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COMPARATIVE EFFECTS OF FOLIAR- OR SOIL-APPLIED NITRATE ON VESICULAR–ARBUSCULAR MYCORRHIZAL INFECTION IN MAIZE

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SUMMARY

Various concentrations of nitrate were applied either to the rooting medium (soil) or to the foliage of maize (*Zea mays* L.) plants inoculated or not inoculated with the vesicular–arbuscular mycorrhizal fungus *Glomus mosseae*.

Certain levels of foliar-applied nitrate and of soil-applied nitrate had similar effects on growth of non-mycorrhizal plants and on concentrations of nitrogen and phosphorus in their shoots. However, when the effects of these concentrations on inoculated plants were compared, plants with soil-applied nitrate always developed much less infection.

These results are further evidence that soil nitrate influences mycorrhizal development by acting on the establishment of infection rather than on spread within the root.

INTRODUCTION

The establishment of vesicular–arbuscular mycorrhizas (VAM) is influenced by the level and types of soil nutrient. Addition of phosphate and nitrate fertilizers may have particularly important effects (Hayman, 1978). A great number of papers describe the effects of phosphate on VAM formation, plant growth and nutrient uptake (e.g. Jasper, Robson and Abbott, 1970; Sanders, 1975; Tinker, 1975; Menge *et al.*, 1978; Asimi, Gianinazzi-Pearson and Gianinazzi, 1980). The influence of combined nitrogen on VAM and the consequent plant responses has also been studied (Mosse, 1962; Lanowska, 1966; Hayman, 1975; Kruckelmann, 1975; Chambers, Smith and Smith, 1980). The current literature indicates that additions of inorganic nitrogen compounds often decrease VAM infection and this may have wide implications for nutrient uptake from soil.

As far as we know there is no information concerning the possibility that combined nitrogen acts differently according to whether it is applied to the rooting medium or sprayed onto the foliage. This difference in mode of application could help us to distinguish whether the effect of nitrogen, on mycorrhizal development, is mainly the result of a direct effect on the pre-infection stages (including propagule germination, hyphal growth and establishment of successful entry points) or the nitrogen inside the host plant which influences the spread of VA infection within the root system. The aim of this paper is to examine these possibilities using a nitrate salt as nitrogen source.

MATERIALS AND METHODS

Two experiments were done: (A) comparison between effects of soil or foliar

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applied nitrate (a range of concentrations) on growth and nutrition of non-mycorrhizal plants given extra phosphate and (B) the effect of nitrate applications on VAM infection. Both experiments were done with the same soil, plant species and growth conditions.

Soil

The soil used was a 'reddish brown calcareous' type, pH = 7·6 containing 12 mg kg^{-1} bicarbonate-soluble phosphorus (Olsen *et al.*, 1954). It was mixed with 25 % sand by volume and steam-sterilized at 100 °C (1 h daily for 3 days) and then rewetted with filtrate from non-sterile soil. The filtrate contained soil micro-organisms but no propagules of mycorrhizal fungi.

Plants and growth conditions

Maize (*Zea mays* L.) was the test plant. Seeds were germinated in moistened sterile sand. Two-week-old seedlings were transferred to pots containing 1 kg of the soil plus sand mixture which was also throughly mixed with 89 mg phosphorus kg^{-1} as K_2HPO_4. Two maize plants per pot (five replicate pots per treatment) were grown for 2 months in a glasshouse at 19 to 25 °C. They were watered from below and fed every 2 weeks with 10 ml of Long Ashton nutrient solution (Hewitt, 1952) lacking nitrogen and phosphorus.

Experiment A

Nitrogen (NO_3^-) treatments. Several concentrations of KNO_3 were prepared and 5 ml per pot of each added to the corresponding pot every day for 20 days, starting 5 days after seedling transplantation. The solutions were either injected into the soil with a syringe ('soil application') or sprayed onto the foliage with a hand sprayed ('foliar application'). After 20 days each pot had received 40, 80, 120, 160 and 240 mg nitrate kg^{-1} ('soil application') or 35, 70, 140, 210 and 280 mg nitrate kg^{-1} ('foliar application') respectively. These doses had been established in previous (unpublished) experiments and are termed S_1, S_2, S_3, S_4 and S_5 ('soil application') or F_1, F_2, F_3, F_4 and F_5 ('foliar application'), respectively.

Measurements. At harvest shoots and roots were separated, weighed and analysed separately for nitrogen, phosphate and potassium content, as before (Azcón and Ocampo, 1981). In addition, subsamples (2 g) of fresh root tissue were homogenized using the Bligh and Dyer (1959) method modified by Donaire (pers. comm.) and aliquots of the supernatant were analysed for total sugar content (Ratnayake, Leonard and Menge 1978) and the results expressed as μg equivalents of glucose per g fresh root weight.

Experiment B

In this experiment the same soil and growth conditions were used but the maize seedlings were inoculated at the transplantation stage with a standardized inoculum of the VA fungus *Glomus mosseae* which contained spores, hyphae and infected root fragments. This inoculum was placed in the planting hole at transplantation. The plant received, by foliar or soil application, the range of nitrate concentrations as before (Experiment A), following a similar schedule.

