Effects of vesicular–arbuscular mycorrhizal inoculation on the yield and phosphorus uptake of field-grown barley

A. Khaliq*, 1, F.E. Sanders

Department of Plant Sciences, University of Leeds, Leeds LS2 9JT, UK

Accepted 20 April 2000

Abstract

In a field experiment on barley, the effects of soil application of phosphorus fertilizer and inoculum of Glomus mosseae on the mycorrhizal colonization of roots, crop yield and P uptake were evaluated in a natural or methyl bromide fumigated soil. Soil fumigation raised the amount of available nitrogen (NH₄–N+NO₃–N) in the soil by 13 mg kg⁻¹. Although both soils were equalized for the N status by adding N fertilizer to non-fumigated soil, fumigated soil gave higher crop yield. The applied P increased the dry matter yield significantly but suppressed mycorrhizal infection. An overall small increase of 3% in total P uptake and a decrease of up to 2% in grain and straw yield were observed as a result of inoculation, which were statistically non-significant at P < 0.05. These trends in results were most likely due to the status of available soil P higher than the threshold limit for a positive mycorrhizal growth response. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: VA-mycorrhiza; Barley; Crop yield; P uptake; Fumigation; Phosphorus; Field study

1. Introduction

Mycorrhizal roots due to their extramatrical hyphae that are capable of absorbing and translocating nutrients, can explore more soil volume than the non-mycorrhizal roots (Joner and Jakobsen, 1995), and thus increase the supply of slowly diffusing ions, such as phosphate to the plant (McArther and Knowles, 1993). In return, the plant meets the carbon requirement of the fungus (Jakobsen and Rosendahl, 1990). Although the roots of barley crop in the field are always colonized with vesicular–arbuscular mycorrhizal (VAM) fungi, the effect of this association on the crop is variable perhaps due to complexity of the interaction between symbiont and environment.

To measure the plant response to VAM infection, mycorrhizal fungi need to be eliminated from the control plants. This can be achieved by the use of certain crop rotations or various soil sterilization techniques (Jakobsen, 1994). It has been reported that VAM inoculation enhanced the growth of barley significantly in soils with low available P (Clarke and Mosse, 1981; Powell, 1981), while such improvement in barley growth was not significant in an irradiated soil containing moderate amounts of available phosphorus (Jensen, 1984). Black and Tinker (1979) produced different sizes of VAM fungal populations in an arable field by different crop rotations, and on the following barley crop they found a negative relationship between mycorrhizal infection and grain yield. However, the results were considered inconclusive owing to possible differences in soil properties between the treatments. In a pot experiment conducted in a field environment, Khaliq and Sanders (1998) used methyl bromide fumigated soil with adequate available P and noted that mycorrhizal fungi decreased barley yield. Fay et al., (1996) in a sand culture study also observed that when P was applied in the range of 50–800 mg kg⁻¹, inocu-
loration with the mycorrhizal fungus *Glomus mosseae* consistently depressed the growth of barley plants.

Even very carefully carried out pot experiments can not truly predict the behavior of the field crops. The objective of our study was, therefore, to evaluate the effect of VAM inoculation and P application on the yield and P uptake of field-grown barley in a natural or methyl bromide fumigated soil.

2. Materials and methods

This experiment was conducted in Field 426 (Leeds University Farm, Headly Hall, Tadcaster) in a sandy–clay–loam soil comprising (g kg$^{-1}$) coarse sand, 133; fine sand, 374; silt, 165 and clay 328 of the Wotheral some series (Crompton and Matthews, 1970). The soil had available P (0.5 M NaHCO$_3$ extractable), 25 mg kg$^{-1}$; nitrogen (2 M KCl extractable), 30 mg kg$^{-1}$ (13.3 mg kg$^{-1}$ as NH$_4$–N and 16.6 mg kg$^{-1}$ as NO$_3$–N); potassium (1 M NH$_4$NO$_3$ extractable), 136 mg kg$^{-1}$ and pH 7.4. Extractable N, P and K contents in soil were determined as given by Gough (1973). The field had been under fallow for 1 year after a kale crop, hence the soil had a low indigenous VAM population (*Glomus* spore density 0.01–0.02 spores g$^{-1}$).

