Role of Mycorrhizas in the Assessment of Phosphorus Efficiency in Cereals

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ROLE OF MYCORRHIZAS IN THE ASSESSMENT OF PHOSPHORUS EFFICIENCY IN CEREALS

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Thesis submitted for the degree of
DOCTOR OF PHILOSOPHY
in
Faculty of Agricultural and Natural Resource Sciences
The University of Adelaide

Department of Soil Science
Waite Agricultural Research Institute
The University of Adelaide

February 1994

Awarded 1994

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SUMMARY

Baon J B 1994 **Role of mycorrhizas in the assessment of phosphorus efficiency in cereals**. Ph D thesis, The University of Adelaide, Australia.

Selection of phosphorus (P)-efficient cereals is very important for agricultural production, especially on marginal soils with low P concentration, due to the possibility of reducing the use of P fertilizers and minimizing the loss of P from the fields. Much effort has been devoted to the identification and selection of P-efficient cultivars of cereals. However, research on P efficiency has generally taken little account of the possible effects of soil microorganisms, including particularly the role of the vesicular-arbuscular (VA) mycorrhizal symbiosis, which is well known to affect both uptake of P and concentration of P in plant tissues. The studies reported in this thesis aim

1. to investigate the role of mycorrhizal infection in cereals and to define its mechanism in affecting the assessment of P efficiency,
2. to identify and characterize cereal cultivars in terms of mycorrhizal responses in relation to P efficiency, and
3. to assess factors that affect mycorrhizal contributions to the determination of P efficiency in cereals.

A general review of literature concerning the importance of mycorrhizal infection on the P nutrition of plants is presented. In this study, P efficiency is defined in three ways. 1) P utilization efficiency is defined as the amount of dry matter yield produced per unit of P in the tissues under a certain P concentration or stress. 2) P uptake efficiency is defined as the amount of P taken up per plant or per unit of roots under a certain P concentration or stress. 3) Agronomic P efficiency is defined as dry matter yield produced at a certain P concentration or stress. Factors influencing the uptake of P from soil including plant root characteristics and mycorrhizas are reviewed.

The first series of experiments were conducted to examine the influence of indigenous VA mycorrhizal infection on the determination of P efficiency in each one cultivar of wheat (*Triticum aestivum* L. cv. Spear), barley (*Hordeum vulgare* L. cv. Galleon) and rye (*Secale cereale* L. cv. S. A. Commercial). The experiments were set up with two soils which had low concentrations of plant-available P and which had been used in a previous study of P efficiency in cereals. Numbers of spores and infective propagules were significantly different in the two soils. The cereals were all colonized by VA mycorrhizal fungi, even at a soil temperature of 10°C and the extent of infection varied with plant species. Root infection of the cereals by VA mycorrhizal fungi was much reduced by increasing the amount of P applied to soil. Results of these experiments have revealed that a high level of mycorrhizal infection by indigenous fungi increased the efficiency of P uptake by barley plants, but it did not affect that aspect of efficiency in wheat or rye. In contrast, mycorrhizal infection of wheat and barley reduced the efficiency of P utilization. This study also showed that mycorrhizal infection does not seem to influence the efficiency of P uptake and efficiency of P utilization in rye. It is clear that VA mycorrhizal infection may change the relative performance of different cereal in terms of several definitions of P efficiency.

The VA mycorrhizal fungi in the soils have been shown to infect the cereals at a soil temperature of 10°C. A glasshouse study was conducted to investigate the effect of soil temperatures of 20, 15 and 10°C on growth and P uptake of barley inoculated with *Glomus intraradices*. The extent of mycorrhizal infection was reduced as the soil temperature decreased. Plant growth depression due to low temperature was more pronounced in mycorrhizal plants than in non-mycorrhizal plants. Concentration of P in roots of mycorrhizal plants was higher than in non-mycorrhizal ones. Significant interactions between mycorrhiza and soil temperature were observed for root dry weight and specific P uptake. Although soil temperature had no effect on specific P uptake by mycorrhizal plants, these results revealed that mycorrhizal plants had a higher specific P uptake than non-mycorrhizal plants and at the same time had lower root-shoot ratios.

Compared with wheat and rye, barley has been shown to be more responsive to mycorrhizal infection. A study was carried out to investigate how barley cultivars which were different in agronomic P efficiency (i.e. they differ in dry matter yield at low P supply) responded to mycorrhizal infection. The experiment was conducted under glasshouse conditions in which soil temperature was maintained at 15°C. Eight barley cultivars were grown. The extent of mycorrhizal infection among the barley cultivars in soil without P amendment varied from 8.6% to 28.6%. Mycorrhizal infection lowered P utilization efficiency (dry matter yield per unit of P taken up) in most of the barley cultivars. P utilization efficiency and agronomic P efficiency among the barley cultivars was negatively correlated with mycorrhizal responses in terms of shoot dry matter yields. However, the response to mycorrhizal infection was positively correlated with the response to P application. Poor correlation was observed between the percentage of root length infected and P concentration when neither mycorrhiza nor P were supplied. Significant interactions between cultivar and P addition, and between mycorrhiza and P addition were observed for shoot dry weight but not root dry weight.

Agronomically P-efficient cultivars responded less both to mycorrhizal infection and to P addition, confirming that mycorrhizas play a role in the allocation of P. A glasshouse experiment was undertaken to investigate the effect of mycorrhizal infection on the allocation of phosphorus in agronomically P-efficient and inefficient barley cultivars. Four barley cultivars differing agronomic P efficiency were inoculated with *Glomus etunicatum* or not inoculated. Cultivars did not differ in percentage of root length infected. Root to shoot ratio of the P-efficient cultivars was lower than that of the inefficient cultivars, and the decrease in the ratio following infection was significant in P-inefficient but not in P-efficient cultivars. The concentration of P in roots of the inefficient cultivars was higher than that of the efficient cultivars. Agronomically P-inefficient barley cultivars responded to VA mycorrhizal infection by reducing the percentage of total plant P which was present in the roots, while efficient cultivars showed no such response. This suggests that mycorrhizal infection increases the relative allocation of P to the shoots of inefficient cultivars. The results also show that in the absence of mycorrhizal infection, inefficient cultivars have a higher proportion of total plant P allocated to the root systems than efficient ones.

Variation in the uptake of P from soil may be due to differences in root hair length among cereal cultivars. An experiment was conducted to investigate the effect of mycorrhizal infection on the growth and P efficiency of two selections of rye (*Secale cereale* L. cv. Imperial) differing in root hair length. Plants with short root hairs had a higher percentage of root length infected by mycorrhizal fungi than the plants with long root ones. In low P soil, mycorrhizal infection increased P concentration of both plants, but decreased shoot dry matter of plants with long root hairs. In the absence of mycorrhiza, concentration of P in roots of plants with short root hairs was higher than those with the long ones, but was not different when the plants were mycorrhizal. P uptake was increased by longer root hairs and by mycorrhizal infection, but the uptake due to longer root hairs was much greater than due to mycorrhizal infection. A negative correlation was also found between response to mycorrhizal infection or to P application and agronomic P efficiency of those plants. Plants with short root hairs, which were agronomically inefficient, showed more response to mycorrhizal infection and to P application. It was also demonstrated that Barber-Cushman

model underpredicted the uptake of P by plants with long root hairs and mycorrhizal plants.

The results of this study clearly indicate the potential contribution from mycorrhizal infection to the determination and selection of P-efficient cereals. Hence breeding programs should take into account the contribution made by mycorrhizal fungi to P nutrition under field conditions and the possible variations in both colonization and response in different cultivars.

DECLARATION

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

I consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying.

February, 1994

John Bako Baon

PUBLICATIONS FROM THE THESIS

The following papers have arisen from work done in this thesis:

Journal Articles:

1. Baon J B, Smith S E, Alston A M and Wheeler R D 1992 Phosphorus efficiency of three cereals as related to indigenous mycorrhizal infection. *Aust. J. Agric. Res.* **43**, 479-491. **(Results of Chapter 4).**
2. Baon J B, Smith S E and Alston A M 1993 Phosphorus allocation in P-efficient and inefficient barley cultivars as affected by mycorrhizal infection. *Plant Soil* **155/156**, 277-280. **(Results of Chapter 8).**
3. Baon J B, Smith S E and Alston A M 1993 Mycorrhizal responses of barley cultivars differing in P efficiency. *Plant Soil* **157**, 97-105. **(Results of Chapter 8).**
4. Baon J B, Smith S E and Alston A M 1994 Phosphorus uptake and growth of barley as affected by soil temperature and mycorrhizal infection. *J. Plant Nut.* **17** (in press). **(Results of Chapter 6).**
5. Baon J B, Smith S E and Alston A M 1994 Growth response and phosphorus uptake of rye with long and short root hairs infected by *Glomus intraradices*. *Plant Soil* (submitted). **(Results of Chapter 9).**

Conference Articles:

1. Baon J B, Wheeler R D, Smith S E and Alston A M 1991 Phosphorus efficiency in cereals: the role of indigenous mycorrhizal fungi. Paper presented at *The Fourth International Symposium on Genetic Aspects of Plant Mineral Nutrition*, Canberra, Australia. Abstracts, p. 54. **(Results of Chapter 4).**
2. Baon J B, Smith S E and Alston A M 1992 Are P efficient plants mycorrhizal responders? Paper presented at *International Symposium on Management of Mycorrhizas*, Perth, Australia. Abstracts, p. 50. **(Results of Chapter 8).**
3. Baon J B, Smith S E and Alston A M 1993 Phosphorus nutrition and growth of barley plants as affected by soil temperature and mycorrhizal infection. Paper presented at *The Ninth North American Conference on Mycorrhizas*, Guelph, Canada. Abstracts, p. 127. **(Results of Chapter 6).**
4. Baon J B, Smith S E and Alston A M 1993 Phosphorus allocation in P-efficient and inefficient barley cultivars as affected by mycorrhizal infection. Paper presented at *The Twelfth International Plant Nutrition Colloquium*, Perth, Australia. Proceedings, pp. 319-322. **(Results of Chapter 8).**

ACKNOWLEDGEMENTS

I am grateful to Assoc Prof S. E. Smith and Dr A. M. Alston, Department of Soil Science, for their guidance, encouragement, constructive comments and stimulating discussion throughout the entire period of my research study.

Thanks are also due to Mr R. D. Wheeler of the South Australian Research and Development Institute for providing his field work data, some seeds and permission to conduct an experiment at Northfield Experimental Station.

Technical assisstance of Mr C. M. Rivers, Mr D. H. Hein and Mr R. D. Abbott at periods of the experimental works is gratefully acknowledged.

I am grateful to Dr A. J. Rathjen, Dr R. C. M. Lance and Dr Herdina for suppling some seeds, Mr P. A. E. Ellis for his guidance in crossing plants, Dr P. Langridge for his advice on molecular analysis, Mr A. Karakousis for his excellent assistance in southern blot analysis, Dr Z. Rengel for providing the program of nutrient uptake model.

Thanks also go to Ms L. C. Giles and Mr I. Nevison (Biometry Section) for advice on statistical analysis of the data. I wish to thank Mr A. J. Dunbar and Ms J. Groom (ETU) for help in taking the photographs.

I would like also to thank other members of the Department of Soil Science, particularly Soil Biology Group, and Plant Nutrition Group of Dr R. D. Graham for providing helpful discussions and an amicable working condition during this study period.

Thanks go to Drs L. K. Abbott, J. H. Graham, I. Jakobsen, R. T. Koide, D. G. Lewis, P. A. McGee, J. A. Menge, R. L. Peterson, D. J. Read, J. P. Thompson, M. L. Van Beusichem and N. A. Walker for helpful discussions.

The financial support of the Australian International Development Assistance Bureau through EMSS scholarship which enable me to further study in Australia is gratefully acknowledged. I also thank the Research Institute for Estate Crops, Jember, Indonesia for granting me study leave.

Finally I would like to offer deep gratitute to my wife, Tutiek, and my son, Ibe, for their patience, understanding and continous support throughout this hard time.

To my parents

Chapter One

GENERAL INTRODUCTION

Phosphorus (P) is of particular interest as one of the macronutrients because of its importance in many metabolic processes in plants. An inadequate supply of P in soil and soil solution leads to reduction in growth and yields of plants. The content of P in seeds and grains of cereals, for example, is in the range of 4 to 5 g kg^{-1} in the dry matter. Growing plants may absorb P from the soil up to the amount of 1 kg ha^{-1} day^{-1} (Mengel and Kirkby 1982). P-containing mineral deposits, which are non-renewable natural resources, are used to supply P in the form of fertlizers for agricultural systems, when P supply inadequate. Since P fertilizer demand has risen sharply and the fertilizer is in short supply, prices have moved up rapidly. The cost of application of P fertilizers and economic problems in many countries have caused reductions in fertilizer usage.

Soils in large parts of the world are low in the "available" P, the form of P that can be taken up by plants. Generally, P deficiency in soil is caused by low concentrations of P in the soil solution, even when the total amount of P in that soil is high. P concentration in the soil solution is usually lower than 10 µM (Bieleski 1973). A fraction of phosphate can be adsorbed to the surface of clay minerals, hydrous oxides, carbonates and apatites which is in rapid equilibrium with the concentration in soil solution. The adsorption of phosphates is frequently combined with precipitation process (Larsen 1967). Most phosphates of Ca, Fe and Al, are relatively insoluble in water which may also account for the low concentration of P in soil solutions. Large amounts of P are also associated with organic materials which are also mostly insoluble and the P may not be available to plants (van Diest 1968).

The concentration and rate of diffusion of P in soil solutions are several orders of magnitude lower than those of other macronutrients. The rate of diffusion of P in soils with high P concentration in solution is much lower than in water, but greater than in P deficient soils. The movement of P in soil is restricted by the presence of soil particles which increase the length of the diffusion path (tortuosity), and by a reversible binding of P to the surfaces soil particles which affects concentration of P in solution.

Application of P fertilizers to soils is one way of correcting deficiency and this has been widely practiced because it is relatively easy. Applied P will promote P supply to the roots by increasing the concentration of P in the soil solution around the roots and by increasing its diffusion rate. The second effect is important and therefore the roots depend on sources of P in soil very close to them. However, the availability of applied P declines with time because the soluble P compounds react rapidly with the soil particles, either by precipitation or adsorption onto surfaces (White and Taylor 1977).

A complementary approach to increasing P supply is to reduce P demand by selection, breeding and development of cultivars which are responsive to the P applied and which are efficient in its exploitation, uptake and utilization. Interest in this approach is increasing (Graham 1984). Cultivars of many species have been shown to have significant differences in uptake and utilization of P and genetic explanation of this variation has been reported (Gabelman and Gerloff 1983, Duncan and Baligar 1990). Species and cultivars with low relative growth rate usually have a high root to shoot ratio and low root absorption capacity (Nielsen and Barber 1978, Lindgren *et al.* 1977).

Plant roots have the potential to take up P from soil faster than diffusion can supply it. Various plant responses to conditions where available P is low could be dependent on morphological and/or metabolic characteristics of their roots. These characteristics may affect the availability of P in soil, and the absorption and utilization of P by plants. Cultivars with long roots or root hairs, for instance, take up more P than those with short roots or root hairs (Caradus 1981). The most obvious adaptation to low P is to have more roots. Species or cultivars or varieties with large amounts of roots generally take up large amount of P. Small diameter results in a large surface area per unit weight and root systems which are able to explore a large volume of soil and increase the uptake of P from the soil. A high ratio of surface of plant roots to soil volume may be the key for the ability of species or cultivars to survive in soils with low concentration of P.

In many programs of plant improvement, especially those aimed at obtaining cultivars which are able to make the most effective use of the limited P available in soil, the term "P efficiency" is commonly used. However, P efficiency can be defined in many ways which are dependent on the aims of the study and the background of the workers. Hence, it is not surprising that many definitions of P efficiency are used. In this study, P efficiency is defined in three ways. 1) P utilization efficiency is defined as the amount of dry matter yield produced per unit of P in the tissues under a certain P concentration or stress. 2) P uptake efficiency is defined as the amount of P taken up per plant or per unit of roots under a certain P concentration or stress. 3) Agronomic P efficiency is defined as the dry matter yield produced at a certain P concentration or stress.

The presence of mycorrhiza can be regarded as a plant characteristic which increases the effectiveness of P uptake. However, many research studies on the selection of P-efficient plants have not included mycorrhiza as one of the plant characteristics that may contribute the effectiveness of P uptake. Vose (1963), Chapin (1988) and Lafever (1981) have pointed out the possible role played by mycorrhiza in the response of plants to nutrient stresses. However, information on the role of mycorrhiza in the assessment of the ability of cultivars within species to growth in soil with low P and still produce high yields is still limited.

Mycorrhizas have been found in most of higher plant species and the importance of mycorrhizal associations in agriculture has been widely recognized (Tinker 1978, Harley and Smith 1983). Several crop plants such as citrus, clover, onion and sorghum absorb more P from soils when colonized with VA mycorrhizal fungi than when non-mycorrhizal. Cereals are also naturally infected by mycorrhizal fungi and beneficial effects of VA mycorrhizal infection have been reported (Khan 1975, Jakobsen 1983, Saif and Khan 1977). This suggest that the association between VA mycorrhizal fungi and cereals does not differ essentially from that with other species. In the association, host plants must provide carbohydrates to the mycorrhizal fungi for development and growth. Association of roots with VA mycorrhizal fungi generally benefits growth particularly of the host plant through enhancement of nutrient uptake, especially the relatively immobile nutrients, such as P (Smith 1980, Koide 1991). Three mechanisms may explain this effect. Firstly, the hyphae explore a large volume of soil; secondly, translocation of P through hyphae is faster than movement through soil; and thirdly, the hyphae may be involved in solubilization of soil P. However, some of P that is taken up by the hyphae has to pass the fungus-root interface before the plant benefits. Hall (1978) has shown that maize cultivar W 415 responded to VA mycorrhizal infection (responder), while PX 610 did not (non-responder). This raises the interesting question of whether the difference in uptake between responder and non-responder cultivars reflects the capacity of the plants to transfer P from the fungal tissues to the plant roots and to distribute P between shoot and roots.

The extent of mycorrhizal infection varies among cereal species and cultivars within species (Bertheau *et al.* 1980, Azcón and Ocampo 1981). Krishna *et al.* (1985) found that the level of mycorrhizal infection was under the control of genetic mechanisms. Benefits that derived from the infection differed between plant species (Gerdemann 1975). This suggest that mycorrhizas may affect the P nutrition of the plants and eventually affect the assessment of P efficiency among plant species or cultivars. A question arises as to whether cereal cultivars which differ in the level of mycorrhizal infection may also differ in growth of their shoots and roots. If they do, then will the difference in infection will be translated to differences in P nutrition of the plants? The number of reports on plant selections and breeding which take into account the possible effects of mycorrhizas on P nutrition is limited.

Winter and spring cereals, such as wheat and barley, are usually grown at relatively low temperatures. Despite the knowledge that temperature greatly influences the growth and development of the cereal plants and its mycorrhizal symbiont (Hayman 1974), there is still lack of information on the effect of temperature on P nutrition of mycorrhizal plants.

The investigations reported here were designed to answer some of the questions raised above. The specific objectives of the study were:

1. to determine the role of mycorrhizal infection and to define its mechanism in affecting the assessment of P efficency in cereals,
2. to examine and characterize cereal cultivars in terms of mycorrhizal responses in relation to P efficiency, and
3. to assess factors that affect mycorrhizal contributions in determining efficiency of P in cereals.

Chapter Two

REVIEW OF LITERATURE

2.1 Introduction

Selection of P-efficient cereals is very important for agricultural production, especially on marginal soils with low P concentration, due to the possibility of reducing the use of P fertilizer and minimizing the loss of P from the soil. Much effort has been devoted to the identification and selection of P-efficient cultivars of cereals (Lipsett 1964, Nielsen and Schjørring 1983, Fageria *et al.* 1988, Jones *et al.* 1989). A P-efficient cereal may be defined as one which can a) take up more P from soil per plant (mg P plant^{-1}) or b) produce more dry matter per unit of P taken up (mg dw mg^{-1} P) or c) produce more dry matter with a given amount of applied P (mg dw µg^{-1} P) or d) have lower concentration of P in grain (mg P g^{-1} grain) under conditions of P stress, than an inefficient cereal. Studies of P efficiency have generally taken little account of the possible effects of soil microorganisms on efficiency, particularly on the role of the mycorrhizal symbiosis which is well known to affect both uptake of P and concentration of P in plant tissues.

In the last two decades there has been much interest in studying mycorrhizas in the context of agricultural research. There are three main reasons for this. Firstly, mycorrhizal fungi have a low-level of host specificity (Gianinazzi-Pearson 1984), so that many possible combinations of hosts and fungi can associate in the symbiosis. Secondly, mycorrhizal hosts are able to make use of nutrients, e.g. phosphorus (P), which are limited in the soil (Tinker 1975), and therefore this symbiosis may reduce the need for fertilizers. Thirdly, many significant aspects related to mycorrhizas are still poorly understood.

This chapter provides a short review of literature on the effects of mycorrhizas on the physiology of plants, with special attention to the effect of mycorrhizas on the uptake of P by roots and on the P concentration in plant tissues. It will also consider the effect of P and plant genotypes on the development of mycorrhizal infection, stressing in particular work on cereals, because they are important economic plants. The emphasis will be on P, because it is a major nutrient, poorly available in many soils, which gives rise problems in plants.

The term 'mycorrhiza' mentioned in this chapter, mostly relates to vesicular-arbuscular (VA) mycorrhiza, because this kind of mycorrhiza is the most common in agricultural plants.

This review integrates the recent results of studies (up to 1990, the begining of this investigation) on VA mycorrhizas and P nutrition of plants, especially in cereals, to provide a focus on the possible interactions of mycorrhiza and the assessment of P efficency in cereals.

2.2 Phosphorus in soil

2.2.1 *Availability of P in soil*

In many soils, the amount of P available to plants is extremely small, in the range of 0.3 to 3 kg P ha^{-1} as found in soil solution (Mengel and Kirkby 1982). P present in the soil can be found either as inorganic or as organic compounds. In the soil, inorganic P may be found dissolved in soil solution, adsorbed on the surfaces of soil particles, or precipitated as separate minerals. Only a small amount of P is present in soil solution, but most of the primary needs of the plant for P are obtained from this source. In acid soils, most of the P is adsorbed on the surfaces of the soil particles or precipitated as Fe and Al phosphates, whereas in alkaline and calcareous soils Ca and Mg phosphates regulate the availability of P (Olsen and Khasawneh 1980, Barrow 1985, Freeman and Rowell 1981). Some Fe and Al phosphates exchange phosphate ions only very slowly and are thus accounted as the insoluble phosphates. The proportion of each form, and their availability to plants generally depends on soil pH (Sample *et al.* 1980, Barrow 1985).

Organic forms of P in soils may be found as soluble P in the soil solution, as insoluble P adsorbed on the surface of soil particles, or as a component of the undecomposed organic matter of the soils. Most of the organic P compounds are easily degraded in soil and the availability of these compounds to plants has been demonstrated (Martin 1973). In most soils, mineralization of the organic P compounds by enzymatic dephosphorylation is needed before they become available to plants (Cosgrove 1977). The contribution of organic P compounds to the availability of P in soil, depends on the rates of mineralization of the compounds.

2.2.2 *Diffusion of P in soil*

There are three processes by which nutrient ions reach the root surface, i.e. root interception, mass flow and diffusion (Barber 1984). Root interception is the process by which the nutrients are absorbed when the root surface comes in direct contact with nutrient. Mass flow is the movement of nutrient to the root surface in the convective flow of water during the absorption of water by plant. Nutrient ions, such as phosphate, which are more readily retained by soil particles are not supplied by mass flow. Following the absorption of P from the soil solution by plant roots, P depletion zones develop around the plant roots. Availability of P in the zone is largely independent of the concentration of P in the soil because the rate of replenishment of P to soil around the root depends on the diffusion of P through the soil (Barber 1984). Diffusion is the movement of a nutrient towards a root surface caused by a concentration gradient. As P in soil is mostly present in immobile forms, the availability of this nutrient to plants is often limited by diffusion.

The rates of diffusion are determined by the characteristics of the ions and of the soil. Nye and Tinker (1977) suggested that diffusion of P in soil is associated with volumetric water content, buffering capacity of the soil, tortuosity of the diffusion path and the diffusion coefficient of orthophosphate in water. Application of more readily available P to soils results in an increase of concentration of P in soil solution, and in the rates of diffusion into the depletion zones, so that the uptake of P may only be limited by the rates at which plants can absorb this nutrient. However, the high increase in total amount of P in soil by addition of sparingly soluble P, such as rock phosphate, is not followed by high amount in soil solution. In this low supply of available P, the uptake of P by the plant is mostly limited by the diffusion to replenish the depletion zone around the root.

2.3 Plant requirement for P

P, as one of the macronutrients, is of particularly interest because of its importance in the structure of deoxyribonucleic acid, ribonucleic acid and adenosine triphosphate which function, respectively, as the carrier of genetic information, in protein synthesis and in energy transfer.

For optimal growth during the vegetative stages, the P requirement is in the range of 3 to 5 g kg^{-1} of the plant dry matter. Total P concentration in plant tissues may vary from 1 to 10 g kg^{-1}, when subjected to variation in P supply (Ozanne 1980). Total P concentration in seeds is slightly affected by the variation of P supply. However, low P supply may reduce the size, number and viability of seeds.

Following its absorption, P is transported quickly to all parts of the plant, and it remains in its highest oxidized form. Considerable amounts of P may leak out of the root (Bieleski 1973), and can be taken into account for predicting P accumulation in the plant tissues. In plants, P is present either in inorganic form mainly as orthophosphate or in organic form mainly as esterified orthophosphate. Two main phosphate pools are present in vacuolated cells of higher plants. Firstly, there is the metabolic pool including cytoplasm and chloroplasts in which P esters dominate. Secondly, there is the non-metabolic pool represented by vacuoles where inorganic P (Pi) is the dominate form. About 90% of the Pi is found in the vacuole, if the supply of P is adequate and growth rate of P deficient plants is determined by the rate at which P was transported out of the vacuole. Plant growth was stopped immediately after this reserve pool was depleted (Bieleski 1968).

Growth of plants suffering from P deficiency is hindered, and they exhibit symptoms of reddish or dark green colours of their leaves. Although the quantity of chlorophyll per unit area of leaf is higher in a P deficient plant (Hecht-Buchholz 1967), the photosynthetic efficiency per unit of chlorophyll is lower (Tombesi *et al.* 1969).

Due to the importance of P in the metabolism and growth of plants, an insufficient supply of P restricts many metabolic processes, such as photosynthesis, respiration and growth (Terry and Ulrich 1973, Heldt *et al.* 1977, Dietz and Foyer 1986). Avdeeva and Andreeva (1974) found that the rate of photosynthesis was decreased more severely in maize, a C-4 species which has a four atom carbon photosynthetic pathway, than in bean, a C-3 species in P deficient soil. The function of Pi in regulating the processes of photosynthesis and carbohydrate metabolism of leaves implies that its main effect is in limiting plant growth, especially at generative period. During this stage the P supply regulates the ratio of starch to sucrose in leaves, and the partitioning of photosynthate between leaves and reproductive organs (Giaquinta and Quebedeaux 1980).

In seeds, most of the P is bound in an organic compound called phytin, which forms salts with Ca and Mg during the formation of the seeds. Phytin is believed to be the reserve form of P which can be mobilized and used by seedlings during germination.

Compared with older leaves, the proportion of organic P in the form of nucleic acids is higher in younger leaves. Michael (1939) and Hart (1972) have shown that content of Pi in plants was decreased by an inadequate supply of P, while organic P in the form of phospholipid and nucleic acid were little affected. A similar result also was obtained in oat grains. It seems that Pi in vegetative tissues functions as the reserve form, while in reproductive tissues this function is taken by phytin.

2.4 Assessment of P efficiency

Considerable attention has been given in recent years to the possibility of improving efficiency of fertilizer use either by exploiting genotypic differences among plants in absorption and utilization of mineral nutrients (Elliot and Läuchli 1985, Gerloff 1976) or by manipulating the symbiosis of plants and microorganisms (Tinker 1984) to obtain higher productivity on nutrient deficient soils. However, better criteria to define an efficient plant are still required to clarify some contradictory definitions (Clark and Brown 1974, Loneragan and Asher 1967, Chisholm and Blair 1988). For example, a cultivar grown in low P soils cannot be an efficient cultivar in terms of both higher P concentration in tissues and higher dry matter produced per unit P taken up. This suggests that the definitions of P efficiency used depend on the aims of the investigations (Smith *et al.* 1990b). The parameters to be measured for specifying the efficiency can be determined from the objectives. With this background and for further understanding the role of the mechanisms in the determination of P efficiency, it is necessary to compare several definitions of P efficiency in plants that have been put forward.

