is, in symbiosis with *Rhizobium* sp. However, the N rates used were inhibitory for nodulation, which is why our results refer to non-nodulated *Erythrina* sp. plants, although this was not the original aim.

The effect of mycorrhizal colonization on *E. poeppigiana* growth and nutrient uptake was strongly influenced by the source and level of N supplied as fertilizer. Under the experimental conditions used here non-mycorrhizal plants were able to use the inorganic N sources with different degrees of efficiency. Control plants supplied with NH_4^+ grew better and took up more N in particular and nutrients in general than the control plants supplied with NO_3^-. In the case of mycorrhizal plants, a similar N uptake was obtained with the application of either N source.

Much of the evidence concerning the effect of VAM fungi on the N nutrition of host plants is indirect, and little research has been published on VAM symbiosis and N nutrition. Johnson et al. (1980) demonstrated that N fertilization could be reduced by using VAM fungi in woody plant production. In the present work we have demonstrated assimilation of NO_3^- by mycorrhizal fungi using different fungal species, all able to assimilate NO_3^-, and a tropical woody plant. The finding that mycorrhizal fungi can improve the uptake of NO_3^--N is in contrast with the general statement that VAM does not affect absorption of the more mobile ions (like NO_3^-) which can readily diffuse through the soil (Rhodes and Gerdeman 1980). Under the experimental conditions used here, nutrients did not limit plant growth and in the presence of a non-adsorbing medium like the sandy substrate used in this experiment, the increased acquisition of nutrients might be attributed to an efficient mechanism which actively contributes to the nutritional advantage of plants associated with VAM fungi. In a medium which allows mobility of poorly diffusing ions (the major VAM effect being related to mineral uptake), the VAM activity defines supplemental mycelial abilities with regard to NO_3^- absorption. These data are consistent with previous findings (Ho and Trappe 1975; Sundaresan et al. 1988; Azcón et al. 1992) of increased assimilative NO_3^- reductase activity in VAM plants. Mobile ions are free to move into the soil and hence one would not expect VAM mycelium to be very important in increasing uptake.

In the present work NO_3^- reductase activity was not measured, but our results indicate that VAM might transfer N as NO_3^- from the growth medium to the plant, in

which case it would be accompanied by an increase in NO_3^- reductase activity in VAM plants compared with non-mycorrhizal plants.

A recent study by Johansen et al. (1993) has raised doubts about the transport of NH_4^+ by VAM hyphae. In this study almost all the NH_4^+ applied appeared to be nitrified during the first 7 days of the experiment and assimilated in the form of NO_3^-. In the present study, there is no doubt about the effect of the different N sources applied. Fresh nutritive solutions were applied three times a week and therefore the presence of NH_4^+ ions in the relevant treatments was guaranteed.

N use, defined in terms of dry weight mg^{-1} N, was considerably increased in the mycorrhizal plants except when N was added as 3 m*M* NH_4^+. As NO_3^- ions predominate in agricultural soils (Rhodes and Gerdeman 1980), under such conditions absorption of NO_3^- may be directly increased by VAM infection (Harley and Smith 1983). The present results also show that *E. poeppigiana* seedlings preferred NH_4^+ as the N source. It could be interpreted as an adaptive characteristic in a species which belongs to climax deciduous forest. Also, some authors have shown that shaded plants have low levels of NO_3^- reductase and show little capacity to use NO_3^- even when these ions are readily available (Stewart et al. 1988). Theoretically, NO_3^- assimilation implies a much higher energy cost to the plant than NH_4^+ does (Chapin et al. 1987). Under light-limiting conditions, NO_3^- assimilation is thought to require ca. 15% of the energy processed within a plant (Penning De Vries et al. 1974). In contrast, when light is not limiting, leaf assimilation of NO_3^- can be driven largely by photosynthetic electron transport with only minor C costs to the plant (Bloom et al. 1989). As *E. poeppigiana* can be considered a shade plant in its seedling phase, our finding that it prefers NH_4^+ instead of NO_3^- seems logical. Our results also indicate that this species can overcome the inherent difficulty of using NO_3^--N through the mediation of VAM fungi.

