

Effects of nitrate and ammonium nitrogen on vesicular-arbuscular mycorrhizal infection in lettuce (*Lactuca sativa*) grown in sand with nutrient solutions

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SUMMARY

The amount of vesicular-arbuscular mycorrhizal infection in plants fed with nutrient solution was studied in sand culture. Complete nutrient solutions containing 0, 5, 10, 20 and 40 ppm N as NaNO_3 , NH_4NO_3 or $(\text{NH}_4)_2\text{SO}_4$ were added twice weekly to lettuce (*Lactuca sativa* var. Tom Thumb) plants grown in sand and inoculated with *Glomus fasciculatus* similar to the spore type E_3 endophyte of VA mycorrhizae. Plants given up to 20 ppm N as $(\text{NH}_4)_2\text{SO}_4$ had up to about 50 per cent of their roots infected but this fell to 7 per cent with 40 ppm N. The pH's of the nutrient solutions in the pots fell from 6.0 to about 5.0 and 5.6 for those containing $(\text{NH}_4)_2\text{SO}_4$ and NH_4NO_3 respectively, whereas there was little or no change with NaNO_3 . However, all the pH's were within acceptable range for E_3 endophyte. They support the view that levels of VA infection in the field may be controlled as much by nitrogen fertilizer as by phosphate levels in the soil.

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Introduction

Vesicular-arbuscular (VA) mycorrhizae have been shown to improve the growth of most crop plants both in tropical and temperate soils. This increased growth has been attributed to improved uptake of plant nutrients, especially phosphorus (Mosse, 1973a; Tinker, 1975; Owusu-Bennoah & Wild, 1980; Powell, 1984). The usual explanation is that the external mycelium of an infected plant

RÉSUMÉ

OWUSU-BENNOAH, E.: Effets d'azote de nitrate et d'ammonium sur l'infection de la mycorrhizae vesiculaire/arbusculaire dans la laitue (*Lactuca sativa*) cultivée dans du sable avec des solutions de substances nutritives. L'étendue de l'infection de la mycorrhizae vesiculaire/arbusculaire (VA) dans des plantes alimentées avec des substances nutritives a été étudiée dans un milieu de culture sableux. Des solutions de substances nutritives complètes de 0, 5, 10, 20 et 40 ppm d'azote comme NaNO_3 , NH_4NO_3 ou $(\text{NH}_4)_2\text{SO}_4$ ont été ajoutées à deux fois par semaine à des plantules de la laitue (*Lactuca sativa* var. Tom Thumb) cultivée dans du sable et inoculée avec *Glomus fasciculatus* qui est similaire à la spore du type E_3 d'endophyte de la mycorrhizae VA. Les plantules qui ont été données jusqu'à 20 ppm de l'azote comme $(\text{NH}_4)_2\text{SO}_4$ ont environ 50 % de leurs racines infectées mais le pourcentage a tombé à environ 7 % avec 40 ppm d'azote. Les pH's des solutions de substances nutritives dans des pots ont diminué à environ 5.0 et 5.6 pour les solutions qui contenaient $(\text{NH}_4)_2\text{SO}_4$ et NH_4NO_3 , respectivement. Pourtant, tous les pH's étaient acceptables pour l'endophyte E_3 . Les résultats donnent l'appui sur l'avis que le niveau de l'infection de VA dans le champ puisse être contrôlé au tant par l'engrais d'azote que par le niveau du phosphore dans le sol.

is able to exploit a greater volume of soil around each root for plant nutrients, which are translocated and released in the cortex of the host root (Cox, Sanders, Tinker & Wild, 1975). Thus, growth response of crop plants infected with mycorrhizal fungi is related to the rate at which infection develops in the root system (Sanders, Tinker, Black & Palmerley, 1977).

This may be complicated by such factors af-

fecting the spread of infection as soil phosphorus and nitrogen status (Hayman, 1970, 1975; Mosse, 1973b; Sanders & Tinker, 1973; Sanders, 1975), inoculum level (Hayman, Johnson & Ruddlesdin, 1975; Black & Tinker, 1977; Owusu-Bennoah & Mosse, 1979), and pesticides (de Bertoldi, Giovannetti, Griselli & Rambelti, 1977; Haas *et al.*, 1987).