Definitions of P-efficient plants can be categorized into three groups, as follows: 1) P efficiency in utilization, 2) P efficiency in uptake and 3) agronomic P efficiency.

2.4.1 P Efficiency in utilization

In this group, several definitions have been used.

A plant is P efficient :

a. if it accumulates high amount of dry matter per unit P accumulated per unit time (Chisholm and Blair 1988),

b. if it produces high amount of dry matter per unit of P absorbed (reciprocal of concentration) (Loneragan and Asher 1967),

c. if it has a low concentration of P in its yield or harvestable product, such as grain (Huisman 1982).

d. if it has high ratio of total carbon over the total P accumulated in the whole plant during the same period (Koide and Elliot 1989).

Clearly, there are many variations in the definition of P efficiency in utilization, and the appropriate definition will depend on the aim of the investigation (Smith and Gianinazzi-Pearson 1988, Smith *et al.* 1990b). If there is a need to determine the efficiency of P utilization, we have to know the kind of utilization, whether for the dry or fresh weight of the leaves, or for the dry or fresh weight of whole tops, or for the grain yields. Each kind of utilization may result in a different value of P efficiency and will lead to different ranking of species and cultivars.

2.4.2 P *Efficiency in uptake*

Several definitions are suggested in this group:

A plant is P efficient:

a. if it takes up high amount of P per unit weight of roots (mg P g root^{-1}). This is also called specific P uptake (Amann and Amberger 1988) or root efficiency (Jones *et al.* 1989),

b. if it takes up high amount of P per unit length of root (mg P m root^{-1}) (Amann and Amberger 1988),

c. if it takes up high amount of P per unit weight of root per unit time (mg P g root^{-1} day^{-1}) (Williams 1948),

d. if it takes high amount of P per unit length of root per unit time (mg P m root^{-1} day^{-1}) (Williams 1948) which is also called inflow (Brewster and Tinker 1972, Smith 1982).

There is need to take into account the total amount of carbon that has been transported into the below ground part of the plant (Koide and Elliot 1989, Smith and Gianinazzi-Pearson 1988). Photosynthate allocated to the root system is used not only for root biomass production, but also for respiration and production of root exudates, including phytosiderophores. These expenditures for supporting below ground activities will be higher when the roots are in association with soil microorganisms, because of the extra requirement for fungal biomass and respiration, a part of which is outside the root system.

2.4.3 *Agronomic P efficiency*

Definitions grouped in this include the following:

A plant is P efficient

a. if the plant uses the nutrient which is in low supply and grows normally (Brown *et al.* 1977).

b. if the plant produces a large quantity of harvestable dry matter per unit time and area growing in a medium that has less than sufficient P available for maximum yield (Fox 1978).

c. if physiological and biochemical processes of the plant are such that it can successfully cope with limiting environmental constraints (Smith and Gianinazzi-Pearson 1988).

The second definition is preferred by many workers, especially plant selectionists, for identifying P-efficient plants or cultivars. There are possibly two main reasons for this preference. Firstly, the definition focuses on high yield with low input which is highly demanded by agronomists. Secondly, the parameters measured are relatively simple, a feature which is required in selecting large numbers of plants.

In investigating the contribution of mycorrhizal infection to the assessment of P efficiency in cereals, it is important to consider the uptake, utilization and agronomic aspects of P efficiency. Therefore, for the present study P efficiency is defined in three ways. 1) P utilization efficiency is defined as the amount of dry matter yield produced per unit of P in the plant tissues under a certain P concentration or stress. 2) P uptake efficiency is defined as the amount of P taken up per plant or per unit of roots under a certain P concentration or stress. 3) Agronomic P efficiency is defined as dry matter yield produced at a certain P concentration or stress.

2.5 Plant root characteristics affecting P uptake

Due to its immobility and low availability in soil, P has been the focus of many studies of plant nutrition. Consequently we also have to understand plant factors that affect the uptake of this nutrient, so we can manage the P nutrition of economically important plants.

2.5.1 Physical characteristics

Root morphology, which includes primarily root branching, root length, presence of root hairs and root depth, determines the ability of plants to exploit the soil for nutrients. Growth and morphology of roots are known to be genetically controlled, but are also influenced by a number of environmental factors, such as soil atmosphere, mechanical impedance, and plant nutrient status.

2.5.1.1 Root length and branching

Reducing the diameter of roots, while maintaining root weight in a certain volume of soil, results in the increase of root length density and possibly root branching, and this will increase P flux to the root per cm^2 of root surface and hence potential P uptake (Baldwin 1975, Loneragan 1978, Silberbush and Barber 1983). Calculations by Barber and Silberbush (1984) indicate that the smaller the root diameter the more gradual is the depletion zone. High root density and branching are especially important and effective in exploring a given volume of soil and in taking up relatively immobile nutrients, such as P, from poor soil, due to the shorter distance between roots and sources of P in soil.

In predicting the uptake of P, Cushman (1979) and Silberbush and Barber (1983) developed models which calculated P uptake by integrating values for root size and its increase with time, P flux into the root as related to P concentration in the soil solution at the root surface and P supply to the root by mass flow and diffusion. They also considered root competition in their models and found that root diameter and root length were also among the sensitive factors. However, the relationship between uptake rate and root density is not linear. When the root density is high the uptake rates begin to decline (Marschner 1986). Overlapping of depletion zones of individual roots may explain this observation (Nye and Tinker 1977). Nevertheless, comparison of the works of Claassen and Barber (1976) and Schenk and Barber (1979) suggests that competition between roots was not a determining factor in the uptake of P from soil. Similar results was found by Silberbush and Barber (1983).

The importance of root length density has been demonstrated in studies comparing different species. The capacity of ryegrass to acquire P from soil is high compared with red clover, mainly due to the total root length of ryegrass which is five times higher than red clover (Steffens 1984). Similar results have been also obtained as comparisons were made among cultivars of the same species of cowpea (Adepetu and Akapa 1977), white clover (Caradus and Snaydon 1986) and common bean (Schettini *et al.* 1987).

2.5.1.2 Presence of root hairs

Differences in length and density of root hairs may occur among species or among cultivars of one species. Root hair length has been shown to be heritable (Caradus 1979) and positively correlated to P uptake (Barley and Rovira 1970) in white clover and wheat, respectively. Little difference was found, however, in uptake of P among wheat varieties differing in root hair density (Bole 1973). Root hairs are of particular importance because of the close contact they maintain between the soil and the root, forming a water continuum between them. The benefit of having high root hair density and long root hairs is that only a small amount of dry matter needs to be invested to produce a large root surface. According to Champion and Barley (1969) root hairs are capable of penetrating moderately resistant clays and may thus contribute to nutrient exploitation of less accessible soil particles. This suggests that root hairs with smaller diameter permit the exploration of smaller soil pore spaces than coarser ones. The P depletion zone around the roots has been shown to be similar to the zone of the root hair cylinder (Lewis and Quirk 1967, Bhat and Nye 1973, Kraus *et al.* 1987). Thus, root hairs play a special role for nutrients which are transported towards the roots by diffusion. Only P available within the distance of root hair length is easily taken up by the plants.

Root hair length is, however, also a variable that can be affected by nutrient availability. Föhse and Jungk (1983) have shown that the mean root hair length of three plant species decreased as the availability of P increased, suggesting that formation of root hairs may be a mechanism of adaptation to the conditions with low P supply.

2.5.1.3 Root distribution

Root distribution vertically may also differ considerably between species, with perennial plants generally rooting deeper than annuals. For agricultural crops, a rooting depth of 0.5 to 1 m is common, but some species may have rooting depths of 2 m and more. More than 25 cm cm^{-3} of roots of Reed canary grass were found in top 5 cm of soil profile, and this value decreased to 2.5 cm cm^{-3} in layer of 15 to 35 cm (Johnson 1981, cited by Barber 1984). Besides differences between plant species, root distribution is also affected by chemical (Pearson 1974) and physical (Taylor 1974) properties of top and subsoil.

Sharpley (1986) has shown that P moves only a few centimeters down the soil profile, after the application of P fertilizer. Although P concentration of topsoil is higher than subsoil, the most active root growth may be shifted from the top to subsoil after available P resources in that top layer have been exhausted. Under these conditions growth of roots down the profile in later stages of plant development seems to be advantageous.

2.5.2 *Chemical characteristics*

Roots are able to influence chemically their immediate surroundings and hence increase the availability of P to the plant. The rhizosphere extends about 1 to 2 mm from the root surface into the bulk soil. In this region many processes such as root exudation of organic acids and enzymes, root-induced pH changes and microbial activities take place and may influence the availability of P. Solubilization of sparingly soluble P sources in soil by organic acids is considered to be important. Moghimi *et al.* (1978) isolated 2-keto-gluconate from the rhizosphere of wheat roots in amounts that could solubilize a considerable quantity of phosphate from hydroxy-apatite. Formation of relatively stable chelates with Fe^{3+} and aluminium by citric and malic acids also increases the solubility and uptake of P. Certain plant species (e.g. *Lupinus albus*) and many genera of Proteaceae release citrate in large quantity and form ferric hydroxyphosphate citrate polymers which diffuse to the root surface where P is taken up after Fe^{3+} is reduced (Gardner *et al.* 1983, see Marschner 1986).

P may be mobilized in the rhizosphere as a result of acidification and high cation absorption rate. Marschner and Römheld (1983) observed that along the root axis of a single plant, there was a significant pH gradient of more than two pH units. The pH of the rhizosphere may increase or decrease relative to bulk soil, if the plant uses nitrate or ammonium, respectively, as N source (Raven and Smith 1976, Smith 1980). The rhizosphere pH may also differ among plant species. For example, chickpeas (Marschner and Römheld 1983) have a low pH in their rhizosphere compared with wheat. The differences in rhizosphere pH among plant species may reflect differences in cation to anion uptake ratio. High ratio of cation/anion uptake leads to a decrease in pH of the rhizosphere (Smiley 1974). Therefore the ability of plant species in utilizing sparingly insoluble P sources may be related to their cation/anion uptake ratio (Bekele *et al.* 1983) and Ca uptake rate (Baon and van Diest 1989). Hedley *et al.* (1982) observed that the ratio became greater and rhizosphere pH decreased as plants aged.

Graminaceous plants produced a range of phytosiderophores, compounds which are able to mobilize iron in the soil (Marschner *et al.* 1986). Besides Fe, phytosiderophores are also able to mobilize Cu, Zn and Mn compounds which are very low in solubility. Phytosiderophores may contribute to the availability P in soils by releasing the phosphates of iron and Zn.

2.5.3 *Mycorrhizas*

Mycorrhiza has to be considered as one of the plant root characteristics that may contribute to P nutrition of the plants. As mutualistic symbioses between plant roots and certain soil fungi, mycorrhizas play an indisputable role in the uptake of P by plants and in P cycling. Interest in this symbiosis is in part due to the possible reduction of fertilizer requirements especially when the depletion in the world reserves of P sources are increasing.

2.5.3.1 *Occurrence and morphology of mycorrhizas*

The roots of most higher plants are mycorrhizal. Mycorrhizas are widespread under natural vegetation and in agricultural soils and most plants of economic importance live in association with mycorrhizal fungi. Only a few plant species belonging mainly to Cruciferae, Proteaceae, Chenopodiaceae, Cyperaceae and Juncaceae, are not able to form mycorrhizas of any sort (Harley and Smith 1983). The symbiosis is biotrophic; the host plant provides organic nutrients to the fungi, which in turn supply the plants with mineral nutrients acquired from the soil by their external mycelium.

Vesicular-arbuscular (VA) mycorrhiza is the most widely distributed type of mycorrhiza. This study concentrates on VA mycorrhizas, mutualistic symbioses between plant roots with a group of zygomycetous soil fungi belonging to the Glomales (Morton and Benny 1990). Nearly all plants, including economically important crops, form VA mycorrhizas, which are characterized by their typical structures in the symbiosis, viz. vesicles and arbuscules. Vesicles are apical or intercalary swellings of hyphae containing lipid and large numbers of nuclei and it is believed that they function as reserve storage organs of the fungi. Arbuscules, the intracellular tree-like structures, are finely branched hyphae and it is believed that they play a role in the bidirectional transfer of metabolites and nutrients between host plant and fungal symbiont (Cox *et al.* 1975, Cox *et al.* 1980, see Smith and Gianinazzi-Pearson 1988). Recently, Gianinazzi-Pearson *et al.* (1991) suggested that intercellular hyphae may also play important part in absorption of C at the intercellular interface.

2.5.3.2 *Mycorrhizal effects on P uptake by roots*

The beneficial effect of mycorrhizal infection on plant growth is mainly been due to an increase in the absorption of nutrients, especially P, from soil (Abbott and Robson 1982, Smith 1980, Tinker 1978). The increase in uptake of P by mycorrhizal plants has been related to the enlarged surface area for absorption, due to fungal hyphae (Sanders and Tinker 1973), increased rate of translocation of P within hyphae (Harley and Smith 1983, Sanders and Tinker 1973) and efficient transfer of P to plant roots (Smith and Gianinazzi-Pearson 1988).

Mycorrhizal plants have been reported to absorb a higher amount of P from soil per unit length of root per unit time than uninfected plants (Sanders and Tinker 1971, Graham *et al.* 1981). Under conditions where P concentration in the soil solution is low, it was found that P inflow into mycorrhizal roots was four to 150 times larger than that into uninfected roots (Sanders *et al.* 1977, Sanders and Tinker 1973, Smith 1982). The high uptake may be due to the ability of mycorrhizal roots to explore a greater volume of soil and/or to absorb P. Kinetic analysis of VA mycorrhizal roots and germ tubes indicates that uptake into mycorrhizal roots is associated with lower Km values (Cress *et al.* 1979, Thomson *et al.* 1990), which suggests that the affinity of sites of phosphate uptake are also much higher in mycorrhizal than in non-mycorrhizal roots. However, a conflicting result has also been reported (Karunaratne *et al.* 1986) on soybean.

By labelling the labile pool of P in soil with ^{32}P and comparing the specific activities, it was found that mycorrhizal and non-mycorrhizal plants drew P from the same source (Sanders and Tinker 1973, Hayman and Mosse 1972). Although mycorrhizas were able to enhance the uptake rate from sparingly-soluble P sources, they did not render these materials useful (Hayman and Mosse 1972, Mosse *et al.* 1977). In agreement with those results, Pairunan *et al.* (1980) found that increase in the uptake of P by mycorrhizal plants was not different between plants supplied with rock phosphate and soluble P fertilizers, although the uptake of P from rock phosphates was better by mycorrhizal plants compared with non-mycorrhizal plants.

Bolan *et al.* (1984b) applied various fractions of labelled P to soil after addition of iron hydroxide. They demonstrated that mycorrhizal plants were able to take up more P from soil, whereas the addition of iron hydroxide decreased the amount of P in the shoots of non-mycorrhizal plants. However, there was no effect of mycorrhizal infection and application of iron hydroxide on the specific activity of P in the plants. They suggested, therefore, that plants infected by mycorrhizal fungi were able to utilize other sources of P which were not accessible to non-mycorrhizal plants.

2.5.3.3 *Mycorrhizal effects on P concentration*

The concentration of P in plants depends not only on the age of the plant, the tissue and the availability of the nutrient in soil, but also on the plant species and cultivar. In a field and literature survey, Sheppard (1991) also found that P concentration in plant was slightly related to the concentration in soil and increased when the soil had low organic matter. The concentration of P in shoots is usually higher in younger plants than in older ones. Furthermore, many reports have shown that in the presence of mycorrhizal infection the concentration of P in plant tissues is higher than in uninfected plants (Raju *et al.* 1987, see Koide 1991).

A higher concentration of P in plants infected by VAM fungi than in plants of equal size in weight without VA mycorrhiza can be associated with increased P uptake which is not followed by an increase in growth. However, the collected results of the effect of mycorrhizas on the concentration of P in some cereal plants (Table 2.1), suggest that the concentration of P in the mycorrhizal plants is not always higher than non-mycorrhizal plants. This implies that high uptake of P in tissues does not always lead to high concentration of P. These data also suggest that concentration of P in tissues of the cereals plants varies with the kind of the tissues and also the time of harvest.

P in the form of polyphosphate was reported to be accumulated in granules and vacuoles of mycorrhizal fungal hyphae (Cox *et al.* 1975, Ling-Lee *et al.* 1975). Cox *et al.* (1980) suggested that the concentration of P in plants may be determined by the translocation of polyphosphate granules by cytoplasmic streaming from the fungal mycelium to the roots of mycorrhizal plants. Presumably, such regulation could be mediated by bidirectional transfer at the interface between host plant and fungal symbiont. Whether a genotype which is efficient in utilization or in uptake could transfer more P to the shoot remains to be investigated. Recently Jakobsen *et al.* (1992) showed that

Table 2.1 **P concentrations in tissues of mycorrhizal and non-mycorrhizal cereals.**

Cereals	P concentration (%)		Tissue	HT[b]	References
	NM[a]	M			
Barley	0.083	0.088[c]	straw	80	Jensen 1982
Barley	0.291	0.277[c]	grain	80	Jensen 1982
Barley	0.105[d]	0.124[d]	shoot	91	Jakobsen and Andersen 1982
Barley	0.128[d]	0.127[e]	shoot	70	Champawat *et al.* 1987
Barley	0.38	0.37[c]	ears	98	Clarke and Mosse 1981
Maize	0.16	0.16	shoot	42	Jackson *et al.* 1972
Maize	0.083[c]	0.079[c]	leaf	85	Hall 1978
Maize	0.061	0.065	shoot	95	Murdoch *et al.* 1967
Oats	0.235[c]	0.345[c]	grain	n.a.	Koide *et al.* 1988
Oats	0.016	0.032	shoot	32	Koide *et al.* 1988
Pearl millet	0.29[e]	0.26[e]	plant	60	Krishna *et al.* 1985
Rice	0.009[d]	0.035[d]	shoot	122	Sanni 1976
Sorghum	0.065[c]	0.11[c]	shoot	48	Raju *et al.* 1987
Sorghum	0.19[c]	0.20[c]	shoot	50/70	Jackson *et al.* 1972
Sorghum	0.062[c]	0.068[c]	shoot	30/60	Murdoch *et al.* 1967
Wheat	0.16	0.17	shoot	n.a.	Kucey 1987
Wheat	0.117[c]	0.145[c]	shoot	70	Azcón and Ocampo 1981

[a] NM= non-mycorrhizal; M= mycorrhizal.
[b] HT= harvest time (days).
[c] Calculated from mean values.
[d] Transformed values.
[e] Calculated from mean transformed values.

different fungi differ in their abilities to absorb, translocate and transfer P to their host plants. These differences may lead to variation in the ratio of carbon to phosphorus exchange between host plant and fungal symbiont (Pearson and Jakobsen 1993).

2.6. Breeding for P efficiency

Cereals are the major arable crops in agricultural areas in the world. To increase yield of cereals, plant cultivars have been developed to produce high yields by increasing the amount of fertilizer applied. However, economic conditions have resulted in large increases in the price of mineral fertilizers. Furthermore, mineral resources used for manufacture of P fertilizers are being exhausted to the extent which may soon limit food production. For these reasons, efforts should be devoted to creating new cultivars characterized by high potential for utilizing low concentration of available P and sparingly soluble P sources in soils. In addition, the cultivars should have the capacity to utilize the large part of the applied fertilizer P that fixed in the soil. This method can be regarded as a low-input approach.

2.6.1 Efficient and inefficient plants

In the low-input approach, cereal breeding programs aim to develop and select cultivars which are P-efficient. A P-efficient plant has been defined in Section 2.4. Many plant species which are adapted to low P supply are able to mobilize P sources in soil by exudation of organic acids or enzymes and by acidification of their rhizosphere.

Mycorrhizal symbiosis as one of the mechanisms important in mobilizing P soil (Smith 1980), is not yet taken into account by most plant breeders in their programs to breed and select P efficient cultivars. Processes contributed by mycorrhizas to P nutrition of the plants cover the uptake of P by the fungi from soil, the transportation of the nutrient from hyphal fungi to the host roots, its distribution and its utilization in metabolisms. A question arises whether mycorrhizal infection affects P efficiency of a plant. If it does, then to what extent does mycorrhizal symbiosis influence the assessment of P efficiency among species or cultivars differing in P efficiency.

High P concentration of mycorrhizal plants (Stribley *et al.* 1980a, Raju *et al.* 1987, Son and Smith 1988), implies that plants infected with VA mycorrhizal fungi apparently utilize more P to produce a unit dry matter of shoot than non-mycorrhizal plants. Thus, mycorrhizal plants are less efficient in utilization of P than uninfected plants and may show luxury accumulation of P. Young plants (mycorrhizal or not) are also apparently leff efficient than older plants. If the aim of the investigation is to determine the efficiency of P in utilization for cereal plants when young, or for dry matter production, then the statement that mycorrhizal plants are inefficient may be accepted. Cereals, however, are generally harvested as grains. During vegetative growth mycorrhizal plants may have taken up and accumulated more P, which is later used in the generative phase for producing more grains. Numbers of heads and dry weight of barley grain per pot (Jensen 1982) and the weight and number of grains per plants of oats (Koide *et al.* 1988) were increased by the presence of mycorrhizal infection. Koide *et al.* (1988) and Jensen (1982) reported that oats and barley, respectively, infected with mycorrhiza have higher concentrations and contents of P in their grains than uninfected plants. However, only one of the four VAM fungal isolates tested by Jensen (1982) with barley produced higher concentration of P in grain. These differences may be due to the ability of the endophyte to adapt to the soil and climatic conditions, as well as to the identity of the endophyte and the host plant.

Apart from the aim to minimize the transport of P out of the soil system by having P efficient plants, it is likely that the nutritive value of grain is also affected by its P content. Although low intake of P may cause health problems, such as rickets in humans (Reid 1980), this aspect of efficiency has not received much attention. It is probable that human's daily meals have enough of this element. If that is the case, then a low concentration of P in grain may offer a good parameter for determining P efficiency. Therefore, it seems logical to develop cereal plants which are inefficient in utilization of P during vegetative growth, but efficient in the generative stage.

Although, mycorrhizal plants usually have higher shoot P concentration than uninfected plants, Raju *et al.* (1987) found that inoculation with mycorrhizal fungi had no effect on the utilization of P by efficient and inefficient sorghum genotypes. However, in the absence of mycorrhizal infection the concentration of P in the shoots of P inefficient genotypes was higher. Those results suggest that P uptake by the plant may be affected by the interaction of mycorrhiza and the plant genotype (Lambert *et al.* 1980, Raju *et al.* 1987). This interaction may influence the comparisons of plant genotypes in P efficiency study for sites contrasting in mycorrhizal status.

The presence of mycorrhiza tends to increase the amount of carbon transferred to the below ground part of the plant, due to the production of biomass and respiration of both symbionts. There is some evidence showing that 4-20% of the photoassimilates were allocated to the mycorrhizal fungus (Douds *et al.* 1988, Jones *et al.* 1991, Jakobsen and Rosendahl 1990, Eissenstat *et al.* 1993, Pearson and Jakobsen 1993). The high carbon expenditure indicates the increased cost for maintaining the symbiosis. The carbon transported to the roots is used also for the exudation and respiration by both symbionts.

The external hyphae may increase contact surface between soil particles and fungi. The mycorrhizal fungus, through its external hyphae in addition to its host plant roots may explore more of the soil volume than the uninfected plant (Abbott and Robson 1977, Sanders and Tinker 1973). This process helps the host plant in the absorption of P from soil, which may be further enhanced by a higher affinity for phosphate ions and lower threshold concentration of absorption by mycorrhizal fungal hyphae (Bolan *et al.* 1987, Cress *et al.* 1979, Thomson *et al.* 1990).

2.6.2 Responder and non-responder plants

Various plant species or cultivars may respond differently to the supply of P when grown in the same soil conditions. Whiteaker *et al.* (1976) found that cultivars with similar agronomic P efficiency were different in their responses to P application. Gerloff (1976) grouped plants into four groups, according to their P efficiency and responsiveness. Efficient and inefficient plants may either respond (responder) or not respond (non-responder) to application of P. Responders produced greater dry weight as P supply was increased, while non-responders did not. A similar pattern was observed with mycorrhizal colonization. However, there has been no experimental comparison between the P efficiency of the plants and their responses to mycorrhizas.

Hall (1978) has shown that one maize cultivar can grow without P addition or mycorrhiza, resulting in a low concentration of P in the shoot. However, this plant had a dense root system which can be seen as a mechanism of adaptation to low P supply and an indicator of independence from mycorrhiza. On the other hand, the other two cultivars tested had low growth rates and root:shoot ratios and high P concentrations in their leaves, but they were more responsive to VA mycorrhizal inoculation. This suggests that the P concentration in shoots of mycorrhizal maize is also affected by the root:shoot ratio if they are grown with low P supply.

2.6.3 *Cereal genotypes and mycorrhiza*

VA mycorrhizas have been found on almost all cereals, including economically important crops, such as wheat (Khan 1975, Bertheau *et al.* 1980, Manske 1989), barley (Saif and Khan 1977, Jensen and Jakobsen 1980, Champawat *et al.* 1987), rice (Sanni 1976, Ilag *et al.* 1987) and maize (Khan 1975, Hall 1978). Growth responses of cereals to VA mycorrhizal inoculation have been reported. Dry weight of wheat was increased about 200% over controls, following inoculation with the spores of *Endogone (Glomus) mosseae* with the mycorrhizal effect being higher than application of 56 kg P ha^{-1} (Khan 1975). Similar results have been obtained with barley (Saif and Khan 1977) and maize (Khan 1975). Over a wide range of P addition, inoculation of cereals grown on a fumigated soil increased total dry matter yield and grain yield (Buwalda *et al.* 1985). Beneficial effects of mycorrhizal infection of barley grown in the field have been reported (Jakobsen 1983).

Cereals, like other graminaceous plants, usually have a finely branched root system. Plant species and genotypes within species may have different root characteristics, such as root diameter and length, root branching, and density and length of root hairs. A theory that the thinness and length of roots and root hairs are indicative of the degree of mycorrhizal dependency has been proposed (Baylis 1970). Coarser roots and roots with short root hairs are thought to indicate a relatively high degree of mycorrhizal dependency, while fine roots and roots with long root hairs indicate a low degree of dependency. This difference may be associated with the greater surface contact with P sources in soil provided by plant with long root hairs compared with short root hairs. Percentage of root length infected in clover with long root hairs tends to be lower than that with short root hairs (Crush and Caradus 1980). However, for several genotypes with a similar root length, there may be large differences in the extent of VA mycorrhizal infection (Huisman 1982, Krishna *et al.* 1985). This can be related to a piece of evidence which has shown that VA mycorrhizal infection is not only host-dependent but also a heritable trait (Mercy *et al.* 1990).

The biochemical and physiological processes in plants, such as photosynthesis and activities of nitrate reductase and glutamine synthetase, can be affected by mycorrhizal infection and these responses are likely to be dependent on host genotype (Kucey and Paul 1982, Oliver *et al.* 1983, Smith *et al.* 1985). Similar results have also been reported for wheat (Sreenivasa and Rajashekhara 1989) and pearl millet (Krishna *et al.* 1985).

The genotype dependent variation in VA mycorrhizal development may be due to an interaction between the genotype of the host and the VAM strain preference (Smith and Walker 1981, Mosse 1981) and the growth rates of the fungus through the cortex (Mosse 1981). Cultivars of wheat (Azcón and Ocampo 1981, Sreenivasa and Rajashekhara 1989), pearl millet (Krishna *et al.* 1985), triticale (Sreenivasa and Rajashekhara 1989), and barley (Tilak and Murthy 1987) inoculated with VA mycorrhizal fungi show different degrees of mycorrhizal infection. Triticale had a lower percentage of infection than wheat (Young *et al.* 1985). Furthermore, it has been reported that there is some variation in the response to VA mycorrhizal infection between and within plant species (Owusu-Bennoah and Mosse 1979, Hall 1978, Ollivier *et al.* 1983). The variability of the effectiveness of mycorrhizal infection on wheat cultivars has been described by Bertheau *et al.* (1980) and Azcón and Ocampo (1981). In the symbiosis, the development of mycorrhizal fungi depends on the carbohydrate supplied by the plant host (Azcón and Ocampo 1981). Absence of mycorrhizal infection in some wheat varieties was associated with lack of sugar exudation from the roots rather than with the sugar content of the roots.