The observed effect of NO_3^- on root growth was a consequence of the improvement in mineral nutrition in mycorrhizal plants observed in the NO_3^--N treatments. However, it is well established that an increase in N supply not only delays senescence and stimulates growth but also changes the plant morphology in a typical manner (Marschner 1986). Shoot elongation is enhanced and root elongation inhibited. Of the mineral nutrients, N has the most prominent influence on both root growth and the production and export of cytokinin to the shoots (Marschner 1986). All these effects are attributed to N in general but there is some evidence that more cytokinin is exported to shoots when the N source is NH_4^+ instead of NO_3^- (Buban et al. 1978). To determine whether the effects observed in the present study with 3 m*M* NO_3^- are related to the hormonal changes described above, further work is required.

It also appears worthwhile to study the dynamics of VAM infection to determine whether the infection spreads faster with NO_3^- in the growth medium. Previous experience with *E. poeppigiana* suggested that the infection spreads very slowly in the root (in the absence of *Rhizobium* sp.) and that 2.5 months are required to achieve 50% root-length infected (data not published).

The present results show that *E. poeppigiana* is a mycothrophic plant, as P uptake was considerably increased in the presence of VAM fungi, even when nutrients were not limiting for growth. *G. etunicatum* inoculum proved to be the most efficient VAM fungus in promoting growth and nutrient absorption by this plant. This is probably related to the ecological conditions under which the inoculum was obtained. The habitat of our isolate of *G. etunicatum* is the same as that of *E. poeppigiana*, cacao plantations in northern Venezuela, where the climate is very humid and warm and has a very marked dry season. The other two endophytes tested came from Europe and were cultured under very different conditions. Although VAM fungal species are not considered to have any specificity towards different taxa of potential hosts under favorable conditions (Harley and Smith 1983), there are many differences in their effectiveness in particular soil conditions and in particular hosts. Under the present experimental conditions *G. etunicatum* was the most efficient fungus tested, not only in relation to yield and N and P uptake but also for the uptake of other nutrients and micronutrients like Zn. With regard to Zn uptake, our results indicate that physiological characteristics may be different for different species and or isolates of VAM fungi. Better adaptation or greater compatibility with the growth medium or host plant could be involved, since the colonization levels obtained (Table 5) did not justify differential behavior between the endophytes. Tobar (unpublished) found that assimilative NO_3^- reductase and glutamine synthetase activities differed between *G. mosseae*- and *G. fasciculatum*-colonized plants under NO_3^- or NH_4^+ fertilization. Supraoptimal micronutrient plant uptake might also be regulated by VAM fungi (Bethlenfalvay and Franson 1989); the lower Zn content observed in VAM plants under NH_4^+ fertilization in the present study may be an expression of this mechanism.

Acknowledgements G. Cuenca was the recipient of a postdoctoral fellowship from the Venezuelan Council for Science and Technology (CONICIT) and IVIC, during her permanence in the Estación Experimental del Zaidín in Granada, Spain. We thank R. Herrera for critical comments on the manuscript, and E. Meneses and G. Escalante for help with the chemical plant analyses.

References

Ames RN, Reid CP, Porter LK, Cambardella C (1983) Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by *Glomus mosseae* a vesicular-arbuscular mycorrhizal fungus. New Phytol 95:381–396

Ames RN, Porter LK, St John TV, Reid CPP (1984) Nitrogen source and "A" values for vesicular-arbuscular and non-mycorrhizal sorghum grown at three rates of ^{15}N-ammonium-sulphate. New Phytol 97:269–276

Aranguren J, Escalante G, Herrera R (1982a) Nitrogen cycle of tropical perennial crops under shade trees. I. Coffee. Plant and Soil 67:247–258

Aranguren J, Escalante G, Herrera R (1982b) Nitrogen cycle of tropical perennial crops under shade trees. II. Cacao. Plant and Soil 67:259–269

Azcón R, Gómez-Ortega M, Barea JM (1982) Comparative effects of foliar or soil-applied nitrate on vesicular-arbuscular mycorrhizal infection in maize. New Phytol 92:553–559

Azcón R, Rubio R, Barea JM (1991) Selective interactions between different species of mycorrhizal fungi and *Rhizobium meliloti* strains and their effects on growth, N_2-fixation (^{15}N) and nutrition of *Medicago sativa*. New Phytol 117:399–404

Azcón R, Gómez M, Tobar R (1992) Effects of nitrogen source on growth, nutrition, photosynthetic rate and nitrogen metabolism of mycorrhizal and phosphorous-fertilized plants of *Lactuca sativa* L. New Phytol 121:227–234

Barea JM, Azcón-Aguilar C, Azcón R (1987) Vesicular-arbuscular mycorrhiza improve both symbiotic N_2-fixation and N uptake from soil as assessed with a ^{15}N technique under field conditions. New Phytol 106:717–725