The experimental area was ploughed, rotavated and divided into two halves separated by a 1 m wide path. One half was used as such (non-fumigated) and the other half was sealed under a polythene sheet and subjected to methyl bromide fumigation (200 kg methyl muriate of potash (K $499$ g kg$^{-1}$) coarse sand, 133; silt, 165 and clay 328 of the Wotheral some series (Crompton and Matthews, 1970). The soil had available P (0.5 M NaHCO$_3$ extractable), 25 mg kg$^{-1}$; nitrogen (2 M KCl extractable), 30 mg kg$^{-1}$ (13.3 mg kg$^{-1}$ as NH$_4$–N and 16.6 mg kg$^{-1}$ as NO$_3$–N); potassium (1 M NH$_4$NO$_3$ extractable), 136 mg kg$^{-1}$ and pH 7.4. Extractable N, P and K contents in soil were determined as given by Gough (1973). The field had been under fallow for 1 year after a kale crop, hence the soil had a low indigenous VAM population (*Glomus* spore density 0.01–0.02 spores g$^{-1}$).

The experimental area was ploughed, rotavated and divided into two halves separated by a 1 m wide path. One half was used as such (non-fumigated) and the other half was sealed under a polythene sheet and subjected to methyl bromide fumigation (200 kg methyl bromide containing 10 g kg$^{-1}$ chloropicrin ha$^{-1}$). After 4 days the polythene sheet was removed and the area was left to aerate. The analysis of soil 15 days after fumigation (i.e., 8 days before sowing) revealed that the extractable N and P were higher by 13 and 5 mg kg$^{-1}$ after fumigation (i.e., 8 days before sowing) revealed that the extractable N and P were higher by 13 and 5 mg kg$^{-1}$, respectively in fumigated compared to non-fumigated soil. Most of the N released was in NH$_4$–N form. The non-fumigated and fumigated areas were each divided into four plots (block size 3 m$^2$), and each block into four plots (plot size 3 m$^2$). The plots were either supplied with VAM free sand (M0) or inoculum of *Glomus mosseae* (M1) and either given no P (P0) or supplied with P (P1) at the rate of 100 kg P ha$^{-1}$ as triple superphosphate (P 196 g kg$^{-1}$). Potassium (31 kg K ha$^{-1}$) was applied to all the plots as muriate of potash (K $499$ g kg$^{-1}$), whereas N, 28 kg N ha$^{-1}$ as nitram (N 345 g kg$^{-1}$) was applied only to the non-fumigated plots to balance for the N released as a result of soil fumigation. No compensation was made for the P mobilized as a result of soil fumigation.

The mycorrhizal inoculum used was produced in a glasshouse where inoculated (with sporocarps of *Glomus mosseae* recovered from the original inoculum brought from Rothamsted Experimental Station) or uninoculated maize plants were grown in dazomet sterilized sand beds. The rhizosphere sand along with maize roots (after necessary homogenization) from the inoculated beds was used as inoculum and that from the controls as VAM free sand for the present experiment at the rate of 20 kg m$^{-2}$ on oven dry basis. Inoculum application at this rate raised the *Glomus* spore density of soil (to a depth of 15 cm) by 1 spore g$^{-1}$.

The factorial combinations of M and P contents constituted four treatments (i.e., M0P0, M1P0, M0P1, M1P1) which were randomized in each block. Statistically this experiment was treated as two independent sets i.e., one on non-fumigated and the other on fumigated area, each with a randomized block design.