Summary

Development of cereal cultivars which are P-efficient in terms of uptake, utilization and agronomic aspects is very important for agricultural systems with low P supply. Programs for identification and selection of P-efficient cultivars have not taken into account the importance of mycorrhizas, although there seems to be a clear appreciation of the contributions of VA mycorrhizal symbiosis to the P nutrition of cereal plants.

In spite of the limited amount of work that has been done on the genetical aspects of mycorrhizal symbiosis, there is an indication that the extent of mycorrhizal infection in cereals is host dependent and heritable.

There is little information on the interaction of mycorrhizal infection and the assessment of P efficiency in cereals. Therefore, the aim of this project was to investigate the role of mycorrhizal infection in cereals and to define its effect on the determination of P efficiency of the plants. How mycorrhizal infection influences the P nutrition of plant species and cultivars differing in P efficiency and responsiveness, and their interactions were investigated.

Chapter Three

GENERAL MATERIALS AND METHODS

In this chapter, the materials and methods commonly used in this study are described, further details and modifications related to specific experiments are presented in subsequent chapters.

3.1 Soil

Two soils from Avon and Peake, in the cereal belt of South Australia, were used. These soils were chosen because they have very low concentrations of P and had been used in previous experiments to study P efficiency in cereals (R D Wheeler unpublished). The soil at Avon was a solonized brown soil (Stace *et al.* 1968) or Xeralf (Soil Survey Staff 1975) under open scrub vegetation (Specht 1972). The Peake soil was a calcareous sand (Stace *et al.* 1968) or Psamment (Soil Survey Staff 1975) under cereal cultivation. Soil samples were collected from 0-20 cm depth, air-dried and passed through a 2 mm sieve. Chemical characteristics of the soils are given in Table 3.1.

Table 3.1 **Some chemical characteristics of the two soils used in this study.**

Soil	pH(H_2O)	Extractable P[a] (mg kg^{-1})	Total N (g kg^{-1})	Organic C (g kg^{-1})
Avon	8.6	4	0.53	7.1
Peake	7.2	5	0.43	7.7

[a] $NaHCO_3$ extraction (Colwell 1963).

3.2 Soil sterilization

The soil was steam sterilized at 100°C for one hour. Air dry soil was placed in a large wooden container (1 m x 2 m) in the bottom of which there are pipes connected to the steam generator. The soil layer in the container was kept less than 50 cm in thickness and covered with gunny sacks.

3.3 Nutrients for plant growth

Pot experiments with cereal plants were designed to ensure that nutrients other than P did not limit plant growth. Each pot was supplied with mineral nutrients containing (all in mg kg^{-1} air dry soil) 133 NH_4NO_3, 145 K_2SO_4, 24.5 $CaCl_2.2H_2O$, 22.5 $MgSO_4.7H_2O$, 14.7 $MnSO_4.4H_2O$, 25 $FeSO_4.7H_2O$, 2.3 $ZnSO_4.7H_2O$, 1.33 $CuSO_4.5H_2O$, 0.83 H_3BO_3, 0.42 $Co(NO_3)_2.6H_2O$, 0.20 $Na_2MoO_4.2H_2O$ mixed into the soil before planting.

3.4 Determination of mycorrhizal population density

Two methods were used to assess the population of propagules of mycorrhizal fungi in the soils.

3.4.1 Spore counting

The method of Gerdemann and Nicolson (1963) was used for the recovery of spores of VA mycorrhizal fungi from soil samples. Each sample of 5 g soil was suspended in 100 mL water in a beaker, stirred and left to soak for one hour. The scum, which contained organic debris and dead spores, was removed from the surface of the beaker. The soil suspension was decanted into a set of four sieves with different mesh sizes (53µm, 91µm, 250µm and 700µm). More water was added and decanting repeated several times until the liquid was clear. The fraction collected in each of the sieves was washed and transfered to a beaker. After adding some water and letting the sand particles settle, the fraction was again passed through the same sieve. Each fraction collected was transferred to a nematode dish and examined under a dissecting microscope at 16x and 40x magnification for the number of spores which were counted with a hand tally counter.

3.4.2 *Most Probable Number*

The method of Porter (1979) and Wilson and Trinick (1982) was used to estimate the number of infective propagules with a trap plant technique. A ten-fold dilution series to 10^{-6} was prepared for each soil sample by thoroughly mixing the sieved, but otherwise untreated, soil with autoclaved (120°C for 40 minutes) soil from the same site. There were eight replicates for each dilution. Each replicate portion (40 g) of each dilution was placed in a compartment of a plastic propagation tray. The dilution series of each soil was put in a separate tray. A seedling of clover (*Trifolium subterraneum* L. cv. Mount Barker) was planted into each compartment which had drainage holes covered with a piece of filter paper. The soil was wetted to field capacity with deionized water. Six weeks after transplanting, the root system from each plant was harvested, washed and stained. The presence of mycorrhizal infection in every compartment was examined under a dissecting microscope. The number of mycorrhizal propagules in the undiluted soils was determined from the number of compartments containing infected roots of each dilution of soil (Alexander 1982), using a program in Basic (Cuthbertson 1986).

3.5 Surface sterilization of seeds

The seeds of the cereals were placed in a glass vial into which 0.5% NaOCl was added to cover the seeds. After two minutes, the solution was decanted and replaced by sterile distilled water. This procedure was repeated once.

3.6 Drying plant materials

At harvest, shoots and roots were separated. After the fresh weight of the shoots and roots were recorded, the plant materials were dried in a forced draft oven for 24 hours at 80°C and weighed to determine dry weight.

3.7 Separating roots from soil

Soil containing plant roots was put onto a 2 mm sieve layered with a piece of 0.5 mm sieve. The sieve with the soil was soaked in a bucket filled with water for at least 5 minutes, when the soil became soft and started to fall apart. Soil particles were carefully separated from roots by washing with tap water. The roots were placed in a plastic tray containing deionized water to remove the remaining soil particles and organic debris, and this was repeated once. The deionized water was changed for every washing.

3.8 Root staining

Clearing and staining of roots were carried out according to the modified method of Phillips and Hayman (1970), to distinguish between host plant and fungal tissues. Root systems were cut into 2 cm segments. Root segments of each sample were transferred to a glass vial, to which sufficient 10% KOH solution was added to cover the roots. The vials were left at room temperature for 1 day for young roots to 3 days for old roots. After clearing, roots were collected onto a fine-mesh sieve, and rinsed with running water. The roots were soaked in dilute HCl solution for 2 seconds, then rinsed again with tap water. The roots were returned to the glass vials and stained by soaking them in 0.01% trypan blue in lactoglycerol for 20 hours at room temperature. After the roots were stained, they were washed with tap water to remove the remaining staining solution. The root samples were covered with 50% glycerol and stored in the vials.

3.9 Measurement of mycorrhizal infection

The grid line intersect method, as described by Giovannetti and Mosse (1980), was used to estimate total root length and the proportion of root length infected. The sample of stained roots was spread on a petri dish under which a grid of lines was marked to form squares of 5 mm sides. Horizontal and vertical lines were scanned with dissecting microscope with x 16 and x 40 magnifications. The presence and absence of infection were recorded for every intersection between roots and lines. An intersection was counted infected when it contained vesicles, arbuscules or internal hyphae. A compound microscope with x 100 and x 400 magnifications was used sometimes to ensure the structures of the fungi. The total number of intersections can be used for calculating total root length using formula generated by Newman (1966),

$$R = \pi NA/2H$$

where R = estimated total root length,

N = number of intersections between roots and straight lines,

A = area in which roots are distributed,

H = total length of straight lines.

Using grid lines with squares of 0.5 cm sides, estimated root length was calculated as R = (11/28) x N (Marsh 1971). Percentage of root length infected can be calculated after counting the number of intersections with infected roots and the total number intersections.

3.10 Determination of phosphorus in plants

To determine the content of P in plant tissues, there are two steps that have to be done.

3.10.1 Digestion

An acid mixture was prepared by blending 500 mL concentrated nitric acid with 83 ml of 70-72% perchloric acid. Plant material (150 mg) was placed in a 50 mL digetion tube and 4 ml of acid mixture added and allowed to stand overnight in the fume hood. Tubes with the plant materials were put into Tecator^{R} digestion block. The temperature and time needed for digestion were programmed as follows.

Step	Temperature (°C)	Ramp (minute)	Time (minute)
1	150	10	20
2	180	10	20
3	225	10	60
4	300	10	5

After finishing the digestion the digests were diluted to 50 ml.

3.10.2 Spectrophotometric analysis

The method used was modified from Hanson (1950). Mixed reagent was prepared by stirring together 1 L concentrated nitric acid, 1 L 0.25% ammonium vanadate and 1 L 5% ammonium molybdate solutions. Standard curves of P were obtained by preparing standard solutions over the concentration range 0 - 5 µg P mL^{-1}. A 20 mL aliquot of diluted digest was transferred to a 50 mL volumetric flask, and 4 mL mixed reagent was added. The solution was diluted to volume with distilled water and mixed. The reading on a spectrophotometer (LKB Biochrom- Ultrospect 4050) at 390 µm was taken 30 minutes after mixing.

3.11 Calculation of plant growth response

Response of plants to mycorrhizas was expressed in % as follows.

$$\text{Response} = \frac{\text{Shoot dry weight (M) - Shoot dry weight (NM)}}{\text{Shoot dry weight (NM)}} \times 100$$

where M and NM stand for mycorrhizal inoculated and non-inoculated plants, respectively. A similar expression was used to calculate plant response to P addition. The formula is different from the one for 'mycorrhizal dependency' (Menge *et al.* 1978a) which is determined by the percentage of dry weight of mycorrhizal plants over the dry weight of non-mycorrhizal plants.

Chapter Four

PHOSPHORUS EFFICIENCY OF THREE CEREALS AS RELATED TO INDIGENOUS MYCORRHIZAL INFECTION

4.1 Introduction

Endomycorrhizal fungi can infect many agricultural crops, including cereals (Saif and Khan 1977, Buwalda *et al.* 1985). Beneficial responses of cereal crops, in terms of shoot or grain yield, to inoculation with VA mycorrhizal fungi in P-deficient soils have been reported for both wheat (Khan 1975, Bertheau *et al.* 1980, Thompson 1990) and barley (Clarke and Mosse 1981, Jakobsen 1983).

There is much evidence to show that the VA mycorrhizal symbiosis may increase the amount of phosphorus taken up by cereal plants (Jakobsen 1983, Thompson 1990), implying that the mycorrhizal plants are more efficient in uptake of P than non-mycorrhizal plants. Furthermore, VA mycorrhizal infection may also result in higher P concentration in plant tissue (Buwalda *et al.*1985, Thompson 1990), suggesting that mycorrhizal symbiosis results in less efficient utilization of P by the plants. VA mycorrhiza may also influence other parameters of P efficiency, *e.g.* agronomic P efficiency.

Experiments to identify and select for P-efficient cereal cultivars are often done at a number of field sites. It might be expected that differences in mycorrhizal infection of the cereals tested at different locations could lead to inconsistent results in the assessment of P efficiency in the same cultivars. This chapter presents results of preliminary investigations on the influence of mycorrhizal infection on the determination of P efficiency in cereals. The aims of the experiments were to determine a) whether two soils that have been used previously to test the P efficiency in cereals have the same mycorrhizal propagule density, b) whether the extent of the infection affects the determination of P efficiency in soils from these sites, c) whether wheat, barley and rye are similarly infected by the indigenous mycorrhizal fungi, and d) the extent to which addition of P affects the proportion of root length infected. The cereals used in this experiment, differed in the efficiency of P uptake and P utilization, on the basis of previous experiments (Wheeler unpublished).

4.2 Materials and Methods

4.2.1 Experiment 4.1: Populations of VA mycorrhizal propagules

This experiment aimed to compare the density of VA mycorrhizal propagules in the two soils from Avon and Peake in the cereal belt of South Australia. The populations of propagules of mycorrhizal fungi in the soils were assessed using the spore counting (Gerdemann and Nicolson 1963) and most probable number methods (Porter 1979, Wilson and Trinick 1982), as described in Chapter 3. The trays of plants for MPN were placed in a controlled environment room (14 h light and 10 h dark photoperiod; 22°C day/17°C night thermoperiod; 300 $\mu E\ m^{-2}\ s^{-1}$ light intensity).

4.2.2 Experiment 4.2: Rates of infection in the three cereals

4.2.2.1 Experiment 4.2a.

This experiment was conducted to study the rates of infection of indigenous VA mycorrhizal fungi in cereals. Three cereals, *viz* wheat (*Triticum aestivum* L. cv. Spear), barley (*Hordeum vulgare* L. cv. Galleon) and rye (*Secale cereale* L. cv. S. A. Commercial), were grown in both Avon and Peake soils with three replications. These cultivars differ in response to applied phosphorus

(Wheeler, unpublished). The seeds of the cereals were surface sterilized and germinated on moist filter paper at room temperature. After three days one seedling was planted per pot containing 200 g of soil. The pots were placed in the same growth chamber and same conditions as Experiment 4.1 and randomized periodically. Plants were harvested 20, 30 and 40 days after transplanting. Fresh and dry weights of shoots and roots were recorded. Percentage of root length infected was determined as described in Chapter 3.

4.2.2.2 Experiment 4.2b.

An experiment similar to 4.2a was set up with three replications to investigate the rates of VA mycorrhizal infection of wheat and barley at low soil temperature. Plants were grown in pots containing 300 g of Peake soil, placed in a water bath thermostatically controlled at 10°C. The mean air temperature during this experiment was 26.6/12.6°C (day/night). The cereals were harvested 14, 28 and 42 days after transplanting.

Soils were maintained at a gravimetric water content of 110 g kg^{-1} by adding deionized water every day. At harvest time, the shoots were weighed and dried to determine the dry weight. The roots were washed and the fresh weight was measured. Subsamples of fresh roots were taken and stained for determination of percentage of root length infected using methods described in Chapter 3.

4.2.3 Experiment 4.3. Response to P applied to soil

The aim of this experiment was to study the effect of rates of applied P on P efficiency, VA mycorrhizal infection and the growth of wheat, barley and rye in Avon and Peake soils. The three cereals used in this experiment were the same as in Experiment 4.2a. The P treatments were 0, 5, 15, 30 and 60 mg P kg^{-1}

soil, added as KH_2PO_4. A completely randomized design with three replications was used for the combination treatments (3 cereals x 5 P x 2 soils).

Mineral nutrients (composition detail in Chapter 3) were added to the soil to ensure that nutrients other than P did not limit plant growth. The seeds were germinated, and three three-day old seedlings were planted into plastic-lined PVC pots containing 3 kg of soil. All pots were placed in temperature controlled waterbaths maintained at 10°C. Deionized water was added daily to bring the water content of the soil to field capacity (11%).

Plants were harvested 7 weeks after transplanting. Fresh and dry weight of shoots and roots were recorded and the proportion of the root length infected by the indigenous mycorrhizal fungi were determined as described in Chapter 3.

4.3. Results

4.3.1 Number of mycorrhizal propagules (Experiment 4.1)

The results in Table 4.2 show that both the MPN and wet-sieving and counting methods gave a higher number of mycorrhizal propagules in Peake soil than in Avon soil. Particularly in the Peake soil, the number of mycorrhizal propagules based on spore number was higher than that based on MPN. Anatomically, the dominant fungus that formed mycorrhiza in the root tissue in both soils was the fine endophyte, *Glomus tenue* (D J Read pers. comm.), while morphologically (observed under dissecting microscope), the dominant spore type present was *Glomus albidum* (P McGee pers. comm.).

Table 4.2. **(Experiment 4.1) Number of mycorrhizal propagules in Avon and Peake soils determined by MPN and wet sieving and counting methods.**

	Method	
Soil	MPN (propagules g^{-1} soil ± SE)	Wet-sieving and counting (spores g^{-1} soil ± SE)
Avon	0.9 ± 0.4	4.0 ± 1.2
Peake	28 ± 12	287 ± 3

4.3.2 VAM infection and plant growth (Experiment 4.2)

4.3.2.1 In the growth room (Experiment 4.2a)

The levels of infection of wheat, barley and rye by indigenous mycorrhizal fungi under growth room conditions are presented in Fig. 4.1. In Avon soil, VA mycorrhizal infection of the three cereals increased with time. However, there was no significant increase in the level of infection of wheat between 30 and 40 days after transplanting. Twenty days after transplanting, there was no difference in the percentage of root length infected among the three cereals tested in Avon soil, but subsequently wheat and barley had a significantly greater percentage of infected root than had rye. Only at 30 days did wheat have a significantly higher percentage of root length infected than barley.

In Peake soil, infection occured more rapidly and the percentage of root length of wheat and barley infected with mycorrhizal fungi was not significantly different at the three times of harvest. However, the level of infection in rye significantly increased with time, and was higher than that of barley at the final harvest.

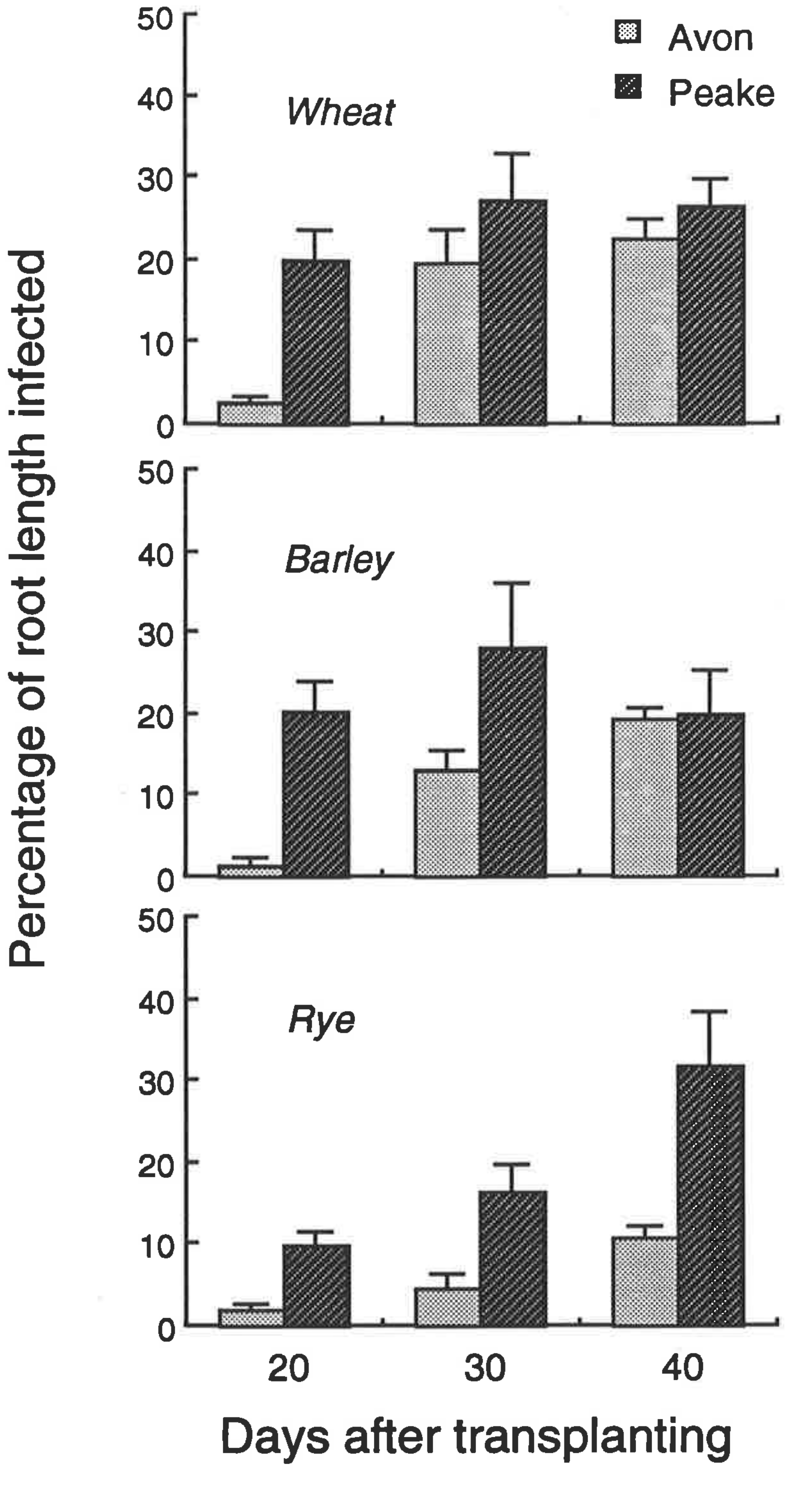


Figure 4.1 **(Experiment 4.2a) VA mycorrhizal infection of wheat (cv. Spear), barley (cv. Galleon) and rye (cv. S. A. Commercial) grown in Avon and Peake soils under growth room conditions when measured at 20, 30 and 40 days after transplanting. Vertical bars represent standard errors of the means, n = 3.**

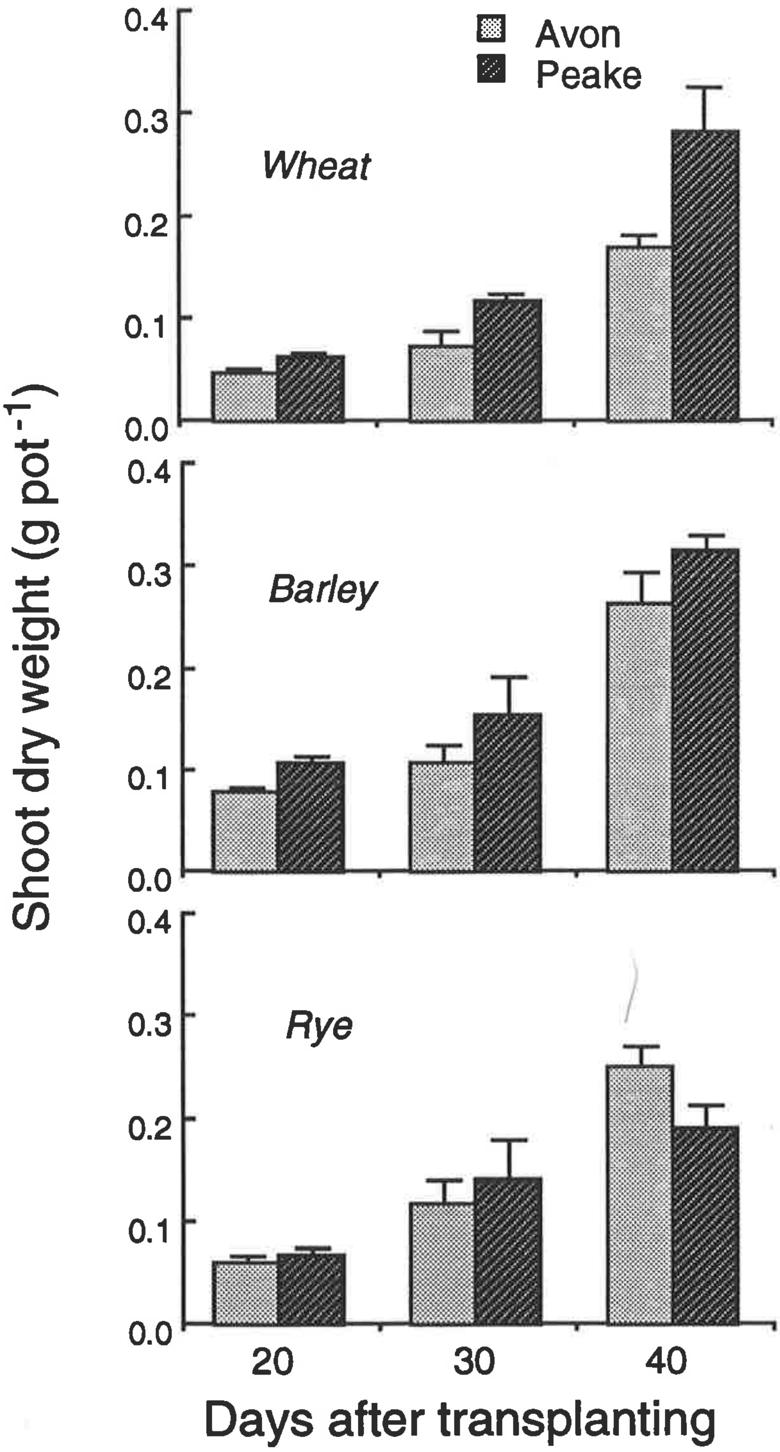


Figure 4.2 **(Experiment 4.2a) Shoot dry weight of wheat (cv. Spear), barley (cv. Galleon) and rye (cv. S. A. Commercial rye) grown in Avon and Peake soils under growth room conditions when measured at 20, 30 and 40 days after transplanting. Vertical bars represent standard errors of the means, n = 3.**

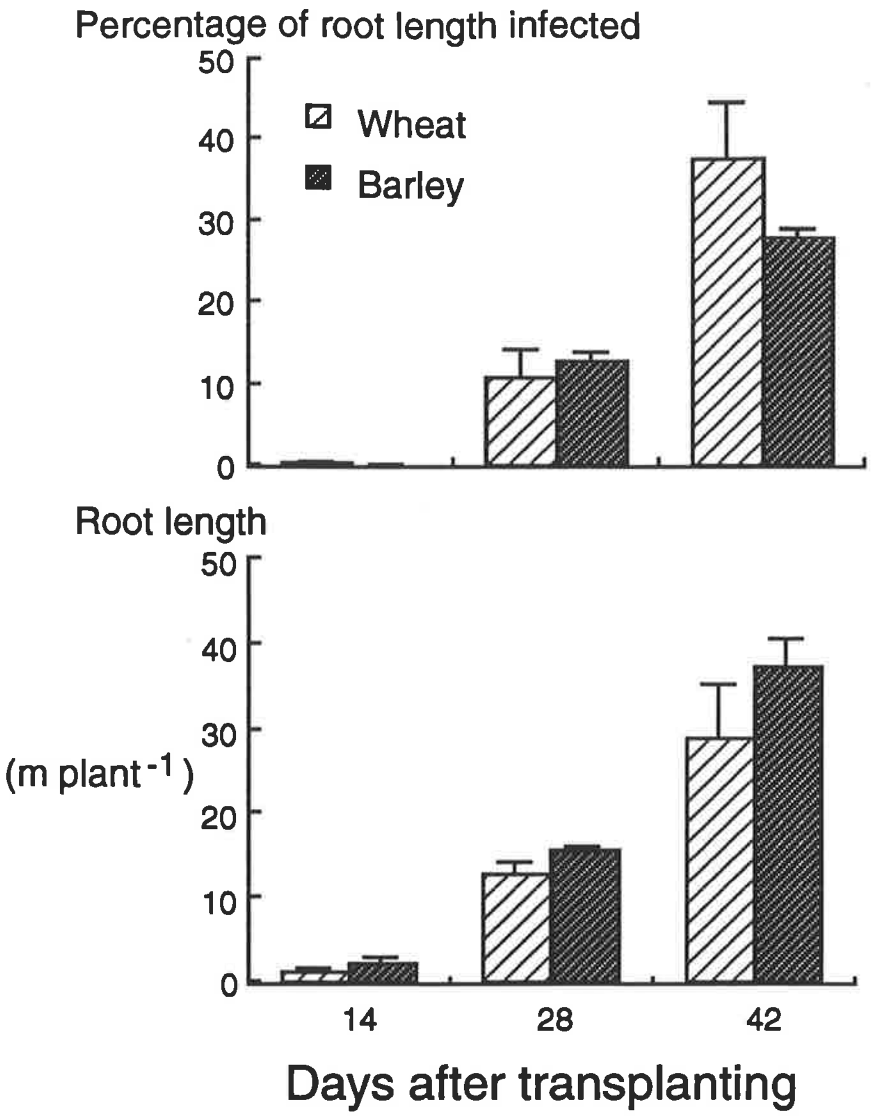


Figure 4.3 **(Experiment 4.2b) VA mycorrhizal infection and root length of wheat (cv. Spear) and barley (cv. Galleon) grown in Peake soil at a soil temperature of 10°C when measured at 14, 28 and 42 days after transplanting. Vertical bars represent standard errors of the means, n = 3; if no bar is shown, standard error is smaller than the line.**

Figure 4.2 shows that, except for wheat, plant growth during 20 and 30 days after transplanting was not significantly different between plants grown in Avon and Peake soils. By 40 days, however, the three cereals showed differential growth responses. Wheat and barley grown in Peake soil had accumulated 67 and 20% more dry matter, respectively, than plants grown in Avon soil. Shoot dry matter of rye grown in Peake soil, however, was 25% less compared with the plants grown in Avon soil.

