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Effects of vesicular-arbuscular mycorrhiza on the availability of iron phosphates to plants

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Key words Colloidal iron phosphate *Glomus fasciculatum* *Lolium rigidum* Mitscherlich equation Phosphate sources Response curves Ryegrass Strength clover *Trifolium subterraneam* Vesicular-arbuscular mycorrhiza

Summary The effect of inoculation with a mycorrhizal fungus on the growth of subterranean clover and of ryegrass was measured using three sources of phosphorus with different solubilities. These were (in order of decreasing solubility): potassium dihydrogen phosphate, colloidal iron phosphate and crystalline iron phosphate. Mycorrhizal infection increased growth more for subterranean clover than for ryegrass for all sources of phosphorus. For both species the greatest benefit from mycorrhizal inoculation was obtained with the least soluble source of iron phosphate. It is suggested that the mycorrhizas were able to explore the soil more thoroughly and hence were able to locate and use the point sources of phosphorus in the insoluble iron phosphates.

Introduction

When phosphorus (P) supply in soils limits growth, plants infected with vesicular-arbuscular mycorrhizal fungi are able to take up more P and grow better than uninfected plants[2,14,18,31]. It has been concluded that mycorrhizal fungi cannot help plants to take up P that would otherwise by chemically unavailable to non-mycorrhizal plants. Rather mycorrhizal infection increases the rate of P uptake by shortening the distance that P ions must diffuse to plant roots. These conclusions are mainly based on two types of studies. One type of study involves the labelling of soils with radioactive P and then comparing the specific activity of P in mycorrhizal and non-mycorrhizal plants[16,19,22,25,29]. In such studies a similar specific activity of P in both mycorrhizal and non-mycorrhizal plants has been often observed. This led to the conclusion that both mycorrhizal and non-mycorrhizal plants obtain their P requirement from the same 'source' of P in soil. However, recent studies have suggested that this conclusion does not necessarily follow because, forms of P

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in soils that differ in their availability to mycorrhizal and non-mycorrhizal plants are uniformly labelled by the addition of ^{32}P[7].

In another type of study, the effectiveness of P sources of different solubilities for the growth and P uptake of mycorrhizal plants relative to that of non-mycorrhizal plants have been compared[5,13,20,23,26,27]. In most of these studies[13,20,26,27] the increase in plant growth due to mycorrhizal infection was found to be larger with poorly soluble P than with soluble P. Thus it has been claimed that there is a disproportionate advantage to mycorrhizal plants when poorly soluble P have been applied. In these experiments, however, comparisons have been made on a particular level of P application between different sources of P. This is not a valid comparison of the effect of mycorrhizas on the uptake of P from various sources of different solubilities because the extent to which mycorrhizas increase growth and P uptake has been shown to vary with the level of P application[1,5,30]. Using complete response curves, Pairunan *et al.*[23] and Barrow *et al.*[5] concluded that although mycorrhizal plants were better than non-mycorrhizal plants in getting P from poorly-soluble rock phosphate or from P that had been reacted with soils, the improvement was in the same proportions as that obtained with soluble P or with freshly-added P respectively.

In contrast to these results, it has recently been observed that adding iron hydroxide to soil decreased the growth and P uptake of non-mycorrhizal plants but had no effect on mycorrhizal plants[7]. Indeed at some levels of P addition mycorrhizal plants were able to take up enough P for near-maximum yield whereas non-mycorrhizal plants took up almost none. In the present experiment, the effectiveness of mycorrhizal plants relative to non-mycorrhizal plants was examined for two iron phosphates of different solubilities. Potassium dihydrogen phosphate was used as a soluble source for comparison.

Methods and materials

Preparation or iron phosphates

Colloidal and crystalline iron phosphates were prepared by the method of Cate *et al.*[9]. Reduced iron powder was dissolved in orthophosphoric acid. The solution was then filtered and the filtrate was oxidised with 30% hydrogen peroxide. When this solution was diluted to fifty times with distilled water, colloidal iron phosphate precipitated out. The precipitate was washed with distilled water to remove any free phosphoric acid. The precipitate was then dried at room temperature. To obtain crystalline iron phosphate, a suspension of colloidal iron phosphate was heated to 90°C in a constant temperature bath for 20 days with constant stirring. It was then washed with distilled water and dried at room temperature. The materials were stored in a dessicator.