The effect of nitrogen fertilizer on mycorrhizal infection in the soil is very important because there is currently much interest in the possibility of inoculating field crops with selected strain of VA mycorrhizal fungi.

Lanowska (1966) reported that NH_4NO_3 , applied at 100 mg N per kg soil, depressed mycorrhizal infection and N content of *Pisum sativum*. Similarly, Hayman (1970) observed that mycorrhizal infection and spore production were highest in field plots of winter wheat which had received no N fertilizer compared with plots fertilized with calcium nitrate. Application of urea to field plots was accompanied by a decrease in VA infection of *Araucaria* at high levels of soil P whereas at intermediate P levels, infection increased with increased urea application (Bevege, 1971). Mosse (1973) and Sanders (1975) have consistently shown in the greenhouse that VA mycorrhizal infection of pot-grown plants is usually decreased by the addition of nutrient solution containing high concentrations of N and P.

None of these investigations distinguished between the effects of ammonium (NH_4^+) and nitrate (NO_3^-) ions. This distinction is important not only because these sources of N differ in their mobility in soil (Fried & Broeshart, 1967; Haynes & Goh, 1978), but also because they have different pathways of assimilation which have very different implications for the organic and inorganic composition of the plant tissues as well as for the biochemical events associated with pH regulation (Raven & Smith, 1976; Raven, Smith & Smith, 1978). It is obvious that the contrasting metabolic events associated with NH_4^+ or NO_3^- assimilation may have important effects on the environment of any root-infecting fungus. The

importance of rhizosphere pH changes with respect to pathogenic root-infecting fungi has been discussed by Smiley (1975), and the approach has been extended to include mutualistic symbionts such as VA mycorrhizae (Chambers, Smith & Smith, 1980).

This paper reports a study into the effects of NH_4^+ and NO_3^- nitrogen on the development and spread of mycorrhizal infection in the roots of *Lactuca sativa* in sand culture.

Materials and methods

Lettuce (*Lactuca sativa* var. Tom Thumb) seeds were sown in a seedling tray in Woburn soil (pH 6.0; NaHCO_3 -P 41 ppm) inoculated with sievings from soil heavily infected with spores of strain E_3 endophyte, a form of *Glomus fasciculatus* (Gerdemann & Trappe, 1974), which had been maintained in pot culture of maize (*Zea mays*). Eighteen days after germination, 10 seedlings were randomly selected from the seedling tray and checked for mycorrhizal infection. Over 90 per cent of the checked seedlings were infected and had a fair amount of external mycelia.

The infected seedlings were then transplanted singly into 7-cm diameter plastic pots containing acid washed sand. A modified complete nutrient solution (Clement, Garbaye & Le Tacon, 1977) of 10 ppm P as $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 85 ppm K as K_2SO_4 , 60 ppm Ca as $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 18 ppm Mg as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ containing 0, 5, 10, 20 and 40 ppm N as $(\text{NH}_4)_2\text{SO}_4$, NaNO_3 or NH_4NO_3 was added to the pots twice weekly and tap water was added in between. Micronutrients were supplied to all pots as follows: 0.02 ppm Mn as $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$; 0.002 ppm Cu as $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$; 0.002 ppm Zn as ZnCl_2 ; 0.05 ppm B as H_3BO_3 ; 0.0005 ppm Mo as $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$; 0.05 ppm Fe as Fe-EDTA. There were three replicates of each treatment. The pH of the sodium and ammonium nitrate and also ammonium sulphate was each adjusted to 6.0 with HCl or NaOH. The plants were grown in a glasshouse for 42 days and received both 16 h light (20 klux) at 25 °C and 8 h dark at 16 °C.

At the end of the experiment, 50 ml of deionized

water was added to each pot to leach the sand. 25 ml of the leachate was collected and *pH* was measured. Fresh weights of the plants at harvest and the dry weights of only the shoots were obtained as the roots were used for measurement of mycorrhizal infection. The total N of the shoot was estimated by Kjeldahl digestion followed by distillation of the ammonium-nitrogen.