4.3.2.2 *At a soil temperature of 10°C (Experiment 4.2b)*

The extent of VA mycorhizal infection and root length of wheat and barley grown in Peake soil with a soil temperature of 10°C is given in Fig. 4.3. No mycorrhizal infection was detected in barley roots 14 days after transplanting. For the two cereals, the percentage of root length infected was a delayed although it increased with time. Only at 42 days after transplanting was the level of infection in wheat higher than that in barley. At all times of observation, root length of barley tended to be greater than wheat, although the difference was significant ($P < 0.05$), only at 28 days after transplanting.

4.3.3 Response to P applied to soil (Experiment 4.3)

Fig. 4.4 shows the percentage of root length infected in wheat, barley and rye grown in Avon and Peake soils at a soil temperature of 10°C as affected by the rates of P. These results support the previous data which showed that the percentage of infection was higher in Peake than in Avon soil. Without added P, there was no difference in the percentage infection among the three cereals when grown in Peake soil, but for Avon soil the percentage of roots infected was higher for wheat than barley or rye. Adding 5 mg P kg^{-1} greatly decreased infection in all three cereals. There was hardly any infection after addition of 30 or 60 mg P kg^{-1} soil. Application of the lowest amount of P tested completely inhibited the mycorrhizal infection in rye grown in Avon soil.

Shoot growth of wheat and rye was not affected by the origin of the soils at any concentrations of P applied (Fig. 4.5). Considerable reduction in dry matter of shoots was only observed in barley grown in Peake soil compared with plants grown in Avon soil.

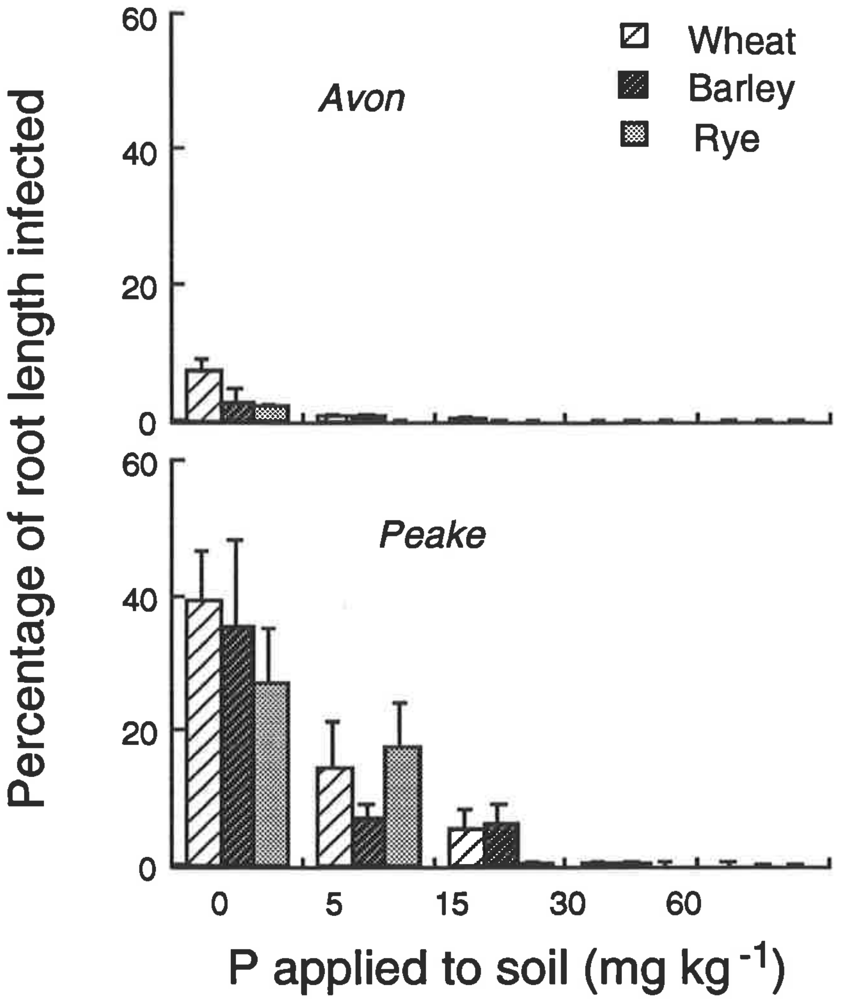


Figure 4.4 **(Experiment 4.3) The effect of P applied to Avon and Peake soils on VA mycorrhizal infection of wheat (cv. Spear), barley (cv. Galleon) and rye (S. A. Commercial) grown at a soil temperature of 10°C for 49 days. Vertical bars represent standard errors of the means, n = 3; where bar is not shown, standard error is smaller than the line.**

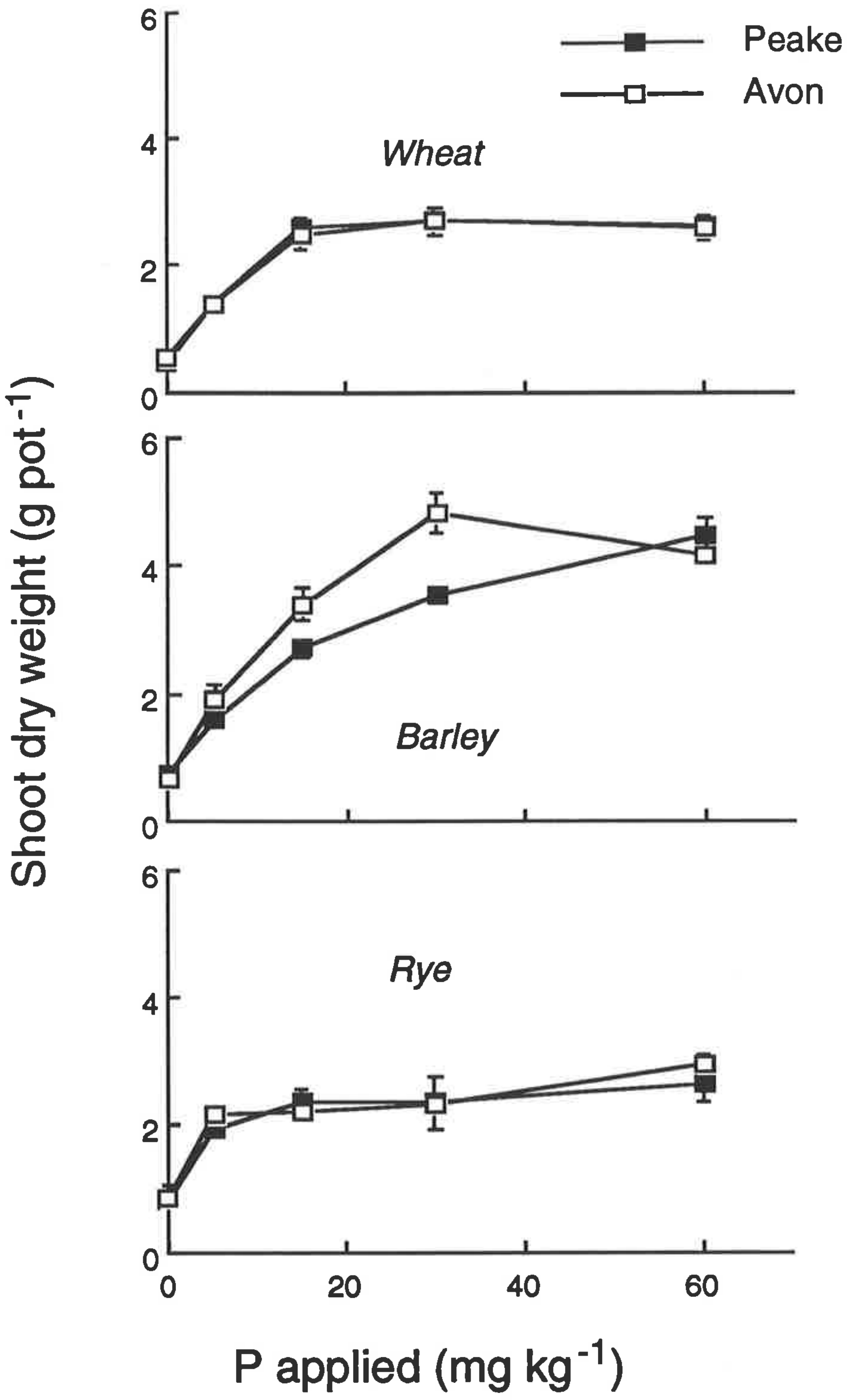


Figure 4.5 **(Experiment 4.3) Shoot dry weight of wheat (cv. Spear), barley (cv. Galleon) and rye (cv. S. A. Commercial) as affected by P applied to Avon and Peake soils. The plants were grown at a soil temperature of 10°C for 49 days. Vertical bars represent standard errors of the means, n = 3; if no bar is shown, standard error is smaller than symbol.**

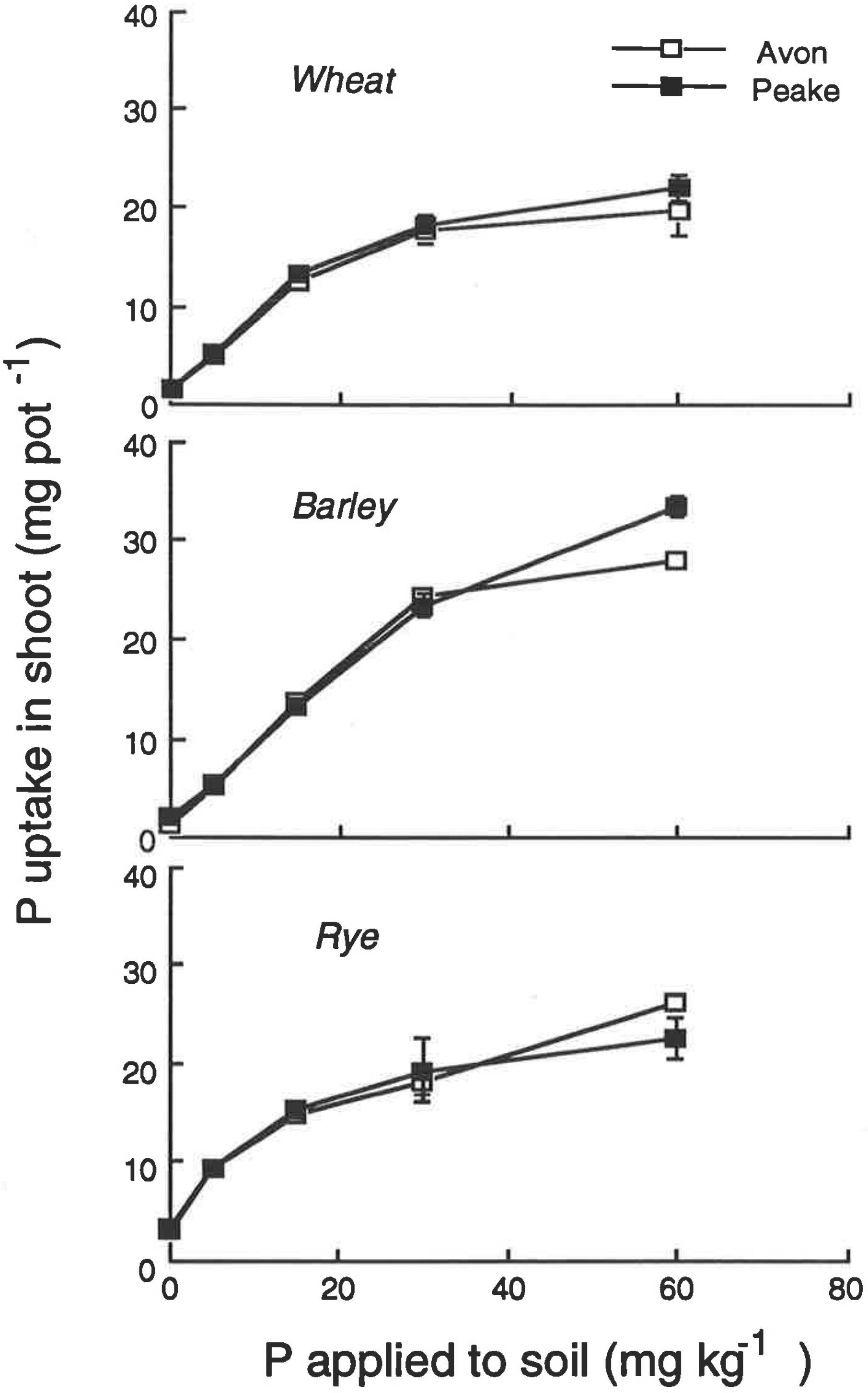


Figure 4.6 **(Experiment 4.3) Uptake of P in shoots of wheat (cv. Spear), barley (cv. Galleon) and rye (cv. S. A. Commercial) as affected by P applied to Avon and Peake soils. The plants were grown at a soil temperature of 10°C for 49 days. Vertical bars represent standard errors of the means, n = 3; if no bar is shown, standard error is smaller than symbol.**

P uptake in the shoots of the cereals was increased by the addition of P to the soil (Fig. 4.6). The uptake began levelling off when the amount of P added was 30 mg kg^{-1}. Barley had higher uptake of P in the shoots than wheat and rye when P was applied at 30 or 60 mg P kg^{-1}. Only when P was applied at 5 mg P kg^{-1} was P uptake by rye higher than that by wheat or barley in both soils. However, when the comparison was based on the concentration of P in the shoots (Fig. 4.7), barley grown in Peake soil had higher values than barley in Avon soil. Concentration of shoot P in rye was generally higher than in wheat or barley.

Fig. 4.8 shows the proportion of root length infected, shoot P concentrations and total uptake of P in wheat, barley and rye grown in Avon and Peake soils without addition of P. In rye, there was no correlation between percent of root length infected and shoot P concentration or total P uptake. However, barley and wheat showed a positive correlation between the extent of mycorrhizal infection and the shoot P concentration. Root lengths of the three cereals grown in growth room for twenty days after transplanting are presented in Fig. 4.9. Barley and rye had longer roots than wheat.

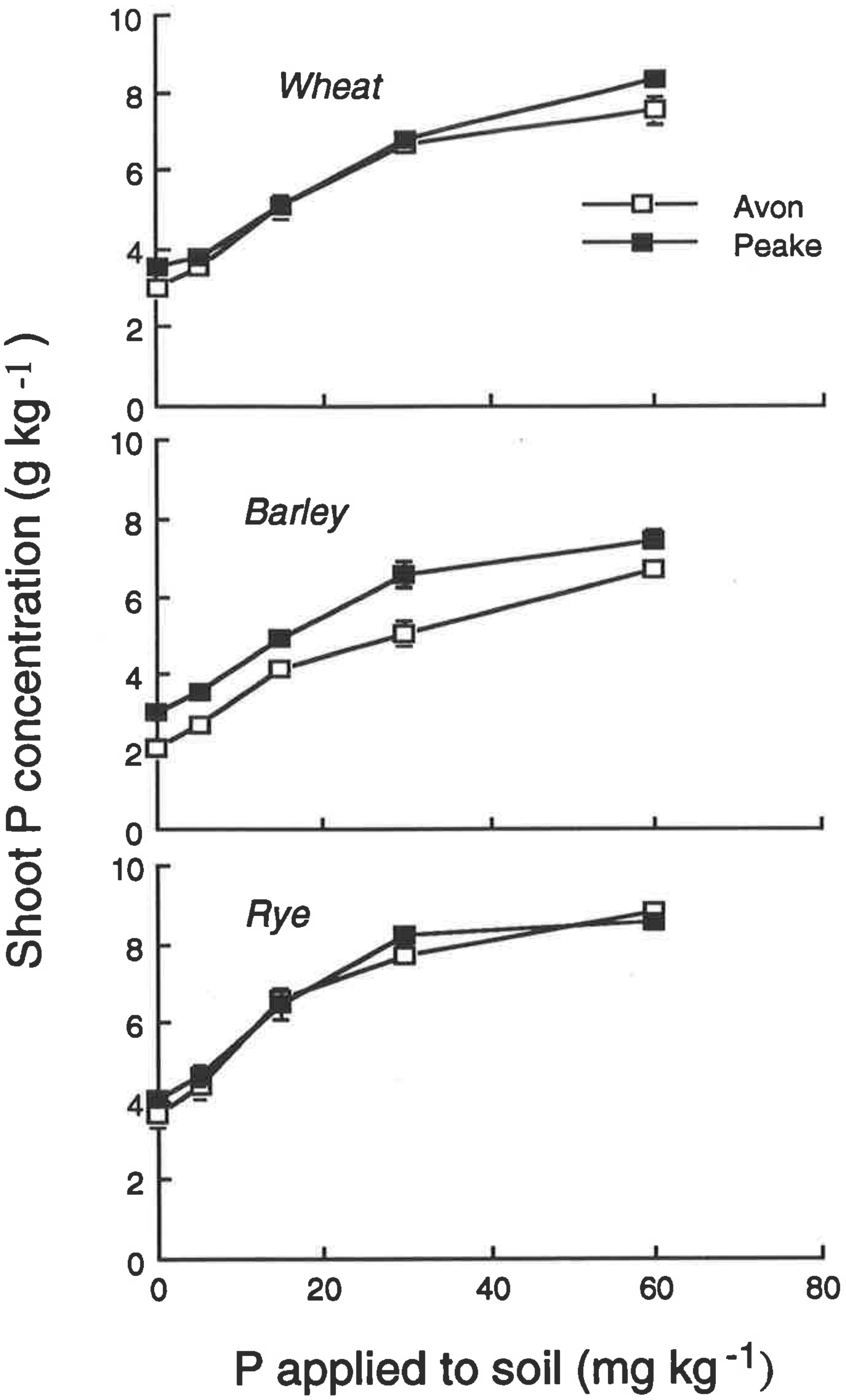


Figure 4.7 **(Experiment 4.3) Concentration of P in shoots of wheat (cv. Spear), barley (cv. Galleon) and rye (cv. S. A. Commercial) as affected by P applied to Avon and Peake soils. Vertical bars represent standard errors of the means, n = 3; if no bar is shown, standard error is smaller than symbol.**

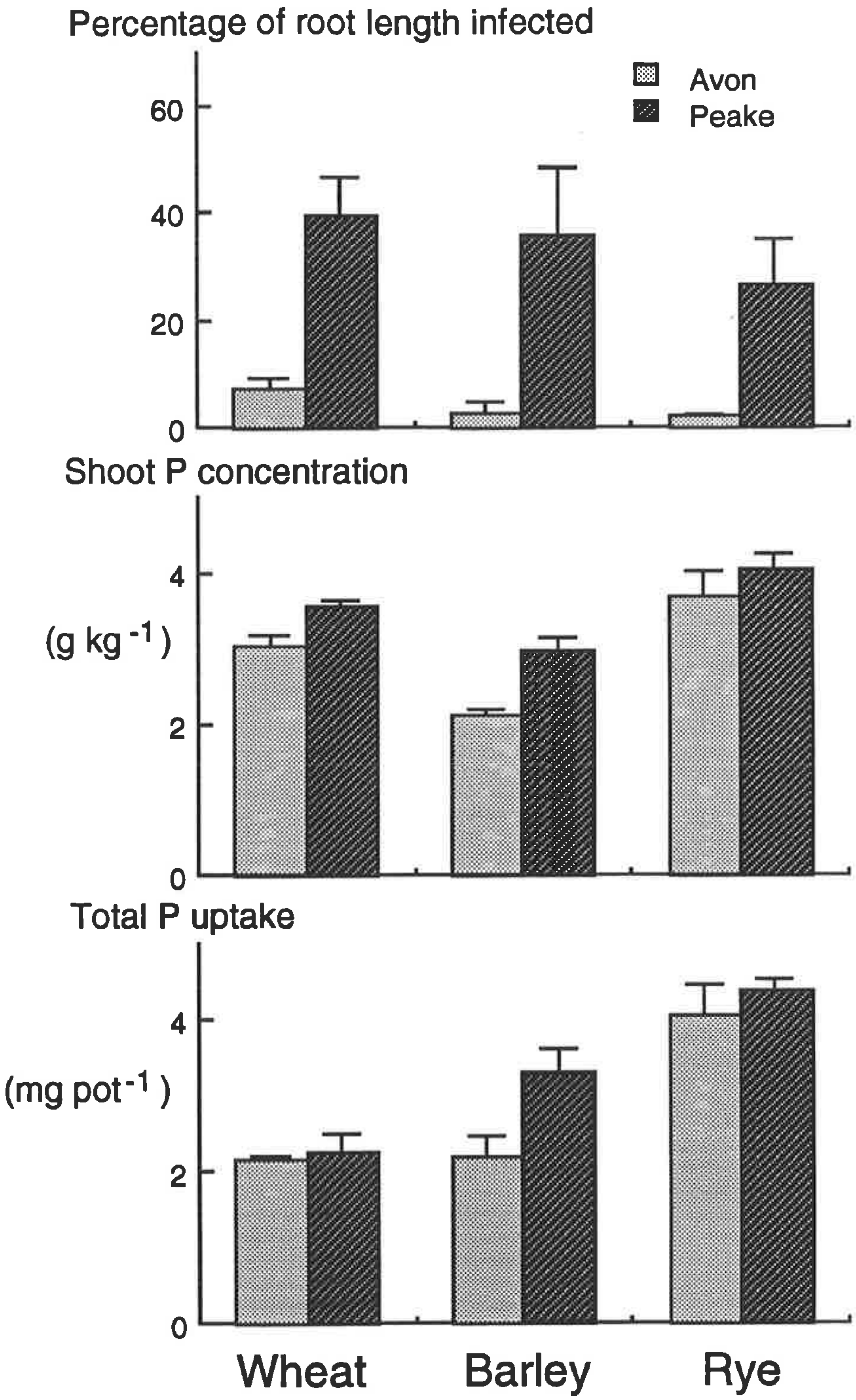


Figure 4.8 **(Experiment 4.3) Percentage of root length infected, shoot P concentrations and total uptake of P in wheat (cv. Spear), barley (cv. Galleon) and rye (S. A. Commercial) grown in Avon and Peake soils without P amendment after 49 days. Plants were grown at a soil temperature of 10°C. Vertical bars represent standard errors of the means, n = 3; if no bar is shown, standard error is smaller than symbol.**

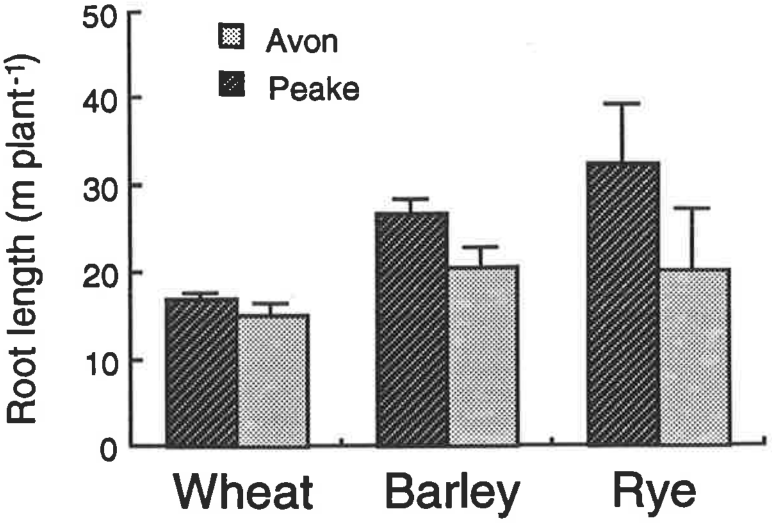


Figure 4.9 **(Experiment 4.2a) Root length of wheat (cv. Spear), barley (cv. Galleon) and rye (cv. S. A. Commercial) grown in Avon and Peake soils after 20 days under growth room conditions. Vertical bars represent standard errors of the means, n = 3.**

4.4 Discussion

In general, the results of these experiments have revealed that mycorrhizal infection by indigenous fungi increased the efficiency of P uptake (mg pot $^{-1}$) by barley plants by about 50 %, but did not affect this aspect of efficiency in wheat and rye. In contrast, mycorrhizal infection of wheat and barley decreased the efficiency of P utilization, but again had no effect on rye.

The extent of the mycorrhizal infection of the three cereals was much less when the plants were grown in Avon soil than in Peake soil. The level of infection was much lower when the plants were grown at soil temperature of 10°C in water baths compared with higher temperatures in the growth room. For the purpose of discussion, the cereals grown in Avon soil at soil temperature of 10°C (see Fig. 4.8) can be regarded as poorly-infected (non-mycorrhizal) plants, whereas the plants grown in Peake soil at the same temperature were highly-infected (mycorrhizal) plants. The high percentage of root length infected in all the three cereals when grown in Peake soil reflects the high density of indigenous mycorrhizal propagules. This high propagule density was convincingly demonstrated by both MPN and wet-sieving and counting methods.

The results of this series of experiments have revealed that MPN and wet-sieving and counting methods for measuring the population of mycorrhizal fungi gave higher numbers of VA mycorrhizal propagules in Peake soil than in Avon soil. The greater number of propagules in Peake soil may be due to the fact that the land at Peake had been used for farming, while the Avon soil is under the natural vegetation of grasses and eucalypts. The number of propagules based on spores was much higher than the number estimated by the MPN method. This suggests that many of the spores found in both soils were either dormant or dead. Moreover, in the Peake soil the number of spores was relatively large, but the dominant fungus infecting the cereal roots was fine endophyte which does not form spores that can be enumerated by the wet sieving technique. Porter (1979) suggested that with the presence of fine endophytes, the MPN method would give a more realistic estimate of infective propagules than spore number and the results of this chapter have confirmed this.

The difference between estimates of populations by MPN and wet-sieving and counting methods emphasize the need for standardization of methods, especially when working with soil samples from the field. In addition to standardization of plant, time of harvest and temperature (see Wilson and Trinick 1982), the method of handling the soil from the field must be considered because physical disturbance of the soil may decrease the infectivity of mycorrhizal fungi in soil (Evans and Miller 1988, Jasper *et al.* 1989). The Peake soil had been subjected to conventional tillage, so that hyphal networks would have been broken up prior to sowing. Estimates of propagule density by the present results are, therefore, likely to be realistic with respect to crops grown at that site. However, where minimum tillage is practiced, MPN values obtained by soil dilution and mixing are likely to underestimate the infectivity of the soil.

The percentages of root length infected in wheat and barley (30-40%) were similar to the percentages reported by Buwalda *et al.* (1985), and Thompson (1990) for wheat and Jensen (1983) and Powell (1981) for barley. However, Tilak and Murthy (1987) observed that infection in roots of hull-less barley was much higher (80-90%) than that obtained in this experiment. The difference may be due to differences in the cereal cultivars or in the population of VA mycorrhizal fungi in the soil, or to a difference in the method of assessment.

The results of this investigation contrast with those of Hetrick *et al.* (1984) who found that winter wheat plants failed to be infected by VA mycorrhizal fungi at 10°C. It is possible that this contradictory result may be due to the difference in the cultivars or fungi used in the experiments. Alternatively, the different methods of controlling the temperature may have affected the results. Hetrick *et al.* (1984) controlled air temperature rather than root temperature, and this may have had more severe effects on plant growth and production of photosynthate, with consequent effects on mycorrhizal formation.

Rate of root growth, as well as rate of infection by the mycorrhizal fungi, affects the percentage of the root system infected. Barley and rye had longer root systems than wheat. In these experiments, and particularly in the Avon soil, it was found that rye had a significantly lower proportion of root infected by VA mycorrhizal fungi than either wheat or barley. These differences between species may have resulted from differences in the rate of root growth, or the rate of fungal growth within the root, or the susceptibility of the species to fungal infection. Further experiments are required to determine the relative importance of the different processes. However, differences in root growth may account for the higher levels of infection in wheat grown in Avon soil since root length of wheat was less than for barley or rye in that soil.