Fertilizers and inoculum were broadcast and incorporated in the soil to a depth of 15 cm by a rotavator; fertilizers to the whole plot i.e., 3 × 3 m but inoculum only to the central 2 × 2 m area of the plot. Barley (*Hordeum vulgare* L., cv. Loja Abed) seed was sown at 2.5 cm spacing in rows 14 cm apart using a Nordsten drill. Rotavator and drill were always run from one direction so that the movement of inoculum and fertilizers across the plots was similar for the whole area. After allowing for soil displacement, the central 2 × 2 m area of each plot was designated as the sampling area. Bird scares were used. The seedlings emerged 10 days after sowing. Both areas were hand-weeded. The fumigated plots had fewer weeds than non-fumigated plots. Plant populations were scored just before tillering. The crop approached anthesis 61 days after sowing (DAS) and maturity at 124 DAS.

Root samples were taken from each plot (sampling area) at 40, 55, 84, 99 and 124 DAS. At each sampling, 10 soil cores (2.5 cm diameter) plot$^{-1}$ to a depth of 15 cm were taken and bulked to give a composite sample from which the roots were washed out, cleared, stained (Phillips and Hayman, 1970) and assessed for mycorrhizal infection by the line-intersect method (Giovannetti and Mosse, 1980). The composite samples of soil were taken at −17, −8, 25, 39, 54, 84, 102 and 124 days from sowing in the same way as that for root and analyzed for extractable soil N. At harvest (124 DAS) the central 2 × 2 m area (2 m × 15 rows) of each plot was harvested and the components of crop yield were recorded. The dried samples of grain and straw after their digestion in a di-acid mixture (HClO$_4$, HNO$_3$; 1:4, v/v) were analysed for P by the vanadomolybdate method (Cavell, 1955).

The statistical analysis of data was carried out by conducting ANOVA. Comparison of the means was made using least significant difference (LSD) or standard error of the mean (SEM). All the data are presented as actual (non-transformed) means. The analysis of data expressing proportion was carried out after arcsine transformation. In that case the attached letters denoting significance are based on the analysis
after arcsine transformation (Snedecor and Cochran, 1980).

3. Results

3.1. Mycorrhizal infection

The mycorrhizal colonization of roots generally increased with time (Fig. 1). Inoculated treatments had significantly ($P < 0.01, 0.001$) higher mycorrhizal infection than uninoculated treatments in non-fumigated as well as in fumigated soil. Mycorrhizal infection did not exceed 0.6 cm$^{-1}$ in uninoculated fumigated plots during the experiment. The plots given phosphate had lower amounts of infection than their respective control plots not supplied with phosphate. The inhibitory effect of P on VAM colonization of roots was significant ($P < 0.001, 0.01, 0.05$) at the first sampling in non-fumigated soil and at the first, third and fifth samplings in fumigated soil. The M × P interaction also suppressed the amounts of mycorrhizal infection in both soils but significantly ($P < 0.01, 0.05$) only at the first sampling in non-fumigated soil and at first, second and fifth samplings in fumigated soil.

3.2. Yield

The application of P fertilizer significantly increased grain, straw and grain plus straw weights m$^{-2}$ in non-fumigated as well as in fumigated soil (Table 1; Fig. 2). Harvest index and number of ears plant$^{-1}$ were also higher in plots supplied with P fertilizer (M0P1, M1P1) than in plots with no added P (M0P0, M1P0). The effect was significant in fumigated soil.