Barea JM, Azcón R, Azcón-Aguilar C (1989) Time course of N fixation in the field by clover growing alone or in mixture with ryegrass to improve pasture productivity and inoculation with vesicular-arbuscular mycorrhizal fungus. New Phytol 106: 717–725

Bethlenfalvay GJ, Franson RL (1989) Manganese toxicity alleviated by mycorrhizae in soybean. J Plant Nutr 12:953–970

Bloom AJ, Caldwell RM, Fruazzo J, Warner RL, Weessbart J (1989) Oxygen and carbon dioxide fluxes from barley shoots depend on nitrate assimilation. Plant Physiol 91:352–356

Buban T, Varga A, Tromp J, Knegt E, Bruinsma J (1978) Effects of ammonium and nitrate nutrition on the level of zeatin and amino nitrogen in xylem sap of apple root-stocks. Z Pflanzenphysiol 89:289–295

Chapin FS III, Bloom AJ, Field CB, Waring RH (1987) Interactions of environmental factors in controlling plant growth. Bioscience 37:49–57

Escalante G, Herrera R, Aranguren J (1984) Fijación de Nitrógeno en árboles de sombra (*Erythrina poeppigiana*) en cacaotales del Norte de Venezuela. Pesqui Agropecu Bras. 19:223–230

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, London

Hepper CM, O'Shea J (1984) Vesicular-arbuscular mycorrhizal infection in lettuce (*Lactuca sativa*) in relation to calcium supply. Plant and Soil 82:61–68

Ho I, Trappe JM (1975) Nitrate reducing capacity of two vesicular-arbuscular mycorrhizal fungi. Mycologia 67:886–888

Jackson ML (1976) Análisis Químico del Suelo, 3th edn. Ed Omega, Barcelona

Johansen A, Jakobsen I, Jensen ES (1993) Hyphal transport by a vesicular-arbuscular mycorrhizal fungus of N applied to the soil as ammonium or nitrate. Biol Fertil Soil 16:66–70

Johnson CR, Joiner JN, Crews CE (1980) Effects of N, K and Mg on growth and leaf nutrient composition of three container grown woody ornamentals inoculated with mycorrhizae. J Am Soc Hortic Sci 105:286–288

Marschner H (1986) Mineral nutrition of higher plants. Academic Press, London Orlando San Diego New York

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Penning De Vries FWT, Brunsting AHM, Van Laar HH (1974) Products, requirements and efficiency of biosynthesis: a qualitative approach. J Theor Biol 45:339–377

Phillips JM, Hayman DS (1970) Improved procedure for clearing roots and staining parasitic infection and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:159–161

Powell CL, Bagyaraj DJ (1984) VA mycorrhiza. CRC Press, Boca Raton

Rhodes LH, Gerdemann JW (1980) Nutrient translocation in vesicular-arbuscular mycorrhizae. In: Cook CB, Pappas PW, Rudolph (eds) Cellular interactions in symbiosis and parasitism. Ohio State Univ Press, Columbus, pp 173–195

Rice EL, Pancholy SK (1972) Inhibition of nitrification by climax ecosystems. Am J Bot 59:1033–1040

Smith SE, St John BT, Smith FA, Nicholas DJ (1985) Activity of glutamine synthetase and glutamate dehydrogenase in *Trifolium subterraneum* L. and *Allium cepa* L.: Effects of mycorrhizal infection and phosphate nutrition. New Phytol 99:211–227

Stewart GR, Hegarty EE, Specht RL (1988) Inorganic nitrogen metabolism in plants of Australian rainforest communities. Physiol Plant 74:26–33

Stewart GR, Joly CA, Smirnoff N (1992) Partitioning of inorganic nitrogen assimilation between the roots and shoots of cerrado and forest trees of contrasting plant communities of South East Brasil Oecologia (Berlin) 91:511–517

Sundaresan P, Rata NV, Funaseka P, Lakshman M (1988) Studies on nitrate reduction by VAM fungal spores. Curr Sci 57:84–85

Tobar RM (1991) Evaluación del papel de las micorrizas vesículo-arbusculares en la fijacíon y captación de nitrógeno en sistemas suelo-planta, bajo condiciones semiáridas, mediante el uso de técnicas isotópicas. PhD thesis, Univ Granada

Vincent JM (1975) Manual práctico de rhizobiología, 1st edn. Editorial Hemisferio Sur, Buenos Aires