Root infection of the cereals by VA mycorrhizal fungi was much reduced by increasing the amount of P applied to soil. The effect of P addition on the development of mycorrhizal infection in plant roots has been well established (Harley and Smith 1983). As little as 5 mg P kg^{-1} soil applied reduced the proportion of roots infected. However, Bolan *et al.* (1984a) found that additions up to 30 mg P kg^{-1} increased the percentage infection of subterranean clover by indigenous fungus. It is most likely that the differences in the amount of available P in soils used in the experiments may account for the contradictory results. The other possible reasons for this difference are the susceptibility of the mycorrhizal fungi (Bolan *et al.* 1984a) and the less sensitivity of mycorrhizal clover (see data of Oliver *et al.* 1983, C. Botta, pers. comm.) to P addition. In relation to the P nutrition of cereals, it is considered important to investigate and screen the VA mycorrhizal fungi which are still infective in spite of the addition of P to soil (Abbott and Robson 1982). The significance of having that kind of fungus is to minimize the use of P in soil for better plant growth and development.

These experiments assessed the efficiency of P uptake in terms of the amount of P that was taken up by the whole plants (mg P pot $^{-1}$). The method did not permit calculation of the rates of P uptake by the plant roots (inflow; mol cm $^{-1}$ s $^{-1}$) because the plants were harvested only once. Expressing the P uptake in terms of inflow would have given a clearer picture of mycorrhizal effects on efficiency of uptake. It is likely that mycorrhizal plants (particularly in soil without P amendment) would have higher inflows than non-mycorrhizal plants (see Sanders and Tinker 1973, Smith 1982) which can result in greater total uptake.

Previously published reports with the clearly stated aim of studying the effects of VA mycorrhizal infection on P efficiency have mainly been on sorghum (Raju *et al.* 1987, Raju *et al.* 1990). Sorghum is a C-4 plant which, according to Hayman (1983), is photosynthetically more efficient and therefore more strongly dependent on mycorrhizas than C-3 plants, such as wheat, barley and rye. This difference makes it difficult to compare our data with those of Raju *et al.* (1987, 1990). Although Hetrick *et al.* (1990) found that warm-season (C-4) tallgrass prairie grass was more responsive to mycorrhiza than cool-season (C-3) grass in terms of plant dry matter yield, which is in agreement with the present results for barley, they did not show the P status of the plants so that comparisons in terms of P efficiency cannot be made.

It appears from the present study that response of the cereals at low P supply to high early infection in Peake soil depends on the soil temperature. In comparison with barley, wheat may grow better in relatively high temperature in the presence of mycorrhiza. Lack of response to mycorrhiza in cereals may be attributed to low soil temperatures which restrict fungal metabolism (Hetrick *et al.* 1984) and host plant metabolism.

It is clear from results of this chapter that VA mycorrhizal infection may change the relative performance of cereal species in terms of several definitions of P efficiency. Poorly-infected (non-mycorrhizal) wheat and poorly-infected (non-mycorrhizal) barley were not different in their efficiency in uptake of P. However, when both were highly-infected (mycorrhizal) the barley plants were more efficient in the uptake of P than wheat. Fine endophyte has also been reported to increase P uptake and growth of *Lolium perenne* (Crush 1973) and *Podocarpus totara* (Baylis 1969). Therefore, the status of cereal species in terms of P efficiency can vary not only according to the definitions of P efficiency used but also depending on the extent of mycorrhizal infection in those plants.

The present results also show that mycorrhizal infection does not seem to influence the efficiency of P uptake and efficiency of P utilization in rye. Rye clearly has a very low dependency on mycorrhizal infection. Some of the differences among the cultivars can be explained in terms of root characteristics. Although the root length of rye and barley were not significantly different, rye has an extensive development of root hairs, which may explain the low dependency. This is in accordance with the hypothesis of Baylis (1975) that plants with extensive root systems, such as graminaceous plants, are able to exploit much greater volumes of soil and generally benefit less from mycorrhiza than plants with coarse roots.

4.5 Conclusion

In summary, the cereals examined in this chapter were all infected by VA mycorrhizal fungi, even at a low soil temperature. The extent of the infection varied with species, propagule density, and P addition, and affected the P efficiency of the plants. This implies that there is a complex pattern of interaction which impacts on evaluation of P efficiency of uptake and utilization in cereals. Present results emphasize the potential contribution from mycorrhizal infection to the determination and selection of P-efficient cereals. Hence breeding programs should take into account the contribution made by mycorrhizal fungi to P nutrition under field conditions and the possible variations in soils with different density of infective propagules and responses in different cultivars.

Chapter Five

P EFFICIENCY OF BARLEY INFECTED BY *GLOMUS INTRARADICES*

5.1 Introduction

The results in Chapter 4 showed various responses to soil with high early mycorrhizal infection by the three cereals grown in a growth room where the temperature were kept at about 20°C. However, lack of response to VA mycorrhizal infection in plant growth was obtained at low soil temperature. Dry matter of barley, furthermore decreased when grown in Peake soil, although the concentration and the uptake of P in barley grown in the soil was higher. These results lead to the selection of barley as the object of study for this chapter. Growth responses of barley plants to VA mycorrhizal inoculation have been reported (Jensen 1982, Champawat *et al.* 1987). However, Owusuh-Bennoah and Mosse (1979) and Jakobsen and Andersen (1982) have also found that mycorrhizas did not increase growth of barley plants. The discrepancy may be due to different cultivars used.

Most results of other work where mycorrhizal inoculation increased plant growth were derived from studies with coarse fungal endophytes (Jensen 1982, Champawat *et al.* 1987). The mycorrhizal fungi discussed in the experiments in Chapter 4 came from field soils which are dominated by fine endophyte. Furthermore, the soil temperature was kept at 10°C. The lack of growth responses to mycorrhizal infection at low soil temperature could be the result of combined effects of inefficient fine endophytes and low metabolic activity at low temperature. This chapter aims to investigate the effect of mycorrhizal infection by a coarse endophyte on growth and P efficiency of barley plants grown in P deficient soil.

5.2 Materials and Methods

5.2.1 Experiment 5.1

The aim of this experiment was to investigate the effect of mycorrhizal infection by *Glomus intraradices*, a coarse endophyte, on growth and P efficiency of two barley cultivars. This VA mycorrhizal fungus was chosen because it was used also in studying the genetic control of the ability of mycorrhizal fungi to infect barley roots in the Department of Soil Science, University of Adelaide. The source of fungal inoculum was "Nutrilink" which was a commercial inoculum containing ± 900 spores per g of *Glomus intraradices* (L M Haugen pers. comm.).

In this pot experiment Avon soil was used as the medium for growing barley plants. Chemical properties of the soil have been described in Chapter 3. The soil was steamed sterilized and 1.4 kg of the soil was put in each plastic pot.

Two barley (*Hordeum vulgare* L.) cultivars, *i.e.* Galleon and Clipper were used in this experiment. Galleon and Clipper were chosen since they have been shown in field studies (Wheeler unpublished) to be, respectively, inefficient and moderately efficient in terms of agronomic P efficiency. Galleon was used in the experiments discussed in Chapter 4. The barley seeds were surface sterilized before being germinated.

Half of the pots received 3 g of mycorrhizal inoculum which was layered 5 cm below the surface. The other half, for non-mycorrhizal pots, were given 3 g of autoclaved inoculum per pot. Two three-day-old seedlings of barley were transplanted into each pot. The 12 treatment combinations (2 mycorrhiza × 2 cultivars × 3 harvest) were replicated three times. The pots were placed in a completely randomized design in a thermostatically controlled water tank which was set at 20°C which was similar with the temperature for Experiment 4.2a. The water content of the soil was brought to field capacity by watering to weight. Mean solar radiation during this experimental period [mid June to early August (winter)] was 142 μE m^{-2} s^{-1}. The plants were harvested 14, 28 and 42 days after transplating.

Shoot and root dry matter, infection levels and P content of the plants were determined.

5.2.2 *Experiment 5.2*

The aim of the experiment was similar to Experiment 5.1. However, an efficient barley cultivar (cv. Yagan) was used to compare with the inefficient one (cv. Galleon). Although, this experiment essentially repeated Experiment 5.1, it differed in that the soil temperature was set at 15°C, which is a temperature commonly encountered by barley in the field. Preparation of soil and inoculum and the design, were also the same as for Experiment 5.1. The present experiment was conducted from mid August to late September, when the mean solar radiation was 264 μE m^{-2} s^{-1}. Preparation and analysis of plant materials after each harvest were similar to Experiment 5.1

5.3 Results

5.3.1 Experiment 5.1

Barley roots grown in the presence of mycorrhizal inoculum were infected by G. *intraradices* 14 days after transplanting (Figure 5.1). Percentage of root length infected increased with time, so that by 42 days nearly one-third of the root length contained hyphae and arbuscules of mycorrhizal fungi. Generally, the level of infection of Galleon was not significantly different from Clipper.

A dry weight response to mycorrhizal inoculation was not observed until 42 days after transplanting (Figure 5.2). At this harvest, although Clipper tended to have higher dry matter than Galleon, there were no significant differences between dry weights of the two barley cultivars.

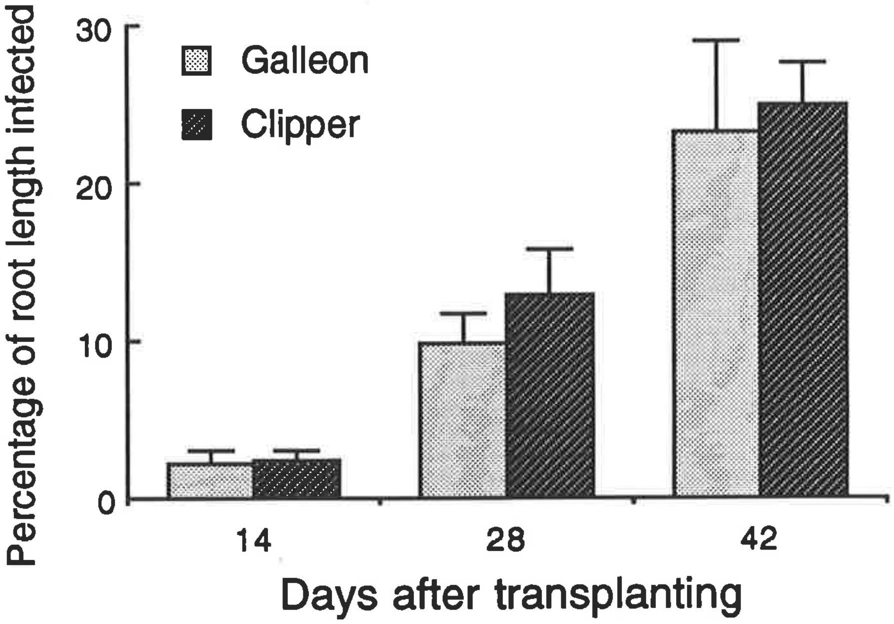


Figure 5.1 **(Experiment 5.1) Percentage of root length of two barley (*Hordeum vulgare* cv. Galleon and cv. Clipper) cultivars infected by *G. intraradices* at 14, 28 and 42 days after transplanting, grown at soil temperature of 20°C. Vertical bars represent standard errors of the means, n = 3.**

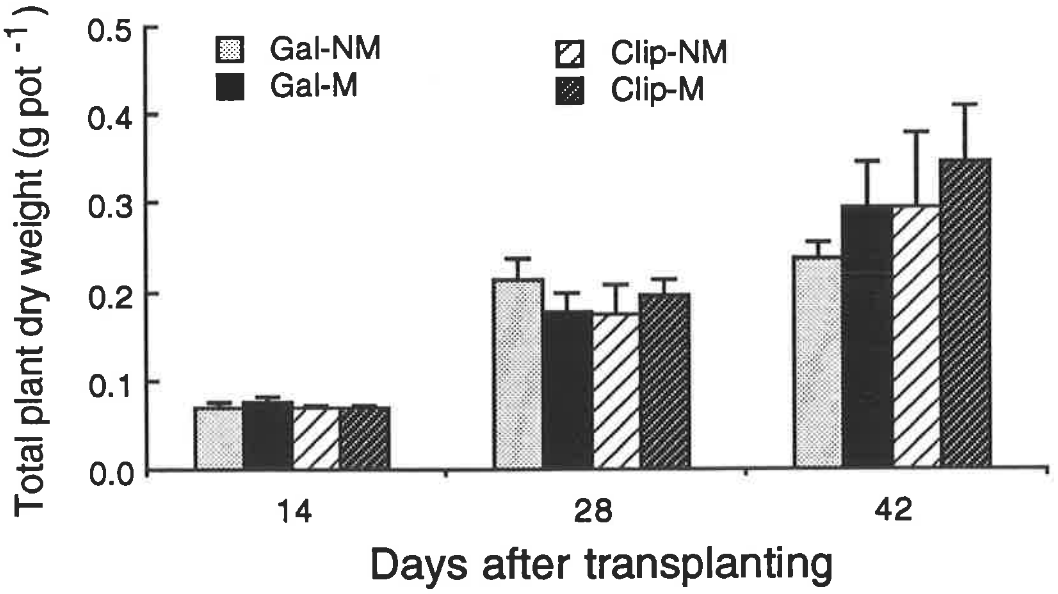


Figure 5.2 **(Experiment 5.1) Total plant dry weight of two barley (*Hordeum vulgare*) cultivars (Gal = Galleon and Clip = Clipper) at 14, 28 and 42 days after transplanting as affected by infection by a mycorrhizal fungus, *G. intraradices* (M = mycorrhizal, NM = non-mycorrhizal). The plants were grown at a soil temperature of 20°C. Vertical bars represent standard errors of the means, n = 3.**

Specific P uptake (mg g^{-1} root) for all treatments between transplanting and 14 days, was higher than at 28 and 42 days (Figure 5.3). Mycorrhizal plants had lower specific P uptake in the first 14 days, but later in 28 and 42 days they were higher than non-mycorrhizal plants. Clipper and Galleon after 42 days were similar in specific P uptake. However, the differences between mycorrhizal plants and non-mycorrhizal plants were more pronounced in Clipper than in Galleon.

Shoot P concentration of all plants decreased with time. Mycorrhizal infection increased shoot P concentration of both barley cultivars 28 and 42 days after transplanting (Figure 5.3). Non-mycorrhizal Galleon and Clipper were not different in their shoot P concentration, but mycorrhizal Clipper had higher shoot P concentration than Galleon.

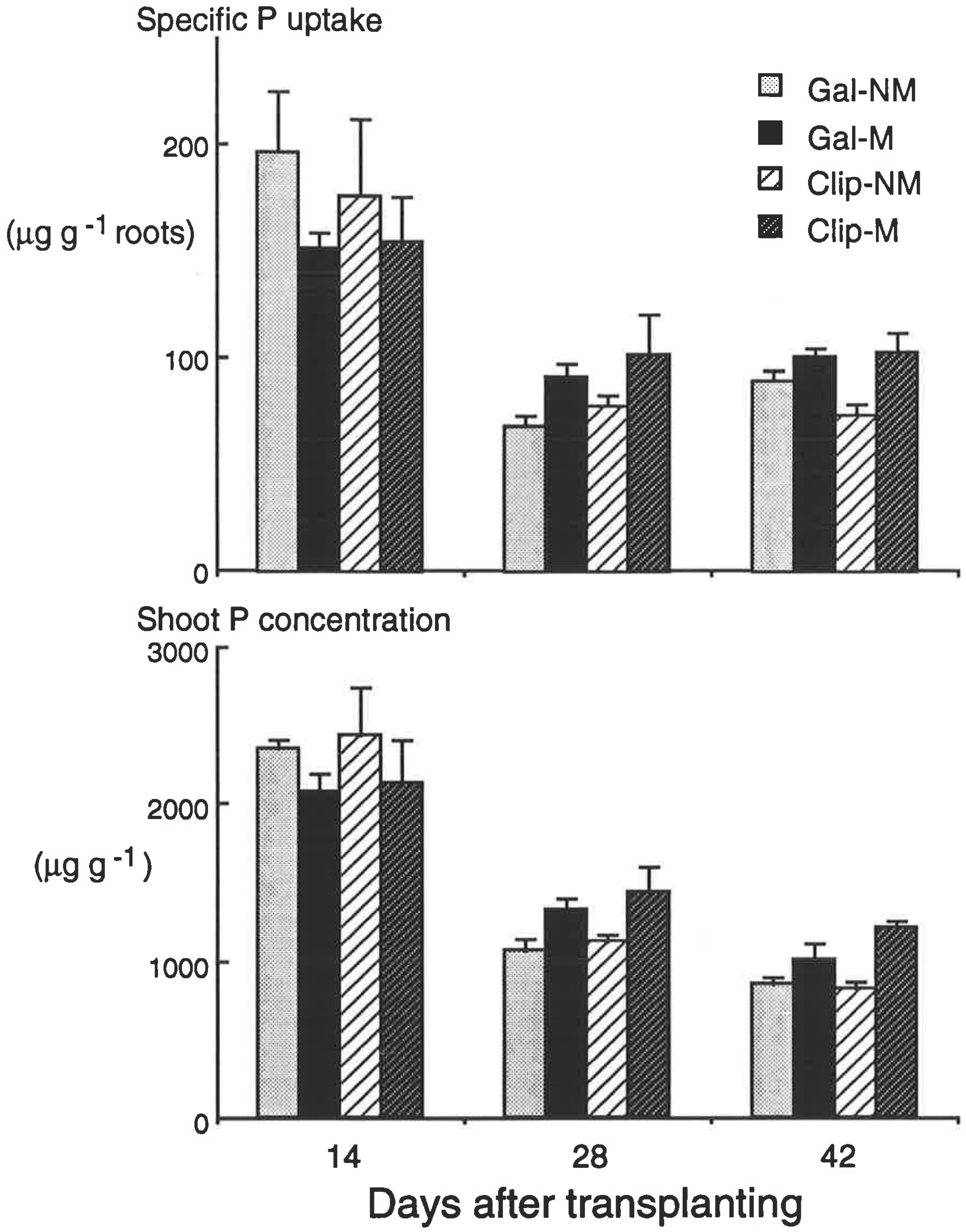


Figure 5.3 **(Experiment 5.1) Specific P uptake and concentration of P in shoots of two barley cultivars (Gal = Galleon, Clip = Clipper) infected by a VA mycorrhizal fungus, *G. intraradices* (M = mycorrhizal, NM = non-mycorrhizal) at 14, 28 and 42 days after transplanting. The plants were grown at a soil temperature of 20°C. Vertical bars represent the standard errors of the means, n = 3.**

5.3.2 *Experiment 5.2*

Infection of the barley plants by G. *intraradices* was detected from 14 days after transplanting. At this stage there was no difference between the two barley cultivars (Figure 5.4). Percentage of root length infected increased to 6% and 8 % for Galleon and Yagan, respectively, after 28 days and it remained relatively constant after that.

Figure 5.5 presents results of shoot and root dry weights. There were no difference among treatments at 14 days after transplanting. The negative effects of mycorrhizas on shoot and root dry weights were observed at 42 days after transplanting, especially on shoot of Galleon. Yagan produced more shoot dry matter yield at low P supply (agronomically more P efficient) than Galleon, but only at 28 days after transplanting. At final harvest, root dry weight of mycorrhizal plants tended to be lower than non-mycorrhizal plants (Figure 5.5).

Concentration of P in plant tissues decreased with time especially in roots (Figure 5.6). For non-mycorrhizal plants, Galleon had higher concentration of P in shoots and roots than Yagan. However, when they were mycorrhizal, P concentration in roots of both cultivars were not different. In the last two harvests, mycorrhizal infection increased the root P concentration of Yagan more than that of Galleon. However, the opposite was observed for shoot P concentration.

In the absence of mycorrhiza, Galleon had a higher percentage of total P content present in roots compared with Yagan. At the last harvest (42 days after transplanting) percentage of total P content in roots were also higher for mycorrhizal plants.

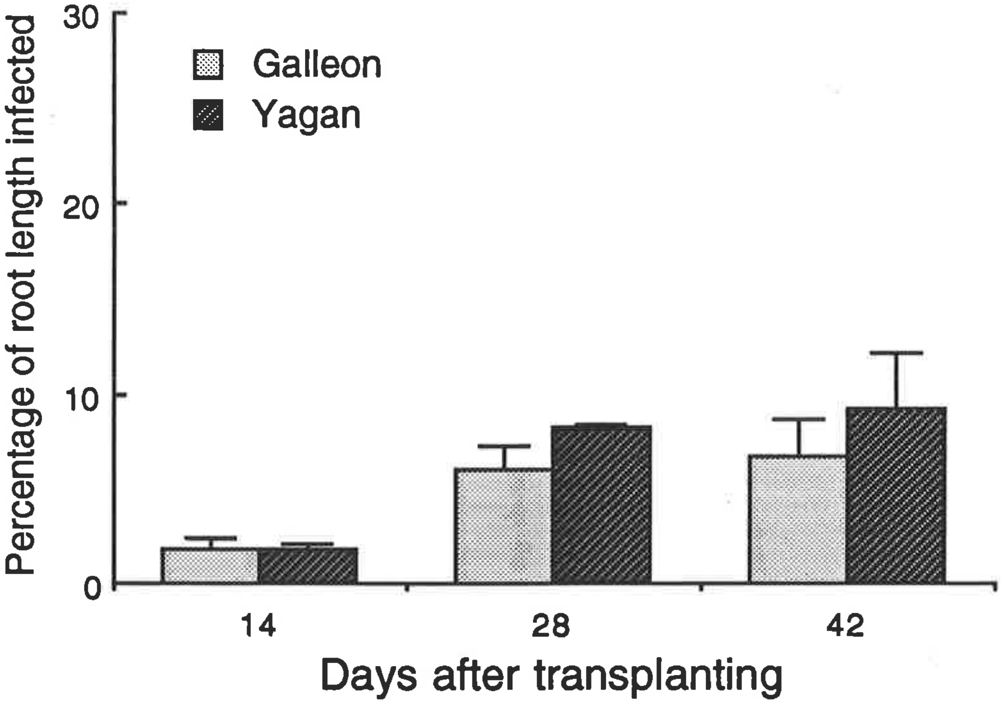


Figure 5.4 **(Experiment 5.2) Percentage of root length infected of two barley cultivars at 14, 28 and 42 days after transplanting. Plants were grown at a soil temperature of 15°C. Vertical bars represent standard errors of the means, n = 3; where no bar appears, standard error is smaller than the line.**

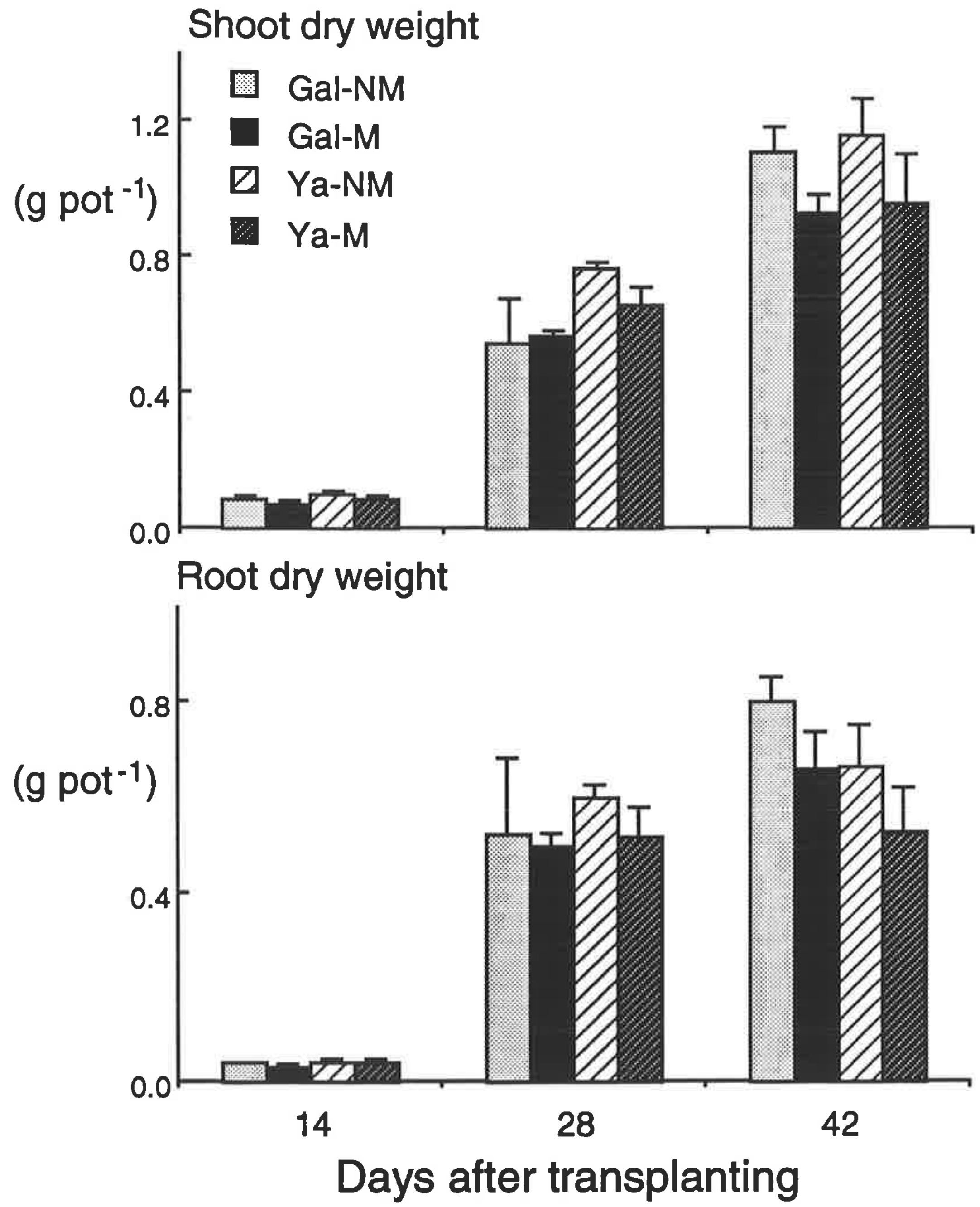


Figure 5.5 **(Experiment 5.2) Dry weight of shoots and roots of two barley cultivars (Gal = Galleon, Ya = Yagan) as affected by mycorrhizal infection by *G. intraradices* (M = mycorrhizal, NM = non-mycorrhizal) at 14, 28 and 42 days after transplanting. The plants were grown at a soil temperature of 15°C. Vertical bars represent standard errors of the means, n = 3; where no bar is shown, standard errors is smaller than the line.**

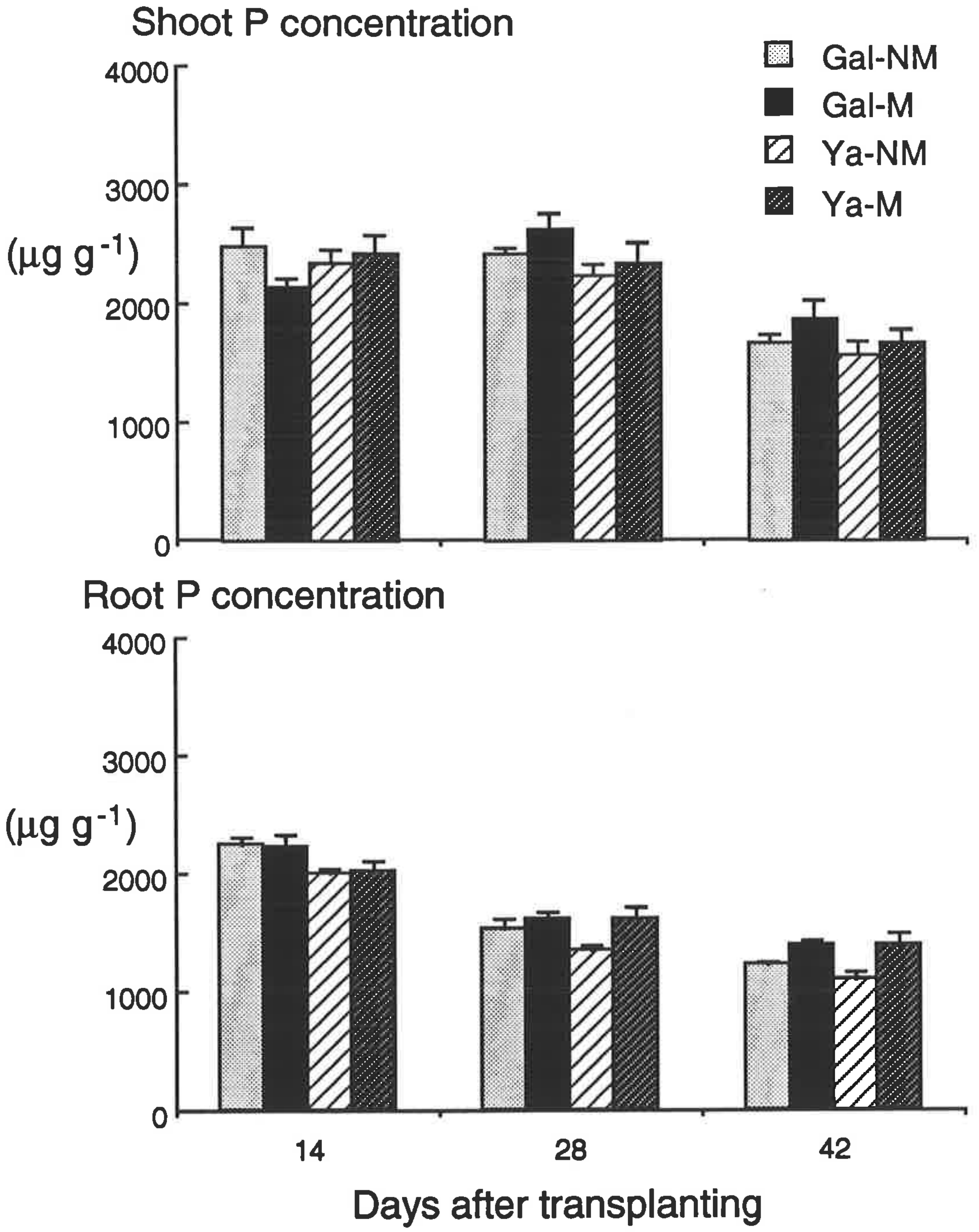


Figure 5.6 **(Experiment 5.2) Concentration of P in shoots and roots of two barley cultivars (Gal = Galleon, Ya = Yagan) inoculated with a VA mycorrhizal fungus, *G. intraradices* (M = Mycorrhizal, NM = Non-mycorrhizal) at 14, 28 and 42 days after transplanting. Vertical bars represent standard errors of the means, n = 3; where no bar is shown, standard error is smaller than the line.**