3.3. Plant phosphorus

Phosphorus concentrations in grain and straw were increased in plants by P application and by mycorrhizal inoculation in both soils, however, the P application effects were significant. Similarly, the P contents of grain and straw were significantly increased by P

---

**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soil</th>
<th>Treatment means, $n = 4$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NF</td>
<td>M0P0</td>
</tr>
<tr>
<td>Grain DW, g m$^{-2}$</td>
<td></td>
<td>477 b</td>
</tr>
<tr>
<td>Straw DW, g m$^{-2}$</td>
<td></td>
<td>721 b</td>
</tr>
<tr>
<td>Harvest index</td>
<td></td>
<td>0.394 a</td>
</tr>
<tr>
<td>Number of plants m$^{-2}$</td>
<td>NF</td>
<td>247 a</td>
</tr>
<tr>
<td>Number of ears plant$^{-1}$</td>
<td>NF</td>
<td>4.07 a</td>
</tr>
<tr>
<td>Number of grains ear$^{-1}$</td>
<td>NF</td>
<td>12.5 a</td>
</tr>
<tr>
<td>1000 grain weight, g</td>
<td>NF</td>
<td>38.1 a</td>
</tr>
</tbody>
</table>

$^{a}$Values sharing the same letter(s) in a row do not differ significantly at $P < 0.05$. DW, dry weight; Harvest index = grain/(grain + straw) dry weight ratio.
application but not by mycorrhizal inoculation (Table 2; Fig. 2).

### 3.4. Extractable soil nitrogen

Inoculation with VAM fungus and application of P fertilizer had no effect on the amounts of total extractable soil N (NH₄⁺-N + NO₃⁻-N) in either of the two soils during the season. The data were, therefore, presented as mean of all the plots in each soil (Fig. 3).

### 3.5. Comparison between soils

A valid statistical comparison between the non-fumigated and fumigated soil could not be made because there was no replication, however, the obvious differences were encountered.

At each P rate the inoculated plants had higher amounts of mycorrhizal infection in non-fumigated than in fumigated soil. The difference was slight at P₀ but considerable at P₁ (Fig. 1). On overall basis, the fumigated soil had slightly higher plant populations than the non-fumigated soil (Table 1). The yield and plant P (concentration and contents) were also higher in fumigated than in non-fumigated soil (Tables 1 and 2; Fig. 2). After the application of additional N to the non-fumigated soil, its total extractable N content remained similar to that of the fumigated soil till the last sampling (124 DAS), however, the NH₄⁺-N remained higher in fumigated than in non-fumigated soil for the period to 39 DAS (Fig. 3).

### 4. Discussion

Negligible mycorrhizal infection in the fumigated uninoculated treatments during the season showed that methyl bromide application at the rate of 200 kg ha⁻¹ effectively eliminated the indigenous VAM population. Generally, the development of VAM infection seemed to follow a three phase growth curve i.e. a lag phase, a phase of rapid growth and a phase of constancy as observed in previous studies (Saif, 1977; Khaliq and Sanders, 1997). The overall higher VAM colonization levels of plant roots in non-fumigated compared to fumigated soil in the respective treatments were most probably due to the presence of an indigenous VAM population in the non-fumigated soil. Moreover, a differential increase (5 mg kg⁻¹) in the availability of P in fumigated soil might be partly responsible for its lower levels of VAM colonization, as mycorrhizal infection was also suppressed in both soils by the fertilizer P. This is in accordance with earlier workers who found that the supply of P to plant by soil (Khaliq and Sanders, 1997; Thingstrup et al., 1998) or by foliar application (Sanders, 1975) decreased VAM colonization. The inhibitory effect of P application on VAM infection was more pronounced in the fumigated soil, indicating that the single population of the introduced fungus (Glomus mosseae) was more sensitive to fertilizer P than the community of indigenous and, indigenous and introduced VAM fungi. VAM fungal species can differ significantly in their tolerance to soil P concentration (Pearson et al., 1994). Abbott and Robson (1978) also observed the better adaptation of native VAM fungi than the added endophyte to higher amounts of soil P. As a result of inoculation, the increase in the fraction of root mycorrhizal (cm m⁻²) over control (non-inoculated) was higher in fumigated than in non-fumigated soil. This could be attributed to the reduction of antagonistic (Warnock et al., 1982) and competitive (Powell, 1979) effects of the native soil microflora by soil fumigation. The comparison of a
single species versus a mixture of species (i.e. how they respond to a factor) is, however, questionable as it does not preclude the genetic differences between the two populations.