Samples of fresh roots, which consisted of about 60 pieces, each 1-cm in length and chosen at random, were cleared in 10 per cent KOH and stained with 0.05 per cent Trypan blue in lactophenol (Philips & Hayman, 1970). The stained roots were stored in lactophenol. Pieces of the stained root were laid on microscope slides and squashed under a cover-slip for examination at $\times 160$ magnification. The fraction of each 1-cm piece containing internal hyphae was estimated visually and the mean percentage infected length of whole root system calculated (Giovannetti & Mosse, 1980).

The mycorrhizal spores in each pot were also obtained by wet sieving and decanting (Gerdemann & Nicolson, 1963) 100 g of moist sand through sieves of decreasing nominal diameter, i.e. 425, 250, 106 and 75 μm . The fractions that were collected on 425, 250 and 106 μm sieves were put together and washed several times through a 106 μm sieve with tap water. The washed spores were counted under a stereoscopic microscope.

Results and discussion

Fig. 1 shows the effect of N application on the growth of lettuce. The control plants grew very poorly and died after 18 days. Hence, there were no data on both fresh and dry weights of shoots, percentage infection and N concentration of the tissue. There was increased growth with increasing addition of N, irrespective of N form added. Table 1 shows N contents of the plants at 42 days. Although the values are based on simple determinations on pooled samples, they give an important indication of the N concentration of the plants under different forms of N. Nitrogen

addition increased the N content in the shoots. The total plant N was highest in plants which received NaNO_3 at the various N concentrations followed by NH_4NO_3 and $(\text{NH}_4)_2\text{SO}_4$ in that order.

The percentage infection was highest at 5 ppm N in all the different forms of nitrogen and decreased with increasing N application; infection was highest with $(\text{NH}_4)_2\text{SO}_4$ followed by NaNO_3 and NH_4NO_3 . The number of spores followed a similar trend.

Notwithstanding the fact that the *pH* of the nutrient solutions was adjusted at the beginning, some changes in the residual solution reaction were observed at the end of the experiment. Table 1 shows that *pH* of the leachate from the bulk sand fell to about 5.4 for pots which received $(\text{NH}_4)_2\text{SO}_4$ whereas the *pH* tended to increase in pots which received NaNO_3 ; there was little or no change with NH_4NO_3 .

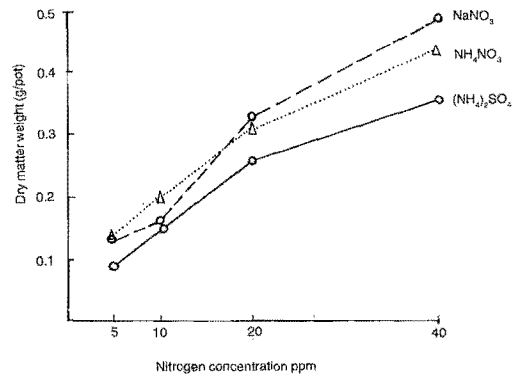


Fig. 1. The effect of N-application on the shoot dry weight of lettuce grown in sand

The type and concentration of nitrogen fertilizers applied clearly influenced the amount of VA mycorrhizal spore numbers and infection in the plant. These results confirm previous findings that N compounds depress mycorrhizal infection of root systems (Lanowska, 1966; Hayman, 1970; Clement *et al.*, 1977). Infection was more sensi-

tive to added NO_3^- than to NH_4^+ . This observation did not appear to be wholly caused by changes in the pH of the rooting medium since, as shown in Table 1, the pH's of leachates from the bulk sand medium were within an acceptable range for satisfactory growth of the endophyte *Glomus fasciculatus* (E₃) (Mosse *et al.*, 1976).