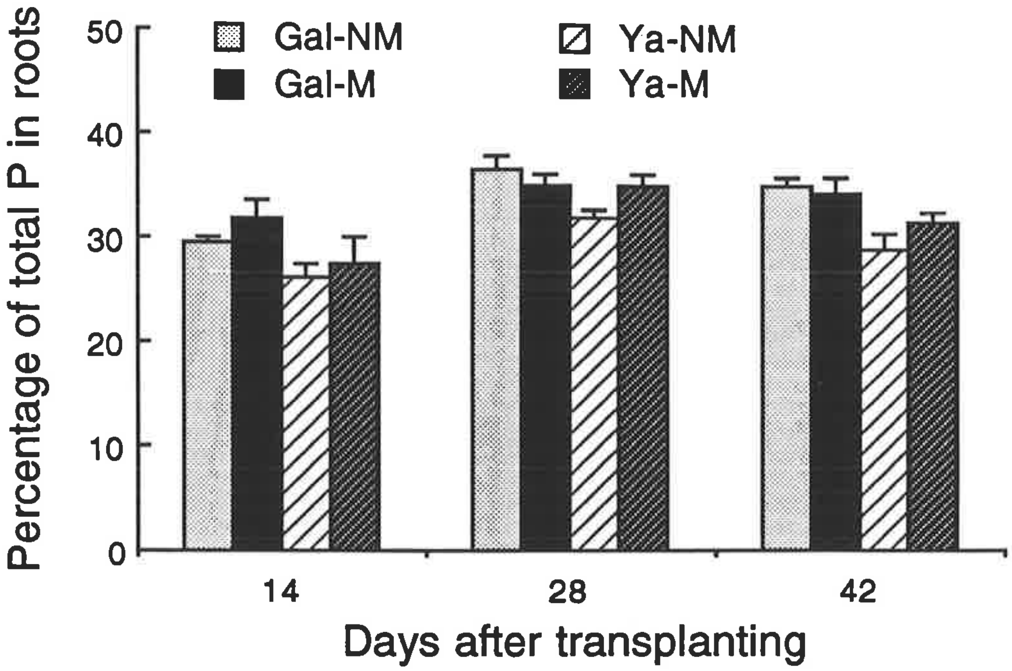


Figure 5.7 **(Experiment 5.2) Percentage of total P content in roots of barley cultivars (Gal = Galleon, Ya = Yagan) inoculated with a VA mycorrhizal fungus, *G. intraradices* (M = Mycorrhizal, NM = Non-mycorrhizal) at 14, 28 and 42 days after transplanting. Plants were grown at a soil temperature of 15°C. Vertical bars represent standard errors of the means, n = 3.**

5.4 Discussion

In view of these experiments, it appears that in the absence of mycorrhizas, an agronomically inefficient cultivar has higher concentrations of P (lower P utilization efficiency) in both shoots and roots and higher percentage of total P content in roots compared to an efficient cultivar. Thus, the efficiency of the barley cultivar can be related to relative allocation of P between root and shoot and to its utilization in plant tissues.

Although the extent of infection was relatively low, an effect of mycorrhizas on growth and P status of the plants was evident. The present results agree with previous work that percentage of root length infected of less than 10% may have significant effects on plant growth and nutrition (Sanders *et al.* 1977, Volkmar and Woodbury 1989).

Results of these experiments clearly showed that *Glomus intraradices*, a coarse endophyte, was able not only to increase but also to decrease growth of barley plants. In Experiment 5.1, total dry matter was about 20% greater in mycorrhizal plants. On the other hand, in Experiment 5.2 mycorrhizal plants had 18% less dry matter compared with non-mycorrhizal plants. This difference was clearly related to differences in growing conditions in the two experiments. Thus with low soil temperature, dry weight of mycorrhizal plants was lower than non-mycorrhizal plants while it was greater when the soil temperature was high.

It is interesting to note that the irradiance of Experiment 5.1 was only 142 μE m^{-2} s^{-1}, which can be regarded as very low. However, a positive response to mycorrhizal infection in terms of total dry weight was observed, especially for Galleon, although it was not significant. The growth increase observed in this study may be associated with a relatively high level of mycorrhizal infection (23 - 25%) for cereal plants which have fibrous root systems. Although there are many studies which have shown that plants with highly infected roots produced higher yields and improved P nutrition of the plants more than plants with poorly infected roots (Chapter 4 of this thesis), it has been suggested (Black and Tinker 1979) that the level of VA mycorrhizal infection and plant growth promotion are unrelated. Ineffective strains of mycorrhizal fungi, grazing by soil animals, inter-plant connections and longevity of roots may attribute to the ineffectiveness of mycorrhizal infection (Fitter 1985).

The results of this experiment showed that the percentage of root length infected of barley plants subjected to a soil temperature of 15°C and photoirradiance of 264 $\mu E\ m^{-2}\ s^{-1}$ were very low (7 - 9%) and growth was slow. However, the plants at a temperature of 20°C and irradiance of 142 $\mu E\ m^{-2}\ s^{-1}$ were highly infected (23 - 25%) and grew quickly. This suggests that mycorrhizal colonization was mainly affected by soil temperature, which was in agreement with the works of Furlan and Fortin (1973), Hayman (1974), Smith and Bowen (1979) and Grey (1991), rather than by photon irradiance as with the work of Son and Smith (1988).

Lingle and Davis (1959) found that the concentration of P in plants was higher at a soil temperature of about 23°C than at 17°C. However, the main difference in shoot P concentration was observed at 4 weeks after transplanting, when plants grown at a soil temperature of 15°C had a higher P concentration in shoots than at 20°C. This contrasts with the observations of other workers (Hayman 1974, Graham *et al.* 1982, Son and Smith 1988) who found that high P concentration was associated with decreasing light intensity. According to Jarrell and Beverly (1981), this is likely to be due to a "concentration effect", where a plant is growing slowly with high seed P content. However, it is also possible there was an interaction between soil temperature and light intensity which contributed to the contradictory results.

The higher light intensity of Experiment 5.2 may have produced more photosynthates than that of the Experiment 5.1. However, with low soil temperature the activity of the plants was not so great so that the plant may have translocated only a small amount of carbohydrate to support the root system. The reverse may have occurred for plants in Experiment 5.1.

That Galleon was agronomically P inefficient, producing a low yield at low soil P, can be observed at 28 and 42 days after transplanting in Experiment 5.2. Comparing the three barley cultivars, the present results showed that there was no difference in the percentage of root length infected, although the level of infection of Galleon, an inefficient cultivar, tended to be lower than Yagan and Clipper, efficient and moderately efficient cultivars, respectively. The high root biomass of Galleon may explain this difference (see Fig. 5.5). It is known that root production is an important factor in mycorrhizal infection (Smith and Walker 1981) and mycorrhizal dependency (Menge *et al.* 1978a).

The barley cultivars showed different responses to mycorrhizal infection in terms of P nutrition of the plants. In general, mycorrhizal infection increased P concentration (and hence decreased efficiency of utilization) in the all cultivars. This increase was greater in the moderately efficient cultivar, Clipper than in the inefficient Galleon, implying that Clipper was less efficient in utilization of P in the presence of mycorrhizal infection.

Specific P uptake of the two barley cultivars, Clipper and Galleon, infected by *G. intraradices* was higher than non-mycorrhizal plants, as shown in Fig. 5.3. This is in agreement with previous results (Sanders and Tinker 1973, Smith 1980). Although the uptake of P by mycorrhizal plants was high, mycorrhizal plants had a higher concentration of P in their roots than non-mycorrhizal plants. It is possible that the fungal tissues in roots accumulate high amounts of P (Cox *et al.* 1975, Ling-Lee *et al.* 1975). It is interesting to note that Galleon, an inefficient cultivar, accumulated more P in its roots than Yagan, an efficient cultivar. This suggest that, regardless of the presence of mycorrhiza, poor translocation of P from root to shoot may explain the inefficiency of Galleon compared with Yagan.

Clarkson and Hanson (1980) suggested that plants cannot regulate the gross concentration of P in their tissues within short periods of time. However, this ability also depends on the species or varieties of the plants, beside other factors that may control the metabolic processes. In this study, the inefficient cultivar which was given access to P via mycorrhizal infection, was less efficient in utilizing P than non-mycorrhizal plants.

Sanders (1975) and Menge *et al.* (1978b) have suggested that high concentrations of P in plant tissues inhibit the infection of VA mycorrhizal fungi. Results from this study showed that in the absence of mycorrhiza, an inefficient cultivar had a higher shoot P concentration than an efficient cultivar (Fig. 5.6). However, the extent of mycorrhizal infection of the two cultivars was similar. The present results suggest that the degree of mycorrhizal infection is not regulated by concentration of P in shoot which support the results of Koide and Li (1990) on sunflower.

5.5 Conclusion

The effect of infection of barley with *Glomus intraradices* on P nutrition and plant growth leads to the conclusion that mycorrhizal infection by the coarse endophyte increased growth of barley plants if the soil temperature was 20°C and decreased it when the temperature was 15°C. The mycorrhizal fungus also reduced P utilization efficiency of the plants. The results also indicate that a possible interaction between soil temperature and light intensity in determining the response of plants to mycorrhizal infection in terms of P nutrition of the plants. Therefore, for further studies on the relationship between mycorrhizal infection and P efficiency, environmental conditions, such as soil temperature and light intensity, should be properly considered.

Chapter Six

PHOSPHORUS EFFICIENCY OF MYCORRHIZAL BARLEY PLANTS IN RESPONSE TO SOIL TEMPERATURE

6.1 Introduction

The consequences of growing plants at low soil temperatures are well known: rates of metabolic processes are slow and plant growth and development are poor (Bidwell 1979). It is also known that temperature has a major effect on colonization of plants by vesicular-arbuscular (VA) mycorrhizal fungi (Furlan and Fortin 1973, Hayman 1974, Hetrick *et al.* 1984, Chapter 5 of this thesis). However, the importance of soil temperature for mycorrhizal symbiosis has received little study. Physiological and nutritional studies have mainly been conducted in glasshouses at soil temperatures of 20-25°C, which are much higher than those commonly encountered in the field (Fabig *et al.* 1989, see Bowen 1991, Jakobsen and Andersen 1982). In most regions of the world, barley is grown at relatively low soil temperatures (10-15°C). It is generally unclear how low soil temperature affects growth of mycorrhizal plants, and little consideration has been given to P nutrition of the plants under these conditions.

It was shown in Chapter 4 that at a soil temperature of 10°C, indigenous mycorrhizal fungi colonized up to 36 and 40% of the root length of barley and wheat respectively, after 49 days. In another study, VA mycorrhizal fungi from Montana, primarily *Glomus macrocarpum*, were found to be tolerant of soils at 11°C to 14°C (Grey 1991). Nevertheless, P nutrition of the mycorrhizal plants has not been considered in relation to the differences in soil temperature.

This chapter describes the effect of low soil temperature on growth and P efficiency of mycorrhizal barley plants. Mycorrhizal fungi depend entirely on carbohydrate from the host, and mycorrhizal roots may use 4-17 % more carbon than non-mycorrhizal roots (Kucey and Paul 1982, Snellgrove *et al.* 1982, Koch and Johnson 1984). As low soil temperature reduces photosynthesis of the host plant, roots may suffer in competition with the mycorrhizal fungus for the carbohydrate translocated to them. The hypothesis tested in this experiment was that partitioning of the carbohydrate between shoots and roots of the plant and P efficiency of the plants will be affected if low soil temperature restricts the extent of mycorrhizal infection of the roots.

6.2 Materials and Methods

6.2.1 Experiment 6.1

The study was conducted in a glasshouse using Avon soil. The soil was collected from 0-20 cm depth, air-dried and sieved to <2 mm and sterilized by steaming.

Barley (*Hordeum vulgare* L. cv. Galleon) was chosen because it was used in the experiments of Chapter 4 and 5 of this thesis and showed a considerable response to mycorrhizal infection. Plants were grown in pots of soil subjected to combinations of 3 (inoculum) x 3 (temperature) treatments. A split-plot design was used, with soil temperature as the main treatment. Each treatment had three replications.

Glomus intraradices Schenck & Smith as 'Nutrilink' was used as inoculum of mycorrhizal fungus. The inoculum was mixed thoroughly with soil in plastic pots. Two inoculum densities were tested, 1 and 3 g 'Nutrilink' per pot. Control pots received no inoculum. Each pot contained 1.4 kg of soil.

To ensure that nutrients other than P did not limit plant growth, mineral nutrients were added as detailed in Chapter 3.

Barley seeds were surface-sterilized and germinated on moist filter paper at room temperature. Two 2-day old barley seedlings were transplanted into each pot. The pots were set into one of three water baths which were thermostatically controlled at 10, 15 and 20°C.

The water content in soil was maintained at field capacity (11%) by daily watering using deionized water. The experiment was conducted during June to August with natural light. Mean solar radiation in the glasshouse ranged from 199 $\mu E\ m^{-2}\ s^{-1}$ in June to 118 $\mu E\ m^{-2}\ s^{-1}$ in August. The minimum and maximum air temperatures inside the glasshouse were 23/10°C (night) and 26/17°C (day), respectively.

Plants were harvested 48 days after planting. After cleaning and washing, fresh and dry weight of both shoots and roots were recorded. Root samples were stained and percentage of root length infected was determined. P contents of the plants were determined.

6.3 Results

Mycorrhizal infection of barley roots by *G. intraradices* was relatively low, averaging up to about 12% (Table 6.1). The percentage of root length infected was low both at low soil temperature and at low inoculum density. No mycorrhizal infection was observed at 10°C, regardless of the density of inoculum. There was a significant difference in percentage of root length infected between two inoculum densities at 20°C. As the level of mycorrhizal infection was low at the density of 1 g pot^{-1}, the remaining data presented are for plants inoculated with 3 g pot^{-1}.

Table. 6.1. **Percentage of root length infected of barley plant as affected by soil temperatures and inoculum density.**

	Soil temperature (°C)		
Inoculum Density	10	15	20
Control	0	0	0
1 g/pot	0	0.6 ± 0.2	1.4 ± 0.4
3 g/pot	0	1.1 ± 0.4	12.3 ± 2.2

Mean ± Standard error of the mean. n = 3.

Fig. 6.1 illustrates the changes in biomass accumulation in roots and shoots of barley plants as affected by soil temperature and mycorrhizal inoculation. At 10°C, root and shoot dry weight of inoculated and uninoculated plants were not significantly different. Increasing temperatures were correlated with increases in shoot growth of both inoculated and uninoculated plants. Poor root growth, which occurred at low soil temperatures, was more pronounced in mycorrhizal than in non-mycorrhizal plants (compare 15°C with 20°C in Fig. 6.1). In all situations, barley plants allocated a higher proportion of dry matter to shoots compared with roots, *i.e.* R/S ratio <1 (Fig. 6.1). Root/shoot ratios were generally higher at low temperatures and non-mycorrhizal plants had higher root/shoot ratios than mycorrhizal plants. Inoculation of plants at 10°C resulted in a slight increase in root/shoot ratio, although the plants were not colonized by mycorrhizal fungi.

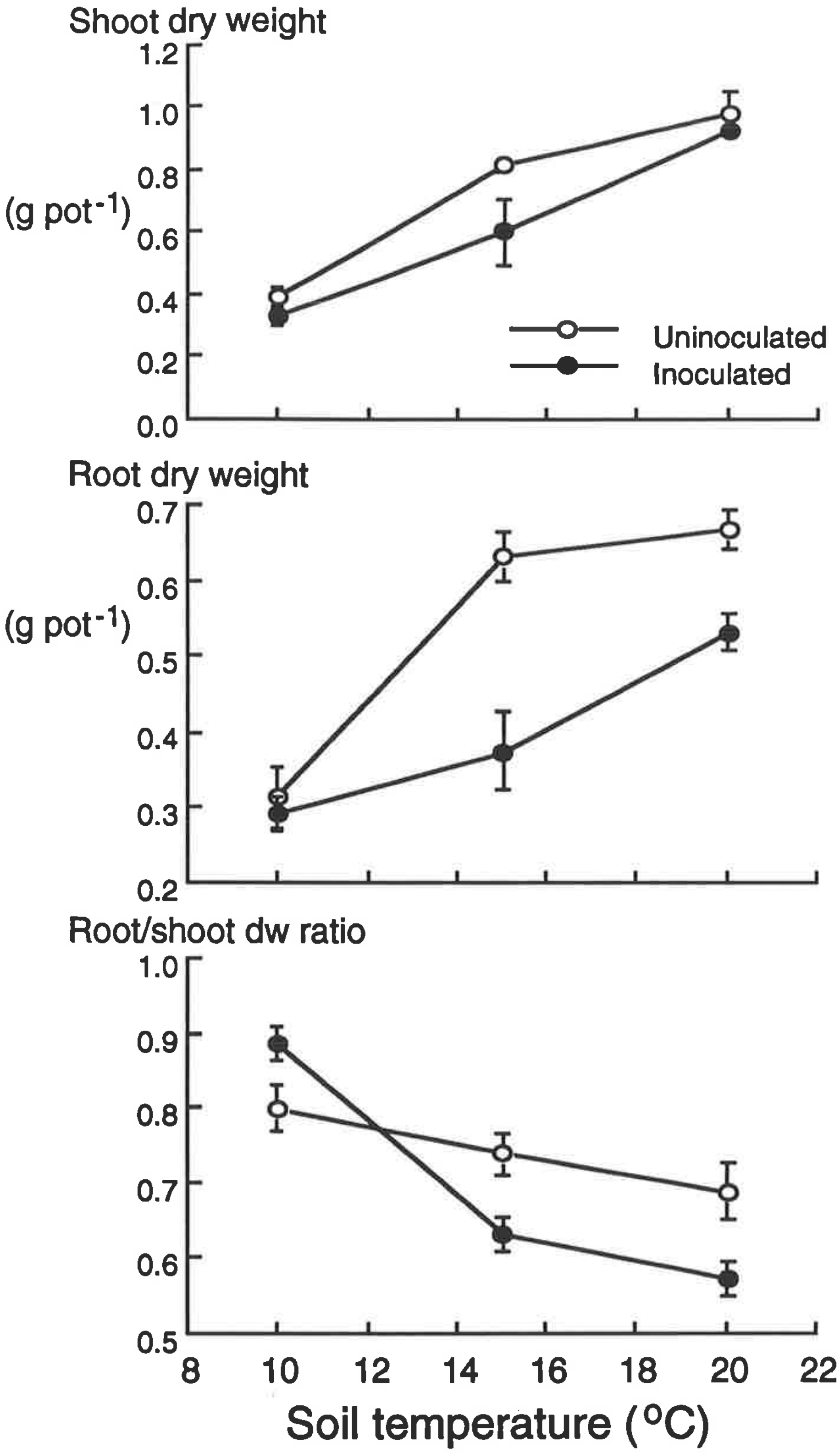


Figure 6.1. **(Experiment 6.1) Dry weight of shoots and roots and root/shoot ratio of barley as affected by mycorrhizal inoculation and soil temperature. Vertical bars represent standard errors of the means, n = 3; where no bar is shown, standard error is smaller than symbol.**

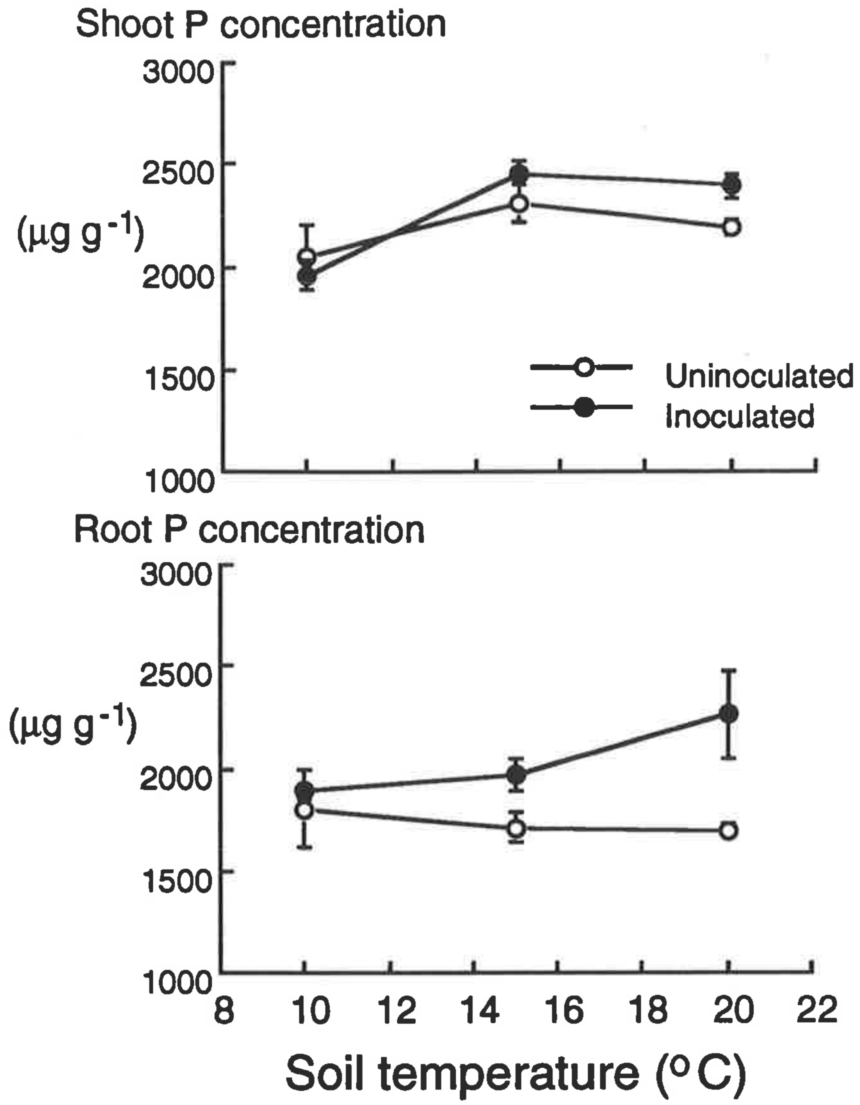


Figure 6.2. **(Experiment 6.1) P concentration in shoots and roots of barley as affected by mycorrhizal inoculation and soil temperature. Vertical bars represent standard errors of the means, n = 3; where no bar is shown, standard error is smaller than symbol.**

In Figure 6.2, the concentrations of P in shoots and roots as affected by mycorrhizal infection are plotted against soil temperatures. There was no difference in P concentration between uninoculated and inoculated plants at 10°C. However, at 15 and 20°C P concentration in mycorrhizal plants was higher than non-mycorrhizal plants especially in roots. Concentration of P in roots of inoculated plants slightly increased with soil temperature increase, while for uninoculated plants the root P concentration tended to decline at high temperatures. High shoot P concentration was observed at soil temperature of 15°C.

Accumulation of P in both shoots and roots of barley was lower at low temperatures (Fig. 6.3), and mycorrhizal plants were more sensitive to low temperature than non-mycorrhizal plants. While the soil temperature of 15°C resulted in lower uptake of P in both shoots and roots of mycorrhizal plants compared with 20°C, this temperature difference did not affect non-mycorrhizal plants. At 10°C there was no effect of mycorrhizal inoculation on uptake of P to either shoots or roots, concomitant with the absence of any root infection. However, at 15 and 20°C mycorrhizal plants had a higher specific P uptake than non-mycorrhizal plants (Fig. 6.3). Specific P uptake of uninoculated plants was not affected by soil temperature.

Agronomic P efficiency is expressed as total dry matter yield obtained when no P was added to the soil. The lower the soil temperature, the lower was this parameter in both inoculated and uninoculated plants (Fig. 6.4).

However, P utilization efficiency (g dm g^{-1} P) was not affected by soil temperature. Plants colonized by *G. intraradices* were less efficient than non-mycorrhizal plants in terms of both agronomic and P utilization efficiencies. Figure 6.4 also shows that the percentage of the total plant P which was present in the roots was higher at 10°C than at the other two temperatures. Mycorrhizal infection had no effect on this parameter.