The non-fumigated soil was supplied with extra N to balance the N concentration in both soils. The analytical results showed that this aim was achieved. An overall higher crop yield from the fumigated compared to non-fumigated area, which was mainly due to the slightly higher 1000-grain weight and plant population of the fumigated area, possibly resulted from the release of P and other nutrients from the lysed microorganisms (Rovira and Ridge, 1979). The presence of higher proportion of NH$_4$ than NO$_3$ nitrogen up to 39 DAS in the fumigated soil might also have contributed to its higher grain and straw yield than that of the non-fumigated soil. This finding supports the results of Camberato and Bock (1990) who obtained significantly higher dry matter yield in wheat by maintaining the NH$_4$ fraction of mineral N in soil higher than in controls. The root system development was observed to be much better in the fumigated than in non-fumigated soil. This was most probably the consequence of the aforementioned nutritional effects of fumigation, while in non-fumigated soil the microorganisms (eliminated by fumigation) might have affected root growth positively (plant growth promoting rhizobacteria — PGPR) or negatively (parasites or pathogens).

The literature shows that barley can respond positively to VAM inoculation at available soil P contents < 10–20 mg kg$^{-1}$ (Clarke and Mosse, 1981; Jakobsen and Jensen, 1981; Powell, 1981; Jensen, 1982). A very small but consistent reduction in the final yield was found in inoculated compared to uninoculated treatments. Adequate amounts of available soil P and the dense and fibrous root system of the plant can make mycorrhiza superfluous, and thus parasitic, because of less benefit to the plant in terms of improved mineral nutrition for a given exchange of photosynthate to the fungus (Pang and Paul, 1980; Bethlenfalvay et al., 1982). The original concentration of available P (25 mg kg$^{-1}$) and the efficient root system of barley probably maintained a supply of P to the plants higher than the threshold limit of positive mycorrhizal growth response in the crop. The adequate P status of the field is reflected from the grain and straw yields comparable to the range of yields obtained under normal agricultural practice. Moreover, a high dose of P (100 kg ha$^{-1}$) gave a rather small overall increase (up to 11%) in the grain + straw yield.

The inoculum used in the present experiment was from the same lot and applied at the same rate as that for our pot experiments in which VAM infection was prolific (70 cm$^{-1}$) in maize, with an associated significant enhancement in plant growth (Khaliq and Sanders, 1997); whereas, the amount of infection obtained was lower in barley (40 cm$^{-1}$) leading to a significant reduction in yield (Khaliq and Sanders, 1998). The differing responses in our experiments were most likely due to differences in the functioning of the symbiosis and not due to differences in the inoculum. The smaller magnitude of the mycorrhizal effect in the present experiment was probably due to the greater field variability, and the overall lower amounts of mycorrhizal infection as a result of higher status of soil P, compared to that of the barley pot experiment. In this context it is important to add that the barley variety (cv. Lofa Abed) used in our experiments was a high root–shoot ratio type, selected on the basis of a previous VAM inoculation study on barley (A. Khaliq unpub. PhD thesis, Leeds University, 1983), indicating that at an adequate concentration of soil P the depression in plant growth resulting from mycorrhizal infection might be greater in a high root–shoot ratio than in a low root–shoot ratio variety. Hetrick et al. (1996) also concluded that this relationship occurred in wheat cultivars.

Most of the published studies on mycorrhizal growth responses in plants have described the usefulness of mycorrhizal symbiosis in P-deficient soils and very few have reported its adverse effects at adequate P levels. This might lead to an unrealistic picture of the effects of mycorrhiza in normal agriculture. Therefore, there is need to develop a broader view of the nature of mycorrhizal symbiosis which apart from being mutualistic can also be null or even negative (Francis and Read, 1995). A further study is warranted to evaluate the effect of VAM inoculation on barley at a wider range of available soil P contents.

References