At harvest, the percentage of infected root tissue was estimated by examining samples (more than 80 segments of root per replicate) of the stained root system (Phillips and Hayman, 1970) under the microscope. Data are given as total

percentage infection and are calculated from records of incidence and of extent of infection at each segment, estimated by multiplying the length by the width of the infected cortex (Azcón, Azcón-Aguilar and Barea, 1978).

Results and Discussion

The results (Fig. 1) confirm previous observations that nitrogen fertilizers influence mycorrhizal infection when applied to the soil (see Chambers *et al.*, 1980 for references) but the present study further suggests that VA infection is also affected by foliar-applied nitrate. Development of infection was increased, particularly at intermediate concentrations, by all additions of nitrate regardless of methods of application. However, plants receiving nitrate soil-applied developed much less infection, at the highest doses (Fig. 1).

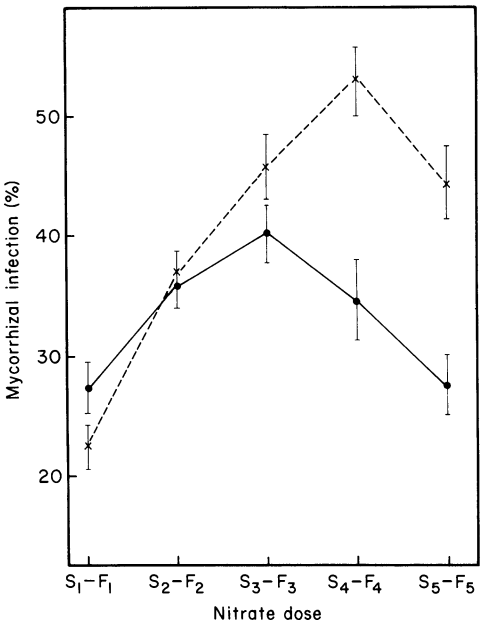

Fig. 1. Mycorrhizal infection (percentage root infected) of maize plants given a range of concentrations of nitrate: (●) 'soil application', (x) 'foliar application'. Confidence limits at the 5% level of significance are given.

Data in Table 3 (calculated from results given in Tables 1 and 2) and Table 4 show that certain soil and foliar treatments (S_3 *vs* F_3, S_4 *vs* F_4 and S_5 *vs* F_5) did not differ significantly in their effect on growth and nutrition of non-mycorrhizal maize plants given extra phosphorus.

However, there were significant differences in the effects on plant growth between soil and foliar applications of nitrate in treatments F_1, S_1 and F_2, S_2 (Tables 1 and 2). Nitrate reductase is present in both root and shoot of maize (Weissman, 1972) so that differences in nitrate assimilation between the two organs, implied by our results, are probably related to differences in the activity of this enzyme. This could be regulated by the supply of organic acids needed to neutralize hydroxyl ions produced during nitrate reduction which, since they are an end

Table 1. *Experiment A. Effects of nitrate applied to the soil on growth and nutrient content of non-mycorrhizal maize plants given extra phosphate**

Nitrate additions	Shoot fresh wt per two plants (g)	Nutrient content (percentage shoot dry wt)		
		Nitrogen	Phosphorus	Potassium
S_1	26·14	0·85	0·060	2·50
S_2	22·70	1·12	0·057	2·93
S_3	28·38	1·47	0·067	3·47
S_4	25·71	1·44	0·067	3·23
S_5	19·43	1·69	0·057	3·53
F value	2·07	8·80	0·88	4·15
L.S.D.				
$P = 0·05$	7·59	0·35	—	0·66
$P = 0·01$	10·79	0·50	—	0·93
$P = 0·001$	15·62	0·72	—	1·35

* Data are given on a per pot basis (two plants per pot; five replicate pots per treatment).
— not significant.

Table 2. *Experiment A. Effects of nitrate applied to the foliage on growth and nutrition of non-mycorrhizal, maize plants given extra phosphate**

Nitrate additions	Shoot fresh wt per two plants (g)	Nutrient content (percentage shoot dry wt)		
		Nitrogen	Phosphorus	Potassium
F_1	38·16	0·91	0·073	2·53
F_2	41·86	1·14	0·060	2·63
F_3	24·17	1·35	0·063	3·77
F_4	24·34	1·68	0·067	3·97
F_5	24·33	1·97	0·073	3·90
F value	19·21	7·67	0·64	6·62
L.S.D.				
$P = 0·05$	6·26	0·48	—	0·87
$P = 0·01$	8·91	0·68	—	1·24
$P = 0·001$	12·90	0·99	—	1·80

* As for Table 1.
— Not significant.

product of photosynthesis, would be more readily available in the shoot. Further, amino acids formed as the product of nitrate reduction would more readily assimilated in the shoot. These mechanisms could account for the greater effectiveness of foliar-applied nitrate (F_1 *vs* S_2 and F_2 *vs* S_2, Tables 1 and 2).