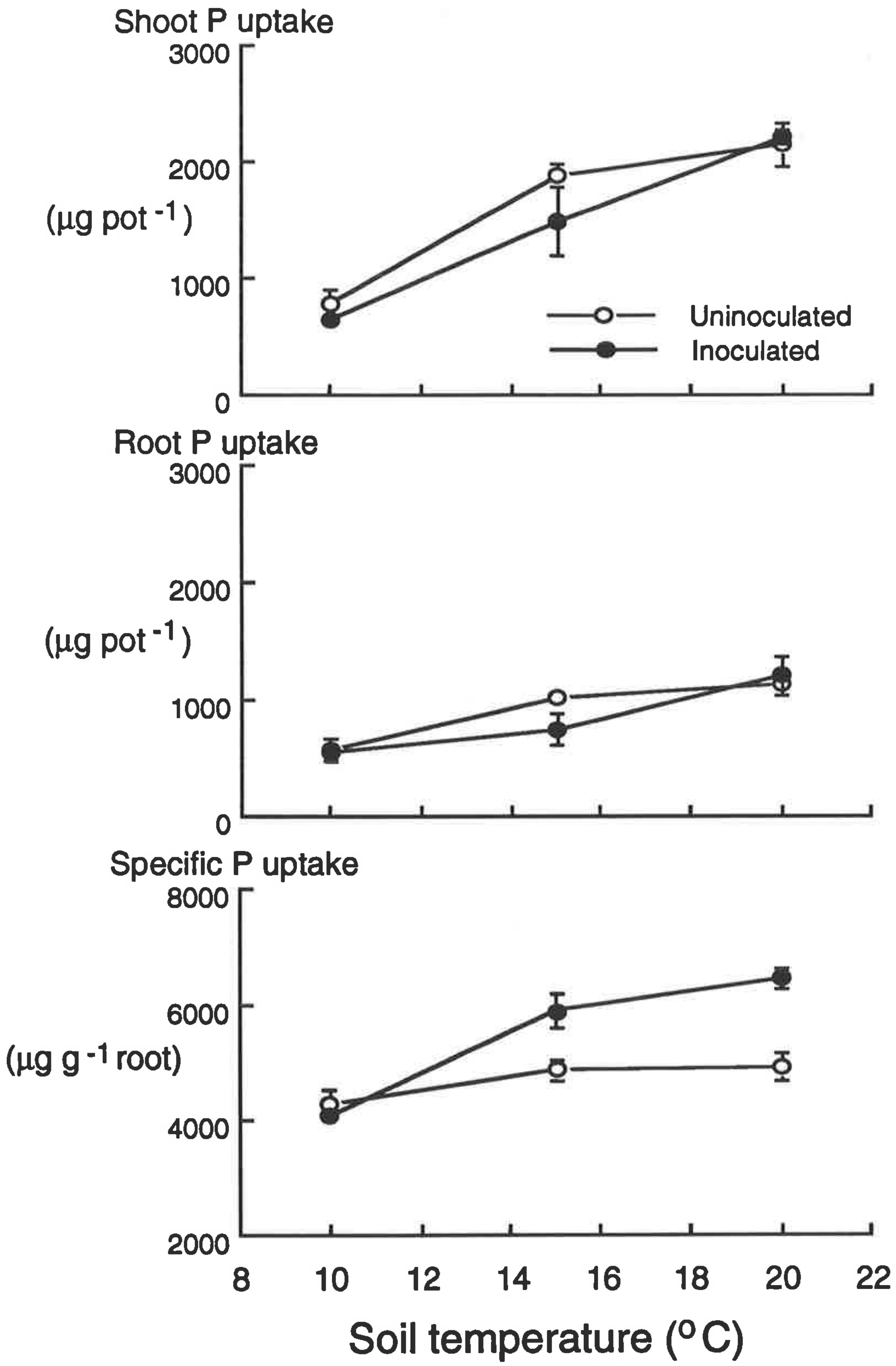


Figure 6.3. **(Experiment 6.1) Uptake of P in shoots and roots and specific P uptake of mycorrhizal barley grown at different soil temperatures. Vertical bars represent standard errors of the means, n = 3; where no bar is shown, standard error is smaller than symbol.**

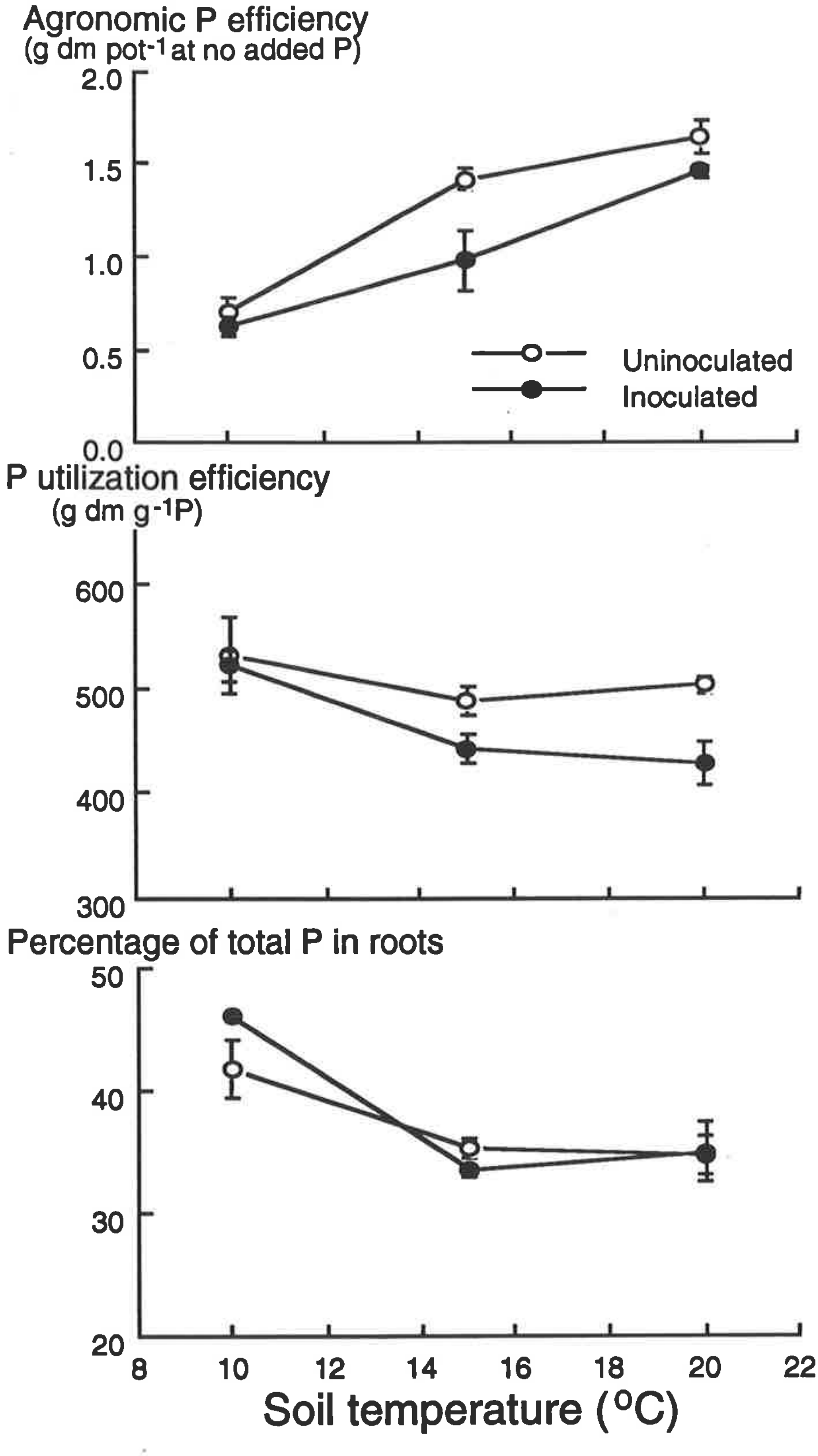


Figure 6.4. **(Experiment 6.1) Agronomic P efficiency, P utilization efficiency and percentage of total plant P in roots of barley as affected by mycorrhizal inoculation and soil temperature. Vertical bars represent standard errors of the means, n = 3; where no bar is shown, standard error is smaller than symbol.**

6.4 Discussion

Low soil temperatures affected both the growth of barley roots and the mycorrhizal symbiosis. The results of this study have shown that P efficiency in both inoculated and uninoculated barley plants was affected by soil temperature, but the responses were different. In inoculated barley plants, growth at low soil temperatures decreased agronomic P efficiency. However, the increase in specific P uptake in inoculated plants at higher soil temperatures was largely due to the effect of mycorrhizal infection, rather than to the effect of temperature.

Previous studies have shown greater VA mycorrhizal infection at higher soil temperatures (Smith and Bowen 1979, Hayman 1974, Graham *et al.* 1982, Grey 1991). Some of these studies indicated that the effects may be mediated either through a direct effect of temperature on the fungus in soil leading to a greater number of entry points (Smith and Bowen 1979) or through an indirect effect following an increase in leakage of root metabolites necessary for fungal activity (Graham *et al.* 1982). In the results of Chapters 4 and 5, the detrimental effects of low soil temperature on infection of barley roots by *G. intraradices* was obvious. At 10°C, barley roots were not infected by mycorrhizal fungi. This suggests that *G. intraradices,* was more susceptible to low soil temperatures than the indigenous fungi from this soil (see Chapter 4) and *Glomus macrocarpum* from Montana (Grey 1991). Clearly there is a need for further investigation into selection of VA mycorrhizal fungi which are well adapted to low soil temperatures and which may benefit barley and other plants normally grown when soil temperatures were low.

The results of this study have also shown that low soil temperature reduced root growth of barley plants infected by *G. intraradices,* but did not affect non-mycorrhizal plants in this way. The ability of non-mycorrhizal barley plants to produce more dry matter yield with no added P (higher agronomic P efficiency) than mycorrhizal plants appears to contradict the findings of many workers (*e.g.* Jensen 1982, Champawat *et al.* 1987). Such a growth depression is probably not due to a high concentration of P in tissues; rather, it may in large part be associated with the low light intensity encountered in this experiment, which was conducted in winter time. The competition for assimilates due to mycorrhizal infection appears to be reflected in lower biomass partitioning to roots in mycorrhizal plants compared with non-mycorrhizal plants. If mycorrhizal symbiosis increases nutrient absorption, the plant may limit energy loss associated with extra root maintenance by decreasing root growth (Hetrick 1989). This result also agrees with other workers (Gerdemann 1964, Baas and Lambers 1988, see Smith 1980) who showed that root-shoot ratios of mycorrhizal plants were lower than non-mycorrhizal plants. Furthermore, root-shoot ratios were generally decreased by increased soil temperature.

When comparing soil temperatures of 15 and 20°C this study also shows that low soil temperature restricted shoot growth more than root growth of non-mycorrhizal plants. However, in mycorrhizal plants growth of both shoots and roots was reduced at low soil temperatures. Nielsen *et al.* (1960) and Power *et al.* (1963) who worked on oats and barley respectively, also found that low soil temperature restricted shoot growth more than root growth, regardless of mycorrhizal status of the plants.

Low soil temperature had a significant effect on P efficiency in both mycorrhizal and non-mycorrhizal barley plants, but the responses were different. P uptake efficiency and agronomic P efficiency of mycorrhizal plants grown at 15°C were lower than at 20°C. However, there was no difference in those two parameters for non-mycorrhizal plants at the same temperatures. The negative effect of low soil temperature was not due directly to the absorption of P, but to indirect effects through the involvement of P in buildup of plant tissues. This is understandable because P uptake efficiency and agronomic P efficiency are functions of plant biomass which was low at low soil temperature.

Although soil temperature had no effect on specific P uptake by mycorrhizal plants, these results showed that mycorrhizal plants had a higher specific P uptake than non-mycorrhizal plants and at the same time had lower root-shoot ratios. This may explain how mycorrhizal plants control the allocation of biomass to plant roots and maintain high P uptake per unit weight of root at low soil temperature. Compared with non-mycorrhizal plants, P uptake efficiency of mycorrhizal plants was more sensitive to decreases in soil temperature. Although specific P uptake of mycorrhizal plants was not affected by low soil temperature, total uptake of P to shoots and roots was reduced which may be due to the differential allocation of biomass to these tissues at different soil temperatures.

6.5 Conclusion

From this study, mycorrhizal plants appear to be more affected than non-mycorrhizal plants by low soil temperatures and that their low agronomic P efficiency results from depression of root growth following mycorrhizal colonization. The results showed that mycorrhizal roots maintained their high specific P uptake even at low soil temperature. The minimal effects of inoculation at 10°C were the result of a failure of mycorrhizal infection. Therefore, an experiment was designed on selection of VA mycorrhizal fungi adapted to low soil temperature and their influences on P efficiency of the barley plants.

Chapter Seven

EFFECTS OF DIFFERENT VA MYCORRHIZAL FUNGI ON P EFFICIENCY AND GROWTH OF BARLEY

7.1 Introduction

Barley has been reported to be infected by a number of different mycorrhizal fungi, for example with *Glomus mosseae* (Jensen and Jakobsen 1980, Jakobsen and Andersen 1982, Powell 1981, Buwalda *et al.* 1985), *Glomus caledonius* (Jakobsen 1983), *Glomus fasciculatum* (Jensen 1982, Powell 1981), *Glomus constrictus* (Champawat *et al.* 1987), *Glomus macrocarpum* (Grey 1991), *Glomus tenuis* (Chapter 4 of this thesis), *Glomus intraradices* (Chapter 5 and 6), *Gigaspora calospora* (Jensen and Jakobsen 1980) and *Gigaspora margarita* (Powell 1981, Jensen 1982). The extent of infection of the mycorrhizal fungi can vary from very low to very high and it depends on crop species (Krishna *et al.* 1985) as well as growth conditions (Hetrick 1984), including situations with low soil temperature where barley plants are mostly grown.

VA mycorrhizal fungi differ in ability to improve plant growth and P uptake by the host plants (Jensen 1982, Secilia and Bagyaraj 1992, Raju *et al.* 1990) and they show a wide range of responses to edaphic and climatic environments. Several studies on mycorrhizal barley have shown different growth responses of both the plant and the fungal symbionts to soil temperatures less than 20°C (Volkmar and Woodbury 1989; Hetrick *et al.* 1984; Grey 1991). This suggests that certain barley cultivars may benefit from the association as long as the VA mycorrhizal symbiosis can develop at the low soil temperature.

Therefore, the objectives of the experiment described in this chapter were to investigate the ability of six mycorrhizal fungi to infect barley plants at a soil temperature of 15°C and to compare their influences on the plant growth response and P efficiency.

7.2 Materials and Methods

7.2.1 Experiment 7.1

This experiment used Avon soil treated as before. The inocula used in this experiment were taken from pot cultures of the mycorrhizal fungi grown with *Trifolium subterraneum* as host plant. The inoculum of each mycorrhizal fungal species was a mixture of spores, hyphae, infected roots and soil. The six mycorrhizal fungi used in this experiment (numbers in brackets are percentages of root length of subclover infected and the ages of pot cultures in storage, in months) were *Glomus mosseae* (86%, 17), *Glomus fasciculatum* (34%, 64), *Glomus versiforme* (not available, 19), *Glomus intraradices* (87%, 16), *Glomus* 'City Beach' (86%, 22), and *Glomus etunicatum* (not available, 18). Each inoculum of 70 g was layered 5 cm below soil surface.

Seeds of barley (*Hordeum vulgare* L.) cv. Galleon were surface sterilized and germinated at room temperature. Two two-day-old seedlings were transplanted into pots containing 1.4 kg of soil. All the pots with plants were set in thermostatically controlled water tanks in which the soil temperature was kept at 15°C. The plants were kept in the glasshouse for 30 days before harvest. The experiment was conducted during mid August to mid September, air temperatures being 23-26/10-17°C (day/night) and the mean of light intensity being 245 $\mu E\ m^{-2}\ s^{-1}$. At harvest, the fresh and dry weights of the plants were determined. Samples of the root systems were taken for determination of the percentage of root length infected. P content of shoot and root samples were determined.

7.3 Results

Root infection of the barley plants by the six mycorrhizal fungi tested was relatively low. However, differences among VA mycorrhizal fungal species in their ability to infect roots of barley were evident throughout this study. Three VA mycorrhizal fungi *i.e. G. intraradices, G. etunicatum and Glomus* spp. 'City Beach' infected barley roots to more than 10% of the root length (Fig. 7.1), whereas percent infection by the other three fungi was less than 4%. *G. etunicatum* was more tolerant to the soil temperature of 15°C and infected barley roots more extensively than other fungi tested. Comparing the percentage of infection in barley infected by *G. mosseae, G. intraradices* and *Glomus* 'City Beach', no relation was found between infection level in the barley plants and the percentage of infection of the inocula (Fig. 7.1).

Fig. 7.2 shows that shoot and root dry weights of plants inoculated with *G. etunicatum* were higher than control plants and plants infected by the other mycorrhizal fungi tested, except *G. fasciculatum*. Furthermore, plants inoculated with fungi other than *G. etunicatum* produced shoot dry matter yield similar to the control.

Specific P uptake of barley plants infected by the six mycorrhizal fungi were not different from control (Fig. 7.3). Comparison among the mycorrhizal fungi, the mean values of specific P uptake by plant infected by *G. fasciculatum* was the least whereas by *Glomus* "City Beach" the highest. The influence of the six VA mycorrhizal fungi on concentration of P in shoots was similar to the influence on specific P uptake of barley plants (Fig. 7.4).

However, Fig. 7.4 also shows that root P concentrations were higher in plant infected by *G. etunicatum* and in *Glomus* 'City Beach' than in control.

Correlations between the concentrations of P in plants and the percentage of root length infected are shown in Fig. 7.5. The slopes of the two lines were low, for both shoots and roots.

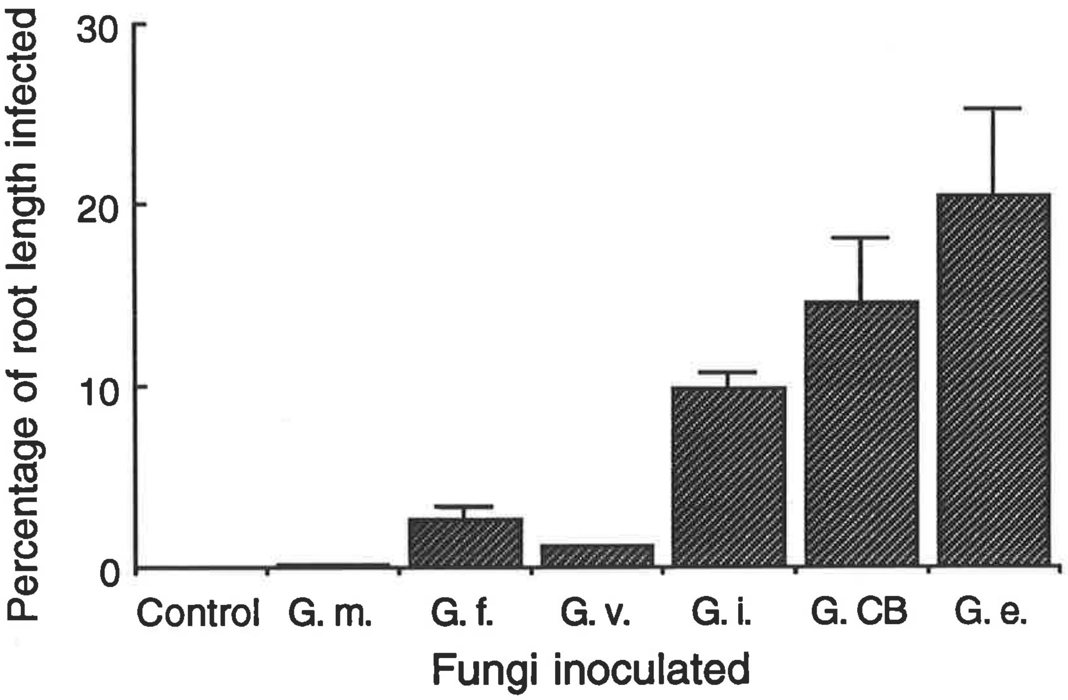


Figure 7.1 **Percentage of root length of barley (*Hordeum vulgare* L. cv. Galleon) infected by six VA mycorrhizal fungi (G.m = *G. mosseae*, G.f. = *G. fasciculatum*, G.v. = *G. versiforme*, G.i. = *G. intraradices*, G.CB = *Glomus* "City Beach", G.e. = *G. etunicatum*) at a soil temperature of 15°C. Vertical bars represent standard errors of the means, n = 3; where no bar is shown, standard error is smaller than line.**

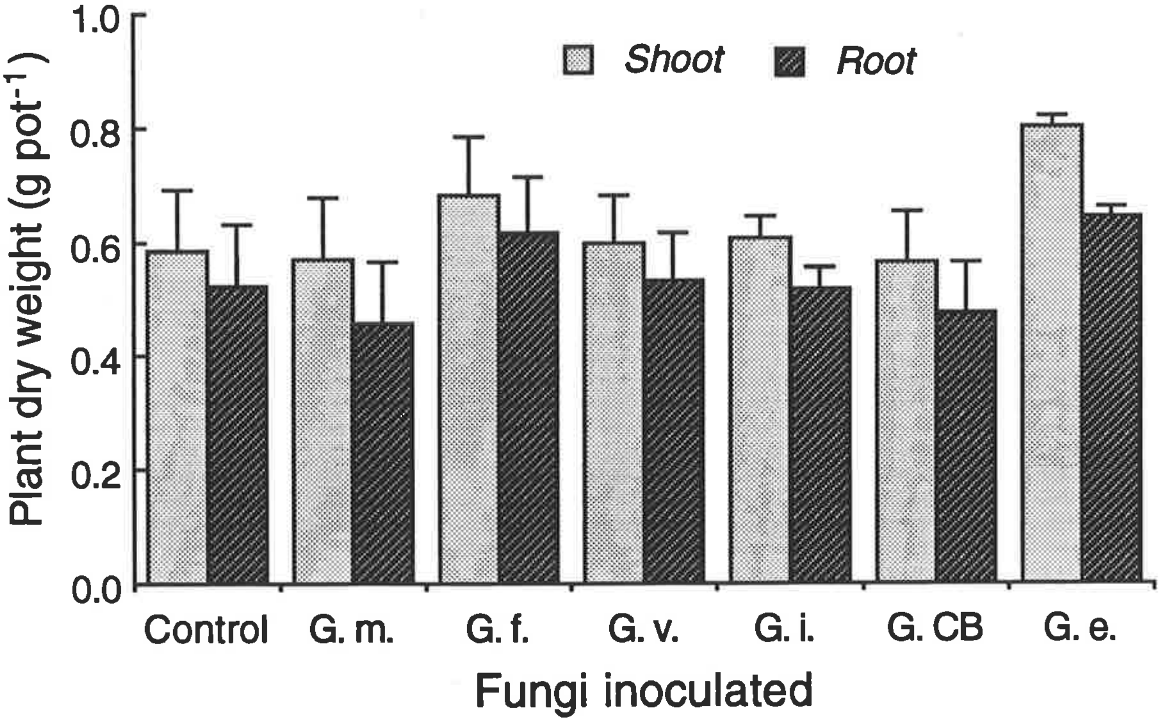


Figure 7.2 **Dry matter of shoots and roots of Galleon barley infected by six VA mycorrhizal fungi (G.m = *G. mosseae,* G.f. = *G. fasciculatum,* G.v. = *G. versiforme,* G.i. = *G. intraradices,* G.CB = *Glomus* "City Beach", G.e. = *G. etunicatum*) at a soil temperature of 15°C. Vertical bars represent standard errors of the means, n = 3.**

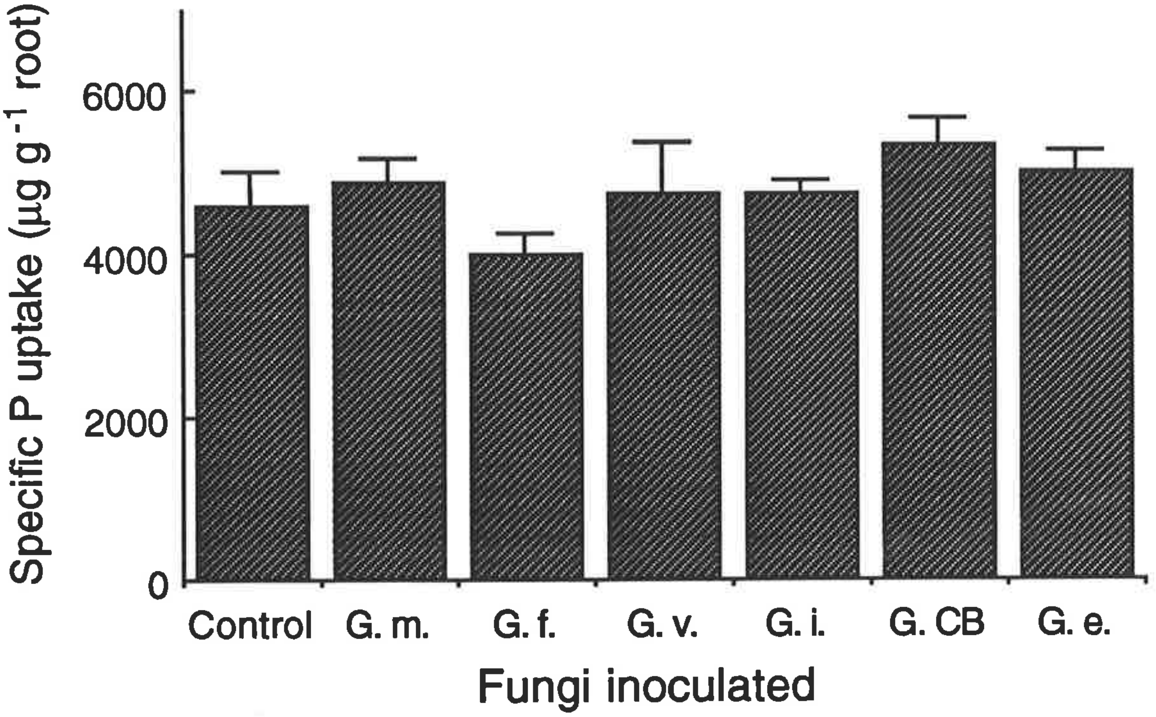


Figure 7.3 **Specific P uptake by barley (cv. Galleon) as affected by infection by six VA mycorrhizal fungi (G.m = *G. mosseae*, G.f. = *G. fasciculatum*, G.v. = *G. versiforme*, G.i. = *G. intraradices*, G.CB = *Glomus* "City Beach", G.e. = *G. etunicatum*) at a soil temperature of 15°C. Vertical bars represent standard errors of the means, n = 3.**

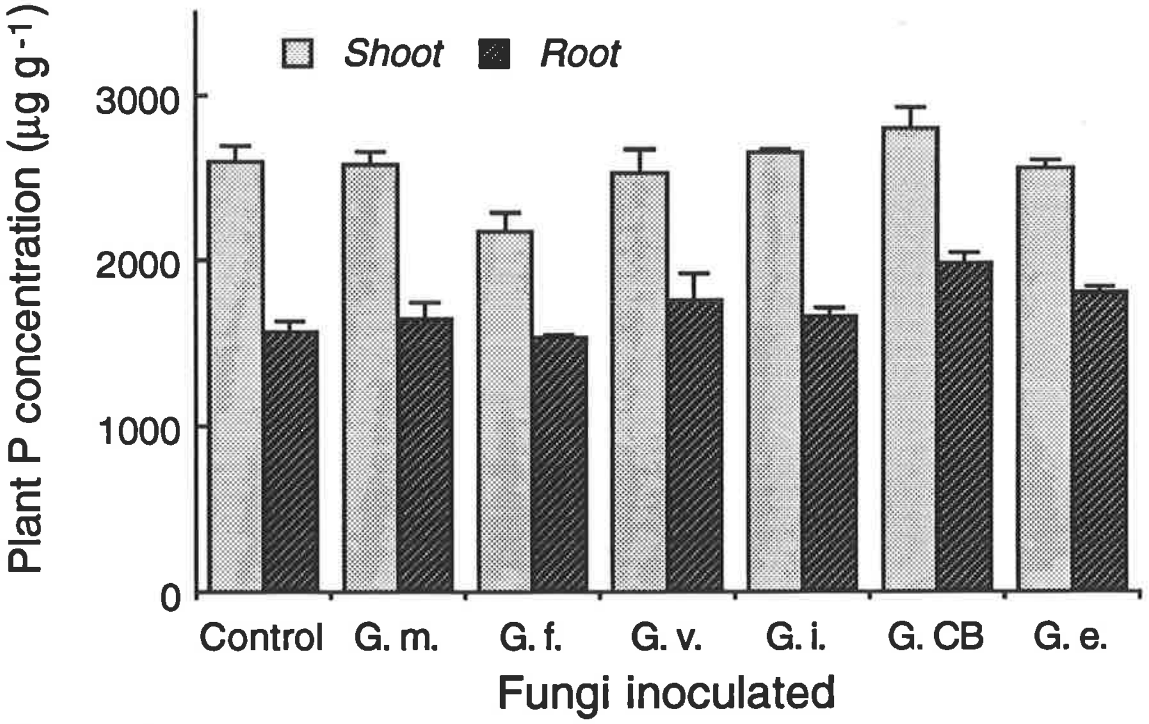


Figure 7.4 **Concentration of P in shoots and roots of barley (cv. Galleon) as affected by infection of six VA mycorrhizal fungi (G.m = *G. mosseae*, G.f. = *G. fasciculatum*, G.v. = *G. versiforme*, G.i. = *G. intraradices*, G.CB = *Glomus* "City Beach", G.e. = *G. etunicatum*) grown at 15°C soil temperature. Vertical bars represent standard errors of the means, n = 3.**

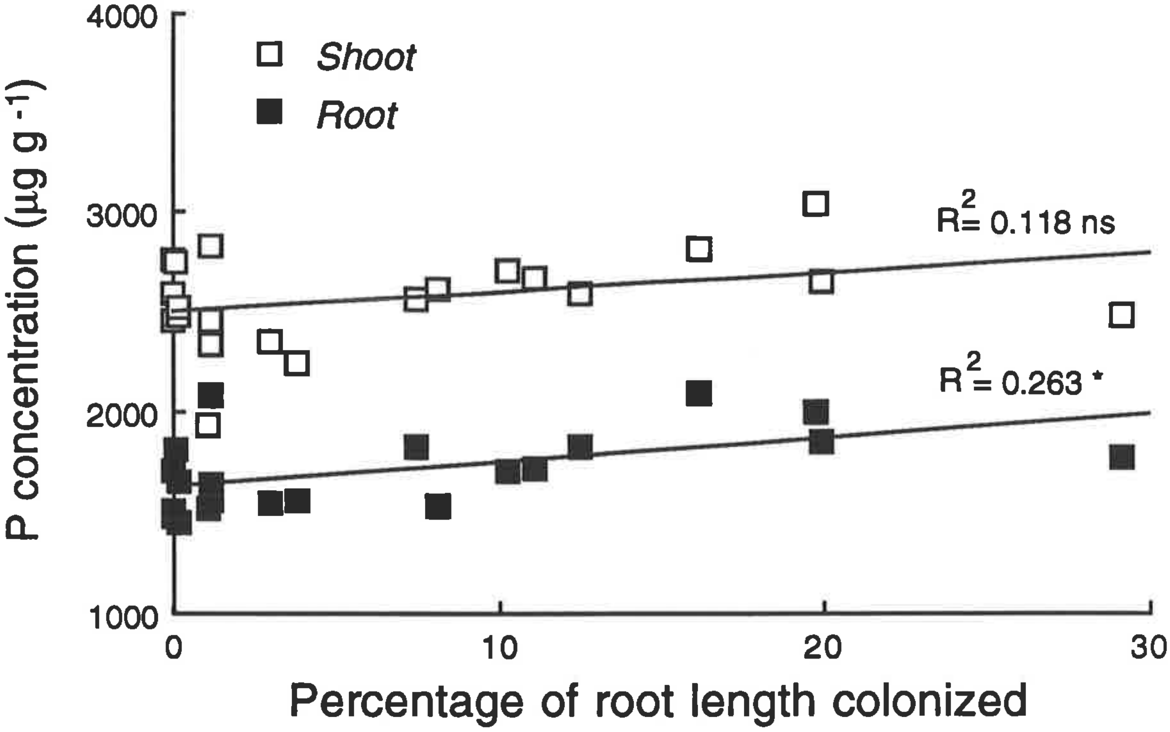


Figure 7.5 **The relationship between percentage of root length infected and concentration of P in tissues of barley plants. Significance at 0.05 level of probability is indicated by *; ns = not significant.**

7.4 Discussion

The main significant result obtained from this experiment was that *Glomus etunicatum* was the only VA mycorrhizal species examined which was able to maintain infectivity and to infect considerable length of plant roots at a soil temperature of 15°C. The results also emphasize the varying degree in infectivity of the different VA mycorrhizal fungi when colonizing barley plants at low soil temperature. However, I recognize that differences in infection were evaluated in this experiment on the basis of weight of inoculum used per pot. This does not take into account possible differences between fungi in terms of the numbers of infective propagules per g inoculum. Although the ages of the inocula were similar, when comparison was made among *G. mosseae*, *G. intraradices* and *Glomus* "City Beach" which had equal level of infection of their previous host plant (subclover), the three VA mycorrhizal fungi infected the barley plants in this experiment to varying degrees. This suggests that the results of this experiment were quite realistic to show the ability of the different VA mycorrhizal fungi to infect barley at a soil temperature of 15°C. Only two fungi, *i.e. G. etunicatum* and *Glomus* 'City Beach', infected more than 15% of the barley roots. Although mycorrhizal symbioses are not regarded as host specific, this result also implies that there may be certain levels of specificity among the mycorrhizal fungi and Galleon barley (see Hetrick 1984).