The influence of form of nitrogen on infection agrees with that obtained by Mosse (personal communication) for soil grown lettuce which showed that infection was more sensitive to added

$\text{Ca}(\text{NO}_3)_2$ than to $(\text{NH}_4)_2\text{SO}_4$. The findings, however, contradict the results of Clement *et al.* (1977) who demonstrated that $(\text{NH}_4)_2\text{SO}_4$ rather than NaNO_3 reduced mycorrhizal infection in soil. It must be pointed out, however, that Clement *et al.* (1977) used *Glomus mosseae* (yellow vacuolate) endophyte which Mosse (1973) has shown to be extremely sensitive to acid medium. According to Green, Graham & Schenck (1976), this fungus (*G. mosseae*) germinates between pH 6.5 - 7.5.

The decreased mycorrhizal infection with in-

TABLE 1

Effects of Nitrogen Application on Mycorrhizal Infection, Spore Numbers and N Content of in Shoot Lettuce Grown in Sand Culture for 42 days

(a) $(\text{NH}_4)_2\text{SO}_4$				
Nitrogen rate (ppm N)	% N shoot	% infection	Spores/100 g sand	pH of leachate from growth medium
0	*	*	5	6.0
5	0.86	53	80	5.8
10	1.21	42	50	5.8
20	1.31	23	24	5.6
40	1.54	7	10	5.4
SD		±25	±30	
(b) NaNO_3				
0	*	*	4	6.0
5	0.97	19	45	6.1
10	1.45	12	26	6.0
20	1.60	5	19	6.2
40	1.93	0	5	6.3
SD		±10	±20	
(c) NH_4NO_3				
0	*	*	7	6.0
5	0.94	16	47	6.1
10	1.35	10	21	5.0
20	1.47	4	12	6.0
40	1.75	0	3	5.9
SD		±8.6	±17.5	

* The control plants grew poorly and died after 18 days.

crease in N concentration in the growth medium to 40 ppm also agrees with field observations made by Porter & Beute (1972), Hayman *et al.* (1975) and Clement *et al.* (1977), but contrary to results obtained by Bevege (1971), who found a trend towards increased VA mycorrhizal infection with increasing concentration of N.

According to Smith & Bowen (1979), mycorrhizae develop in two phases: a pre-infection phase in the soil and fungal growth within the root. Effects of added nutrients on mycorrhizal development may be the result of direct action at the pre-infection stage or mediation via the root. In the latter case, either the pre-infection stage or fungal growth within the root could be affected since both root exudates and root composition may be modified and so affect fungal nutrition. Sanders (1975) showed that fungal growth within the root and development of external mycelium are certainly affected by the internal root phosphate in the case of onions.

The results also show that differences in the nutrition of the host plants grown on NH_4^+ or NO_3^- are likely to be implicated in the changes in mycorrhizal infection. Plants grown on NH_4^+ exude H^+ from their roots with a consequent drop in the pH of culture solution (Kirkby, 1976; Riley & Barber, 1971; Smiley, 1974). Such a drop in pH of the rooting medium, as shown in Table 1, especially where $(\text{NH}_4)_2\text{SO}_4$ was applied at highest concentration, may also be important in the control of root inhabiting fungi, *G. fasciculatus*. Similar observations have been made by Smiley (1975) and Clement *et al.* (1977).

Root exudates other than the H^+ or OH^- may also influence fungal growth in the rhizosphere and bulk soil (Rovira, 1965). However, according to Clement *et al.* (1980), no published reports of differences in the composition of exudates from roots supplied with NH_4^+ or NO_3^- are available in the literature. Nevertheless, the composition of the root does affect the identity and quantity of substances exuded (Boulter *et al.* 1966; Huber & Watson, 1974). It would be expected that amino

acids and amides (particularly glutamate, glutamine, aspartate and asparagine) would predominate in exudates from NH_4^+ -fed plants, while carboxylic acids might predominate in exudates from NO_3^- -fed plants.

It would be useful to have information on the utilization of such compounds by VA mycorrhizal fungi as clearly both growth in the rhizosphere and within the root might be influenced by them.

The present results show the need to consider the level and form of N-fertilizers in any future mycorrhizal field study to obtain maximum benefit from the association between the fungus and host crop plant. Any decrease in infection may affect considerably the high uptake and transfer efficiency of the external mycelium of the fungus.

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