X-ray diffraction analysis showed that the crystalline iron phosphate was strengite. No evidence of crystallinity was detected for colloidal iron phosphate. Total and water- and citrate-soluble P contents of the sources were determined using the AOAC[3] procedures (Table 1).

Table 1. Total and water- and citrate-soluble phosphorus contents (%) of different sources of phosphate

Phosphorus (%)	Potassium dihydrogen phosphate	Colloidal iron phosphate	Strengite
Total	22.7	12.9	14.8
Water-soluble	22.7	2.5	0.2
Citrate-soluble	22.7	5.8	2.6

Table 2. Rates of phosphate application (mg P per kg soil) for subterranean clover and ryegrass

Potassium dihydrogen phosphate		Colloidal iron phosphate		Strengite	
Ryegrass	Sub clover	Ryegrass	Sub clover	Ryegrass	Sub clover
0.8	1.7	1.7	3.3	3.3	6.6
1.7	3.3	3.3	6.7	6.6	13.3
2.5	5.0	5.0	13.3	13.3	20.0
3.3	6.7	6.6	20.0	33.3	33.3
5.0	10.0	13.3	33.3	66.6	66.6
6.7	13.3	33.3	66.6	133.3	133.3
13.3	20.0	66.6	133.3		200.0
33.3	33.3				333.3
	66.6				

Experimental design

The experiment was a randomised block design with factorial combinations of the following treatments: two plant species (subterranean clover, *Trifolium subterraneum* L cv Seaton Park and annual ryegrass, *Lolium rigidum* L cv Wimmera); three sources of P (potassium dihydrogen phosphate, colloidal iron phosphate and strengite); mycorrhizal inoculation (control and inoculated with VA mycorrhizal fungus, *Glomus fasciculatum* (Thaxter) Gerdemann and Trappe); and different levels of P application (Table 2).

The plants were grown in a sandy soil from Lancelin, Western Australia (pH 5.3 in 0.01 *M* $CaCl_2$ and 2 ppm P extractable in 0.5 *M* $NaHCO_3$[10]). The soil was sieved through a 2 mm sieve and steamed at 100°C for 1 hour. After mixing thoroughly, 3000 g of air dried soil were placed in 20.3 cm diameter, undrained plastic pots. Basal nutrients, sufficient to eliminate all nutrient deficiencies except P were applied in solution and allowed to dry before P fertilizers were mixed in as fine solids. The soil was watered to its field capacity from one week before planting until harvest. Mycorrhizal inoculum consisted of roots of subterranean clover infected with *G. fasciculatum* plus the surrounding soil. Control pots received non-mycorrhizal roots of subterranean clover plus the surrounding soil. Seeds of subterranean clover or ryegrass were sown approximately 1.5 cm deep. Eight days after sowing, plants were thinned to leave 9 plants per pot. Plants were harvested 35 days after sowing, dried at 70°C in a forced draught oven and weighed. Roots were washed from soil to assess mycorrhizal infection. Procedures for preparing and placing mycorrhizal inoculum and for assessing root infection were those of Abbott and Robson[1].

Analysis of response curves

For each source of P, we chose levels of application that would enable us to define the complete response curve. As it was intended to describe the responses by fitting curves, many levels of P were applied and there were only two replicates of each level.

An expanded form of the Mitscherlich equation was used to describe the results. The Mitscher-

lich equation may be written

$$\ln(y) = \ln(a)(1 - \exp(-c(x + q))) \quad (1)$$

where y is dry weight of shoots, x is the level of application of P, c is a coefficient that reflects the shape of the response curve, and q is another coefficient that reflects the P supplied in the seeds, in the mycorrhizal inoculum and by the unfertilized soil. Because several combinations of fertilizer and mycorrhizal treatments were to be compared, the exponential term was expanded to

$$c_1(x_1 + q) + c_2x_2 + c_3x_3 \textit{ etc.} \quad (2)$$

where the subscripts refer to different treatment combinations. A further modification was necessary because the mycorrhizal treatments gave different intercepts on the yield axis—that is the mycorrhizal treatments gave different access to the q units of P. Hence, for the plus-mycorrhizal treatments, a term c_1q was used, but for the non-mycorrhizal treatments this was replaced by c_2q. In both cases the c coefficients used were those appropriate to the potassium dihydrogen phosphate treatment and hence q is given in terms of this source of P.