Certain concentrations of foliar-applied and soil-applied nitrate were equal in their effects on growth and nutrient composition of non-mycorrhizal plants (Tables 3 and 4). When the effects of these concentrations of nitrate on development of infection were compared, the level of VAM infection was significantly less when nitrate was given to the soil rather than to the foliage (Fig. 1) i.e. S_3 *vs* F_3, S_4 *vs* F_4 and S_5 *vs* F_5. Further examination of these results implies that high nitrogen concentrations within the plant are not responsible for the reduction of VAM because some foliar nitrate-treated plants (S_5 *vs* F_5, Table 4) even had higher

Table 3. *Experiment A. Statistical comparison between effects of foliar and soil (F_x vs. S_x) nitrate treatments on growth and nutrient uptake of non-mycorrhizal maize plants given extra phosphate* (Refer to Table 1 and 2)

Foliar *vs* soil (F_x *vs* S_x) Treatments	L.S.D. and significance levels			
		Percentage shoot dry wt		
	Shoot fresh wt	Nitrogen	Phosphorus	Potassium
F_1 *vs* S_1	12·02 †	0·06 n.s.	0·014 n.s.	0·03 n.s.
F_2 *vs* S_2	19·16 *	0·02 n.s.	0·003 n.s.	0·30 n.s.
F_3 *vs* S_3	4·21 n.s.	0·12 n.s.	0·004 n.s.	0·30 n.s.
F_4 *vs* S_4	1·37 n.s.	0·24 n.s.	0 n.s.	0·74 n.s.
F_5 *vs* S_5	4·90 n.s.	0·28 n.s.	0·01 n.s.	0·37 n.s.

* $P = 0{\cdot}01$.
† $P = 0{\cdot}05$.
n.s. = not significant.

Table 4. *Experiment A. Comparison between effects of some root or foliar nitrate applications on the growth and concentrations of nutrients and sugars in the roots of non-mycorrhizal maize plants given extra phosphorus**

Nitrate treatments	Root fresh wt (g)	Nitrogen in roots (g)	Phosphorus in roots (g)	Glucose in roots ($\mu g\ g^{-1}$)
S_3	18·46[a]	0·87[a]	0·04[a]	1150[a]
F_3	18·75[a]	1·07[a]	0·05[a]	1165[a]
S_4	16·88[ab]	0·75[ab]	0·03[a]	1250[a]
F_4	13·93[b]	0·98[a]	0·04[a]	1190[a]
S_5	15·30[ab]	0·58[b]	0·03[a]	1320[a]
F_5	13·90[b]	0·96[a]	0·04[a]	1210[a]

Data are given on a per pot basis (two plants per pot; five replicate pot per treatment).
Values sharing a letter in common do not differ significantly ($P = 0{\cdot}05$).

nitrogen concentrations in their roots than those given nitrate to the rooting medium, the percentage VA infection being higher in the former than in the latter. Indeed, these data could suggest that percentage nitrogen in root and percentage VAM infection are positively related and it could still be argued that internal percentage of nitrogen controlled infection. However, in previous (unpublished) experiments we have found negative relationships between percentage nitrogen in root and mycorrhizal development.

Mycorrhizal infection is also influenced, however, by the concentration of root carbohydates (Lewis, 1975; Ratnayake *et al.*, 1978; Jasper *et al.*, 1979; Azcón and Ocampo, 1981). This factor could be of relevance in this study and must be considered for a correct interpretation of the results obtained.

Although the overall pathway for nitrate assimilation is similar in plants whether the process occurs in leaf or root tissue, as in maize (Weissman, 1972), Butt and Beevers (1961) found an increased metabolism of carbohydrates *via* the pentose phosphate pathway in maize roots supplied with nitrite, due to the demand of NADPH for nitrite reduction. Such changes in root carbohydrates could influence development of mycorrhizal infection. Accordingly, the sugar contents of roots

were measured to see if they were affected by the mode of nitrate application. Table 4 shows however, that there were no significant differences in this parameter.

All of these results therefore suggest that nitrate in soil directly affects the pre-infection stage of VAM establishment. This hypothesis agrees with the results of Chambers *et al.* (1980).

Certain amounts of nitrate in the medium are known to depress the infectivity of mycorrhizal propagules (spores or infected root fragments) (Mosse and Phillips, 1971; Hayman, 1970; Chambers *et al.*, 1980).

We do not suggest that our observations necessarily imply that nitrate salts *per se* act directly on infection structures. There may be other, indirect, effects on the plant rhizosphere, namely, changes in the root exudation pattern (Ratnayake *et al.*, 1978; Azcón and Ocampo, 1981), hydroxide secretion (Smith, 1980), qualitative or quantitative changes in rhizosphere microbial populations, or their activities, all of which are known to affect VAM formation (Azcón *et al.* 1978; Azcón-Aguilar and Barea, 1981). None of these possibilities were tested in the experiments reported here but obviously all could account for the effect of nitrate on VAM establishment when such a fertilizer is given to the rooting medium. These possibilities warrant further investigation.

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