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

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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 mM) 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 mM 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.
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\(_4^+\)-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.J. 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 (Esclante 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 & Gerderman, *G. mosseae* Nicola and Gerd. Gerderman and Trappe, or *G. intraradices* Schenck and Smith. These treatments were supplied weekly with N at 3 or 6 mM as NH\(_4^+\) or NO\(_3^-\). Each treatment was replicated three times for a total of 48 pots.

**Plant and soil treatments**

Pregenerated *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, collected from Granada (Spain), had a pH of 7.8, 2.07% organic matter, 0.1% total N, 12 μg P г\(^{-1}\) (NaHCO\(_3\)-extractable P), 311.2 μg K г\(^{-1}\) (NH\(_4\)Ac-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 cultivated in a neutral soil with *Psyeraria phaseoloides* as the host plant. The other two VAM inocula were from Granada, Spain. Five grams per pot of inoculum 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 strain that was isolated from field nodules of *E. poeppigiana* as 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 mM N and K per pot, N being added as Ca(NO\(_3\))\(_2\) or (NH\(_4\))\(_2\)SO\(_4\) and K as K\(_2\)SO\(_4\).

#### 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\(_2\)SO\(_4\) in the presence of vanadium pentoxide. P was analyzed colorimetrically following the method of Murphy and Riley (1962). For N determination, digestion with H\(_2\)SO\(_4\) 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 mM) 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 mM 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-