The effectiveness of any given treatment in increasing the log yield is given by the appropriate partial differential of the equation. The ratio of the effectiveness of any two treatments is given by the ratio of the appropriate partial differentials. Provided the q term is regarded as an extra contribution to the potassium dihydrogen phosphate treatment, the ratio of the derivatives is the ratio of the appropriate c coefficients.

A similar approach has been used to obtain relative effectiveness for plant growth between two treatments, for example between mycorrhizal and non-mycorrhizal plants[5,23], between freshly-applied P and P that has been reacted with soil[5] or between different sources of P[24].

Results

Plant response to applied P

(a) *Comparison of sources of P.* All sources of P increased plant growth (Figs. 1, 2). However, the sources differed markedly in their

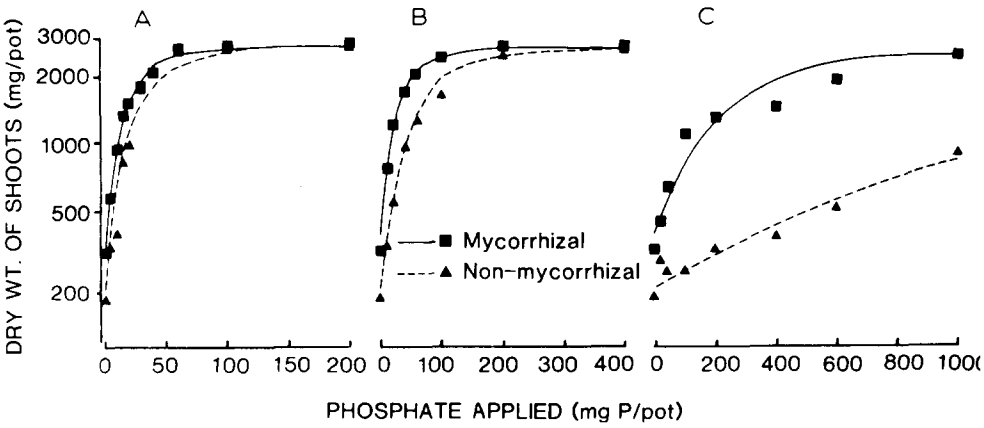

Fig. 1. Effect of phosphate application on the dry weight of shoots of mycorrhizal and non-mycorrhizal subterranean clover supplied with (**A**) potassium dihydrogen phosphate (**B**) colloidal iron phosphate and (**C**) strengite as the source of phosphate. Equation 1 was fitted to the response curves ($R^2 = 0.974$). The estimated value of $\ln(a)$ was 7.86 and of q was 22.9 mg/pot. The values for the c co-efficients are given in Table 3.

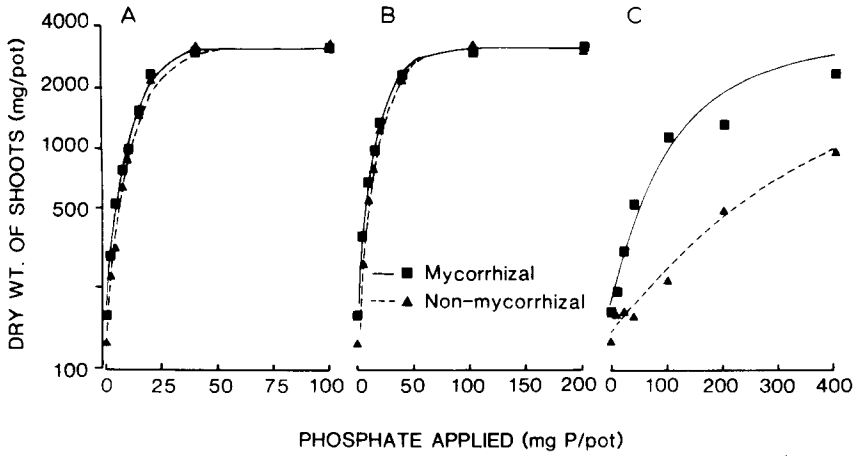

Fig. 2. Effect of phosphate application on the dry weight of shoots of mycorrhizal and non-mycorrhizal ryegrass supplied with (**A**) potassium dihydrogen phosphate (**B**) colloidal iron phosphate and (**C**) strengite as the source of phosphate. Equation 1 was fitted to the response curves ($R^2 = 0.979$). The estimated value of $\ln(a)$ was 8.06 and of q was 10.0 mg/pot. The *c* values are given in Table 3.

effectiveness. When strengite was the source of P for non-mycorrhizal plants, the maximum yields of the other treatments were not reached. However the responses were consistent with the assumption that the same maximum would have been reached if sufficient strengite had been supplied. The estimated values of the c coefficients of Eq. (1) for the various treatments are given in Table 3.

(b) *Comparison of mycorrhizal and non-mycorrhizal plants.* For subterranean clover, mycorrhizal plants had an advantage over non-mycorrhizal plants and the less soluble the source of P, the greater the advantage. Thus the estimate of the relative effectiveness of mycorrhizal plants compared to non-mycorrhizal plants were 1.24 for P added as potassium dihydrogen phosphate, 1.86 for colloidal iron

Table 3. Regression estimates of the values of the c co-efficients for the response in shoot weights of subterranean clover and ryegrass to three sources of phosphate when plants were either mycorrhizal (+M) or non-mycorrhizal (−M)

Species	Source of phosphate					
	Potassium dihydrogen phosphate		Colloidal iron phosphate		Strengite	
	+M	−M	+M	−M	+M	−M
Subterranean clover	0.061	0.049	0.039	0.021	0.0045	0.00077
Ryegrass	0.100	0.090	0.063	0.059	0.0092	0.0026

phosphate, and 5.84 for strengite. That is, to produce the same weight of shoots, the non-mycorrhizal plants required 1.24, 1.86 and 5.84 times as much P respectively as mycorrhizal plants. For ryegrass the advantage of the mycorrhizal plants was small for both potassium dihydrogen phosphate and colloidal iron phosphate. For these sources of P the values for relative effectiveness were both about 1.1. For strengite the advantage of the mycorrhizal plants was larger and the value for the relative effectiveness was 3.5.

Mycorrhizal development

Plant roots from the inoculated treatments were heavily infected with the mycorrhizal fungus. Those of the control treatments were uninfected. The level of application of P from all sources markedly influenced the percentage of root length infected for both ryegrass and subterranean clover (Fig. 3). For initial levels of P there was an increase in the percentage infection but, at higher levels of P, the percentage infection decreased. Effect of P sources on the infection appeared to be related to their effects on plant growth. Hence, while the relationship between P applied and percentage infection varied with the source of P, the relationship between percentage maximum growth and percentage infection was found to be characteristic of the plant species and independent of the source of P (Fig. 4). For ryegrass, the maximum percentage root length infected was reached when the plants produced slightly less than 10% of their maximum growth. By contrast, for subterranean clover, the maxi-

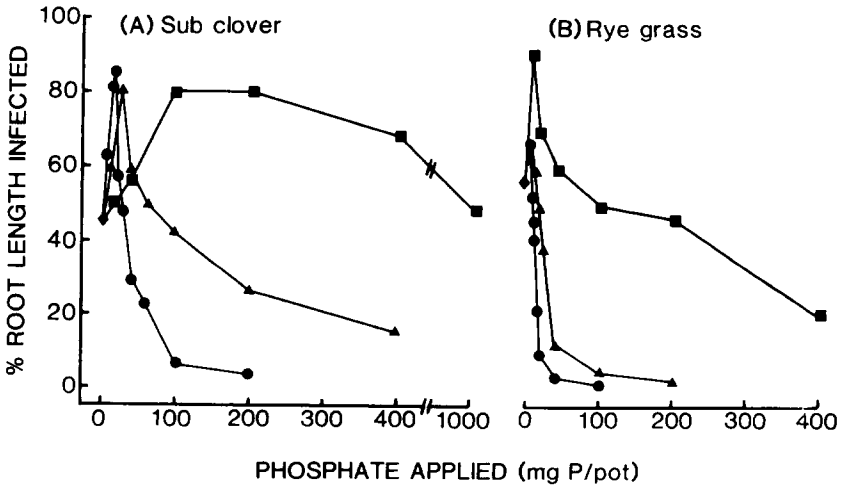

Fig. 3. Relationship between phosphate application and percentage root length infected for (A) subterranean clover and (B) rye grass supplied with (●) potassium dihydrogen phosphate (▲) colloidal iron phosphate, and (■) strengite.

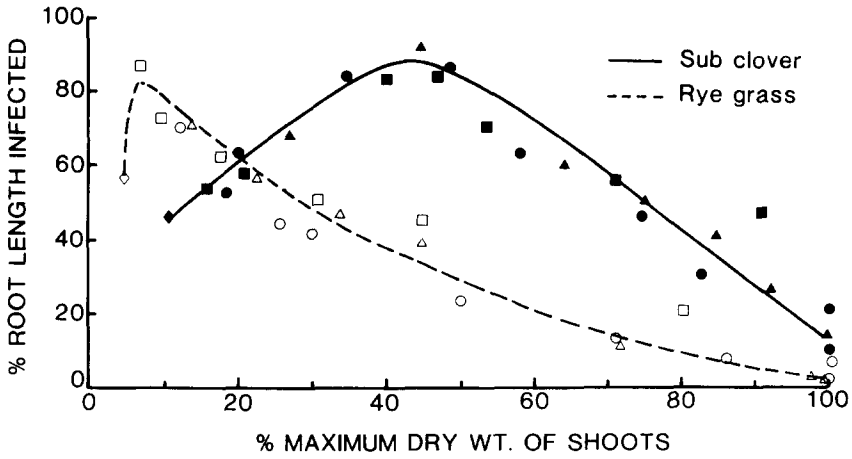

Fig. 4. Relationship between percentage maximum shoot growth and percentage root length infected for subterranean clover and ryegrass supplied with (○, ●) potassium dihydrogen phosphate, (△, ▲) colloidal iron phosphate and (□, ■) strengite.

mum percentage root length infected was reached at about 50% of maximum plant growth.

Discussion

The result of the present experiments contrast with those of our previous experiments[7] in that no threshold effect was observed in the absence of mycorrhizas. In those experiments we found that when iron oxides had been added to the soil, non-mycorrhizal plants were almost unable to take up phosphate until large applications of phosphate had been made. In that case the reaction involved was a sorption reaction between the phosphate and the iron oxide. As a result, the concentration of phosphate in the soil solution would have increased as the level of phosphate application increased. Thus uptake apparently did not occur until some threshold concentration had been reached. In the present study, the phosphate was supplied as particles of iron phosphate. The solubility of the phosphate was low and hence phosphate would not have been able to diffuse very far from the particles. It is therefore unlikely (at least at low levels of application) that the diffusion zones around the particles would have interacted. Increasing the level of phosphate would not have resulted in a higher concentration but rather in a greater number of "point" sources. The concentration of phosphate at these point sources was apparently greater than any threshold for uptake and hence uptake occurred for both mycorrhizal and non-mycorrhizal plants. Nevertheless the uptake by the mycorrhizal plants was greater. Several explanations may be suggested for this.

The simplest explanation for the increased uptake by the mycorrhizal plants is that the mycorrhizas explored the soil more thoroughly and so found more of the point sources. Having found the sources it is possible that they also increased the rate of uptake from the sources by increasing the diffusion gradient either by a close approach to the source or by achieving a low concentration of phosphate at the surface. A further possibility is that mycorrhizal hyphae may be able to chemically modify the availability of iron phosphates by producing organic compounds such as citrate. There is evidence that ectomycorrhizas produce oxalates[11,17] which chelate iron and release P for plant uptake. However there is yet no evidence that endomycorrhizas produce these compounds.

It is not possible to identify which of these two possibilities is the most likely explanation. Moreover it is not possible to differentiate among these alternatives from the existing studies in which radioactive P has been used to compare the specific activity of P in mycorrhizal and non-mycorrhizal plants. Forms of P in soil which differ in their availability to mycorrhizal and non-mycorrhizal plants have been shown to be uniformly labelled by the ^{32}P. This naturally results in a similar specific activity of P in mycorrhizal and non-mycorrhizal plants irrespective of their ability to get access to different forms[7].

The advantage of mycorrhizal infection in increasing plant growth and thus decreasing external P requirement was greater with subterranean clover then with ryegrass for all sources of P. This results is consistent with observations that the response to mycorrhizal infection was poor for most of the fine rooted graminaceous plants[6,12]. However the present result differs in showing that response to mycorrhizal infection could be obtained for ryegrass when a poorly-soluble phosphate source was applied. One of the possible reasions attributed for the poor response to mycorrhizal infection by ryegrass is that its roots are more efficient than subterranean clover roots in exploiting soil P[4]. Another possible reason for the poor response of mycorrhizal infection in ryegrass (especially when soluble P was added) is that the infection of ryegrass roots by the mycorrhizal fungus falls off at much lower levels of plant growth than that of subterranean clover. This may be related to the physiological characteristics of the plant species (for example soluble carbohydrate levels within the roots[28] or differences in root permeability[15]).

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