Combination of both low soil temperature and light intensity resulted in low infection. The present results also support the suggestion in Chapter 5 that light intensity is not an important factor in determining the extent of mycorrhizal infection. With very similar light intensity and soil temperature, the percentage of mycorrhizal infection by *G. intraradices*, plant growth and P status of the Galleon barley of this experiment were in agreement with those of Experiment 5.2. It wil be interesting to examine whether with increasing light intensity but with low soil temperature will result in increasing colonization by mycorrhizal fungi.

Wide variation in the level of mycorhizal infection was not associated with different growth responses. Only shoot dry weight of barley infected by *G. etunicatum* was higher than the control. Nemec (1978) and Secilia and Bagyaraj (1992) showed that citrus rootstocks and rice, respectively, had a high degree of dependency on *G. etunicatum*. As the effects of VA mycorrhizal infection on plant growth depend on environmental conditions (Hetrick *et al.* 1986, Son and Smith 1988), the results from this experiment can not be directly extrapolated to other situations.

The growth increase of mycorrhizal plants is generally attributed to high nutrient uptake, especially P (see Harley and Smith 1983). This experiment showed that none of the fungi examined had either higher specific P uptake or shoot P concentration than controls. However, root P concentration of plants infected by *G. etunicatum* and *Glomus* "City Beach" was higher than control, which was in agreement with previous findings (Secilia and Bagyaraj 1992).

There was a positive correlation between P concentration in plants and percentage of root length infected among all the fungi observed in this experiment. The rather low slopes shown in the relationship verify the lack of response to mycorrhizal infection in the uptake and concentration of P.

7.5 Conclusion

This preliminary investigation demonstrated that among the fungi examined, only *G. etunicatum* was shown to be suitable as a VA mycorrhizal symbiont for Galleon barley at 15°C of soil temperature. Plants infected by *G. etunicatum* produced high dry matter yield at low P supply. The results imply that the level of mycorrhizal infection is not regulated by light intensity. Therefore, an experiment with higher light intensity and soil temperature of 15°C is suggested.

MYCORRHIZAL RESPONSES OF BARLEY CULTIVARS DIFFERING IN P EFFICIENCY

8.1 Introduction

There is an increasing interest in breeding for nutritional traits to improve the productivity of crops on soils with low concentrations of available P and increase the efficiency of fertilizer use (Graham 1984). The physiological and morphological factors responsible for the differences in P efficiency, and the prospects of selecting plants with an improved P efficiency have been reviewed (Clark 1990). Föhse *et al.* (1991) have shown that properties of root systems, such as root hairs, influenced the efficiency of P uptake. Differences in uptake of P have been reported for some barley cultivars and have been attributed to variations in root growth (Schjørring and Nielsen 1987).

While substantial progress has been made in elucidating factors affecting efficiency of P (uptake and utilization) in plants, less attention has been devoted to the role of mycorrhiza in influencing assessment of P efficiency, particularly for cereal crops, which are widely believed to have a low dependency on mycorrhizas. Previously, the interaction between mycorrhizal infection and P efficiency in three cereals was addressed (Chapter 4). P nutrition of barley (cv. Galleon) was found to be more dependent on and influenced by mycorrhizal infection from field inoculum than that of wheat (cv. Spear) or rye (cv. S. A. Commercial). The objectives of the present experiment were 1) to develop the work of Chapter 4, 2) to investigate how barley cultivars which are different in P efficiency respond to mycorrhizal infection, 3) to examine the effect of mycorrhizal infection on the allocation of P in efficient and inefficient barley cultivars and 4) to determine whether this information can be related to differences in yield response to both mycorrhizal infection and P addition.

Few studies of mycorrhizas have been done at soil temperatures lower than 20°C, even though the temperatures in field soils of a large part of the region where barley is grown are below this value (Bowen 1991, Jensen 1983, Fabig *et al.* 1989). Power *et al.* (1963) considered that 15°C is the optimum for barley. Results presented in Chapters 4, 5 and 6 showed that mycorrhizal infection was affected by soil temperature. There is some evidence that growth responses of both the plant and the fungal symbiont are different from high soil temperatures and this suggests that barley cultivars may associate with certain VA mycorrhizal fungi which can grow and develop at soil temperatures lower than 20°C (Volkmar and Woodbury 1989, Hetrick *et al.* 1984, Grey 1991, Chapters 4 and 6). This study was set up at a soil temperature of 15°C.

8.2 Materials and Methods

8.2.1 Experiment 8.1

The objective of this experiment was to investigate P efficiency of eight barley cultivars in relation to mycorrhizal response. The experiment used soil from Avon, South Australia. The soil was air-dried, sieved to <2 mm and steam-sterilized. Nutrients were added to ensure that nutrients other than P did not limit plant growth.

The barley cultivars were chosen to provide a range of dry matter yields at no added P (*i.e.* a range of agronomic P efficiencies). Four cultivars (Yagan, WI 2737, O'Connor and Kaniere) had been shown in field trials (at Peake) to be efficient and four cultivars (Galleon, Shanon, WI 2539 and Skiff) inefficient (R D Wheeler pers. comm.). Galleon has been used in the experiments described in previous Chapters. The experiment had a randomized complete block design with three replicates. The treatments were 8 cultivars, 2 mycorrhiza (inoculated and non-inoculated) and 3 rates of P application in factorial combination. The seeds of barley were surface-sterilized and germinated. Phosphorus as KH_2PO_4 was added at 0, 10 or 20 mg kg^{-1} soil. For mycorrhizal treatments, barley plants were inoculated with 70 g inoculum of *G. etunicatum* which has been shown to be suitable for barley cv. Galleon at a soil temperature of 15°C (Chapter 7). The inoculum which consisted of roots, hyphae, spores was layered 5 cm below the surface.

Two two-day-old seedlings of barley were transplanted into each pot containing 1.4 kg of soil. The pots were placed in a glasshouse in three thermostatically controlled water tanks which were set at 15°C. The water content of the soil was brought to field capacity (11%) by adding deionized water daily.

The experiment was conducted during October to November. Air temperature ranged from 25-31/13-24°C (day/night). Mean solar radiation in the glasshouse ranged from 449 $\mu E\ m^{-2}\ s^{-1}$ in October to 534 $\mu E\ m^{-2}\ s^{-1}$ in November. The plants were harvested 42 days after transplanting. At harvest, the fresh and dry weights of the plants were recorded. Samples of the root systems were stained and assessed for the percentage of root length colonized by the fungus. P content of the plants was determined. Plant response (%) to mycorrhizas was expressed as described in Chapter 3.

Analyses of variance was performed with GENSTAT 5 (Genstat 5 Committee 1987). Data with non-homogenous variances were subjected to logarithmic or to angular transformation before analyses. The data have been retransformed for clarity of presentation in the tables and figures .

8.3 Results

8.3.1 Responses of barley cultivars

There were large differences among the barley cultivars in the percentage of root length infected (Fig. 8.1). Values for this parameter varied from 8.6% for Kaniere to 28.6% for Shannon in soil without added P. No infection was observed in the roots of non-inoculated plants. In general, the extent of mycorrhizal infection was lower following the application of P to the soil and the extent of this effect varied among the cultivars.

Fig. 8.2 shows that mycorrhizal infection had little effect on shoot dry weight and that all cultivars, except Shannon, had similar dry weights. With most cultivars, application of P increased shoot dry weight. The cultivar × phosphorus and the mycorrhiza × phosphorus interactions were significant for shoot dry weight but this was not the case for root dry weight (Table 8.1). Mycorrhizal treatments significantly affected only root dry weight, while phosphorus significantly affected only shoot dry weight.

Total P uptake of mycorrhizal plants was higher than that of non-mycorrhizal plants where no P was added to the soil (Fig. 8.3).

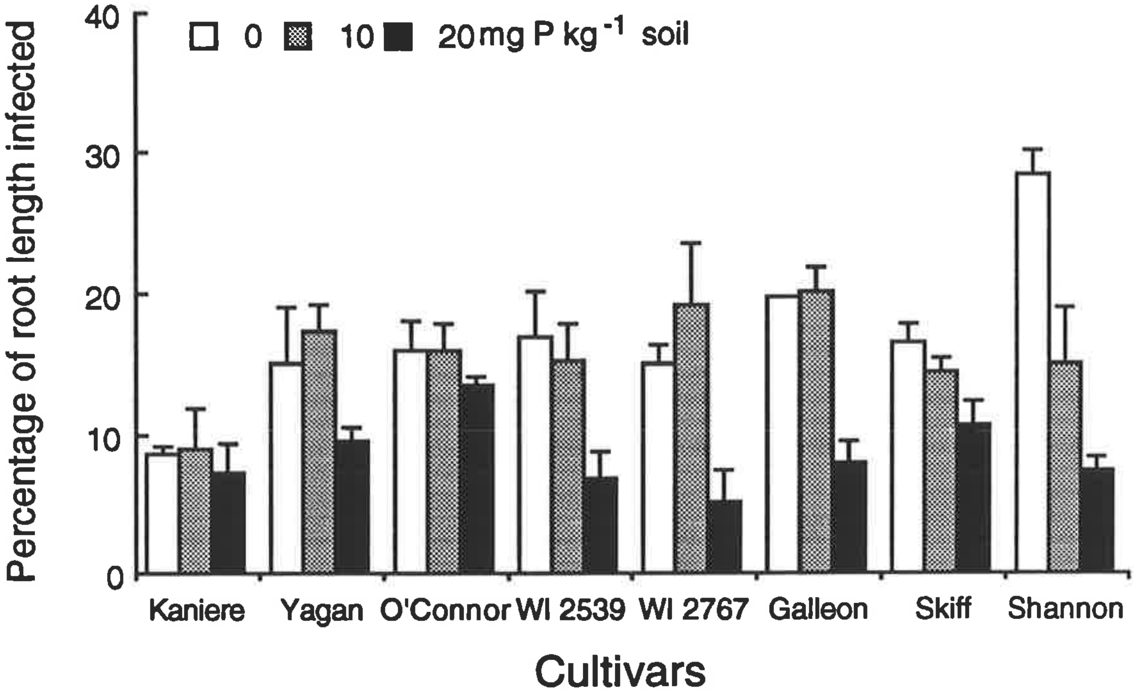


Figure 8.1 **Percentage of root length of barley cultivars infected by *G. etunicatum* as influenced by P application. Vertical bars represent standard errors of the means, n = 3; where no bar is shown, standard error is smaller than line**

Table 8.1 **Mean squares and significance of the effects of barley cultivars, mycorrhizal infection and phosphorus application on some parameters of P status and plant growth. P utilization efficiency (total dry matter yield per total P uptake); Specific P uptake (amount of P taken up by per unit weight of root).**

Source	df	Shoot DW	Root DW	Total P uptake	Infection	P Utilization Efficiency	Specific P uptake
Cultivar (C)	7	9.462***	2.493***	26.87**	23.069***	0.2772***	1.2258***
Mycorrhiza (M)	1	0.000	0.655**	204.7***	16301***	0.3825***	2.0264***
Phosphorus (P)	2	24.021***	0.166	3249***	195.57***	1.2302***	8.4635***
C x M	7	0.054	0.042	2.411	23.112***	0.0035	0.0133
C x P	14	0.439***	0.053	10.11	14.620**	0.0051*	0.0332
M x P	2	1.163***	0.061	33.37*	195.757***	0.0437***	0.0830*
C x M x P	14	0.068	0.077	2.958	14.677**	0.0040	0.0501*
Error	93	0.096	0.067	7.982	5.721	0.0022	0.0249

*, ** , *** Significant at 0.05, 0.01, 0.001 levels of probability, respectively.

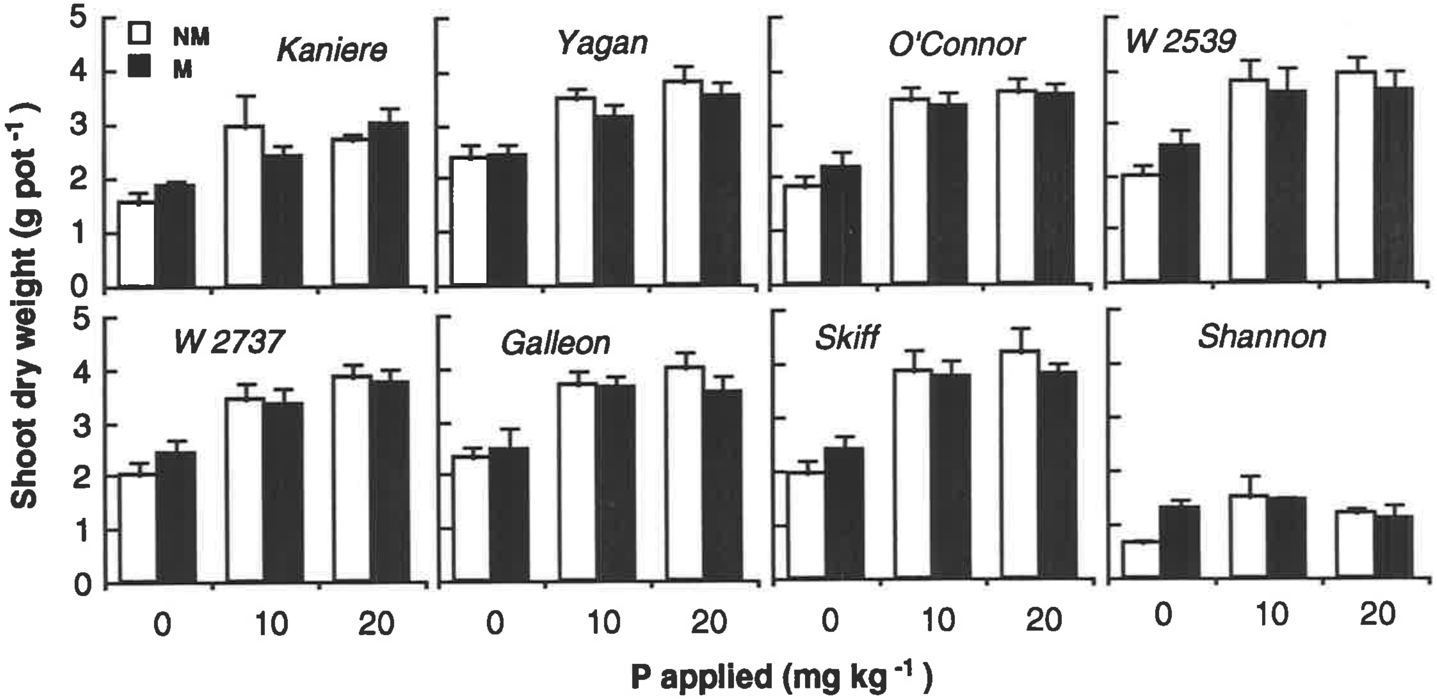


Figure 8.2 **Shoot dry weight of barley cultivars as affected by addition of P and mycorrhizal inoculation (M= mycorrhizal, NM= Non-mycorrhizal). Vertical bars represent standard errors of the means, n = 3; when no bar is shown, standard error is smaller than line.**

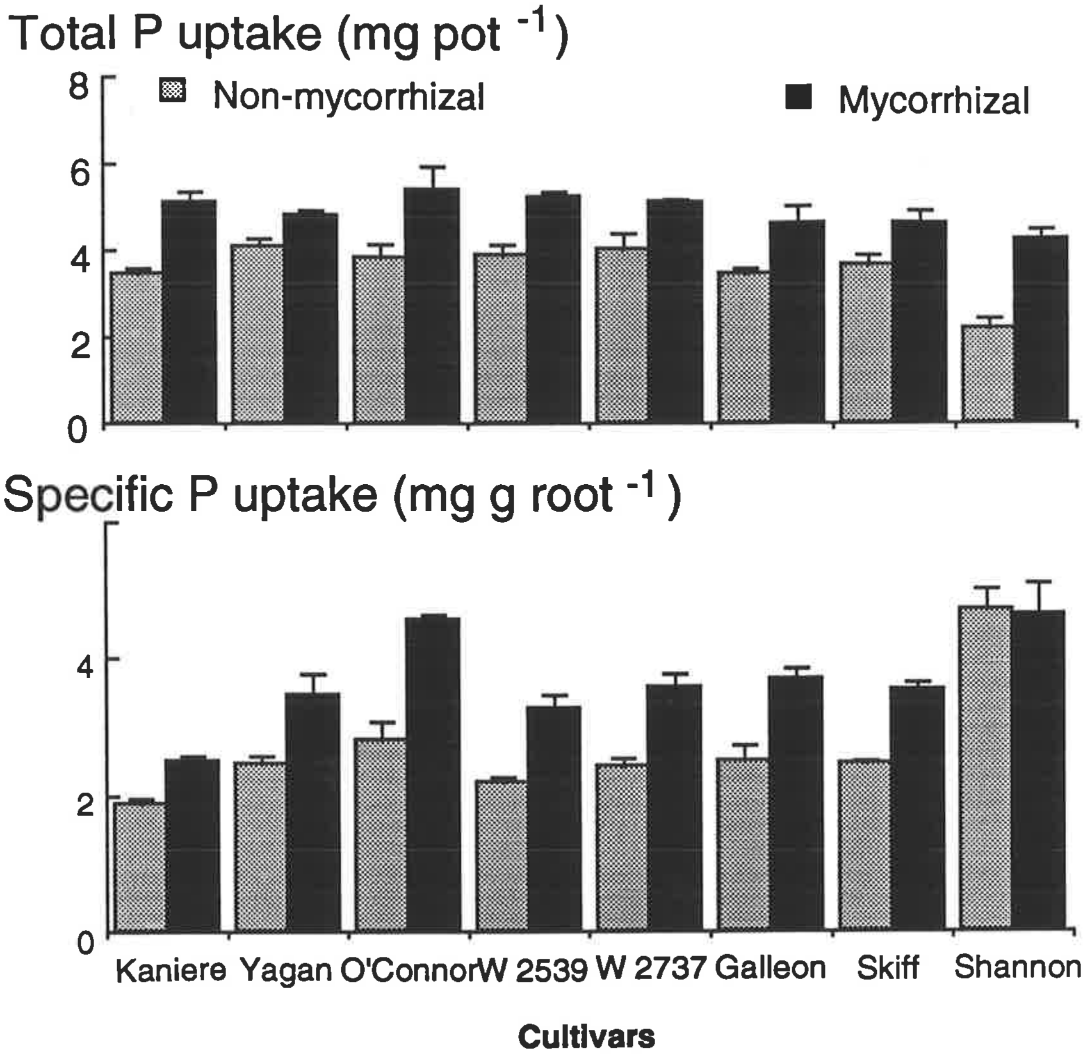


Figure 8.3 **Total P uptake and specific P uptake of barley cultivars as affected by mycorrhizal inoculation where no P was added. Vertical bars represent standard errors of the means, n = 3; where no bar is shown, standard error is smaller than line.**

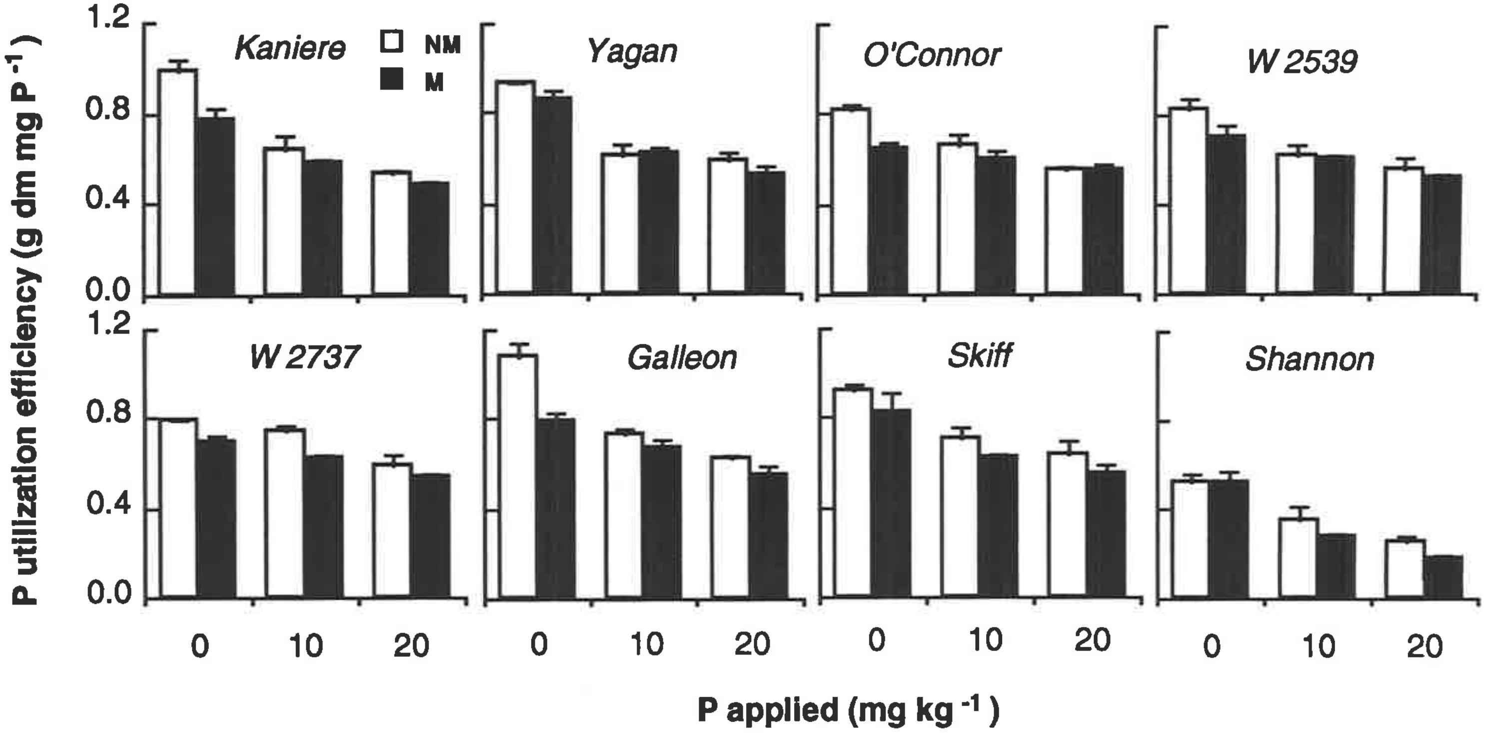


Figure 8.4 **P utilization efficiency of the barley cultivars as affected by mycorrhizal infection (M= mycorrhizal, NM= Non-mycorrhizal) and P addition. Vertical bars represent standard errors of the means, n = 3; where no bar is shown, standard error is smaller than line.**

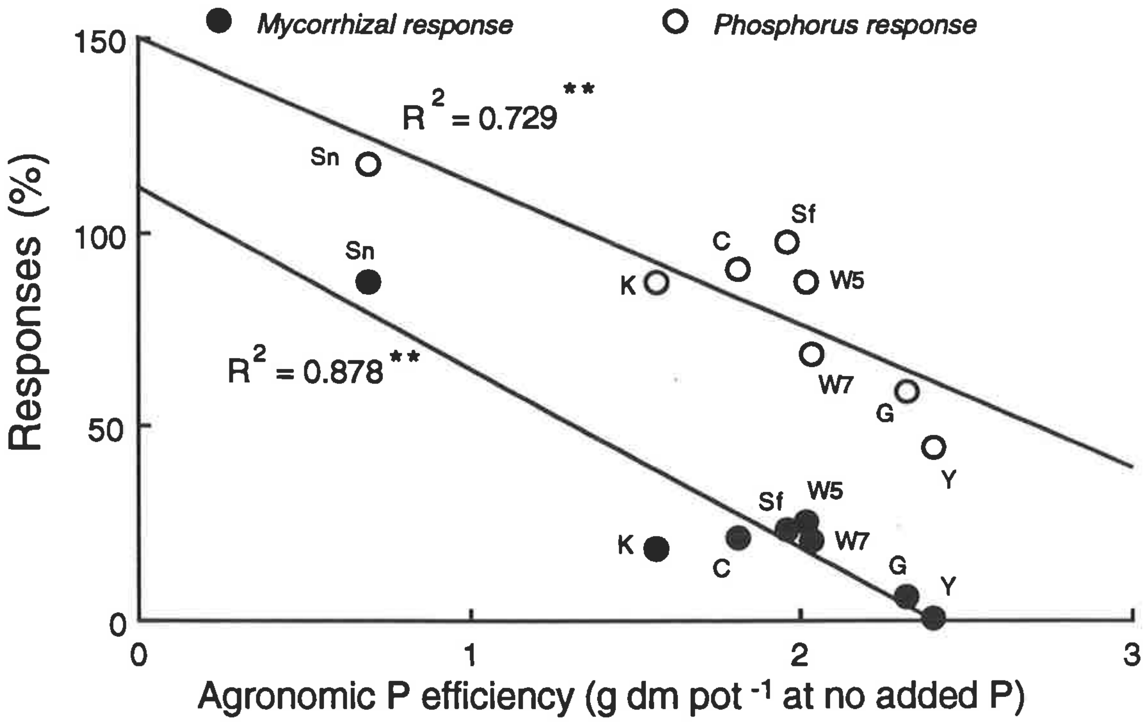


Figure 8.5 **Relationship between agronomic P efficiency of barley cultivars and their responses to both mycorrhizal infection and phosphorus addition (10 mg kg^{-1}). Sn (Shannon), K (Kaniere), C (O'Connor), Sf (Skiff), W5 (WI 2539), W7 (WI 2737), G (Galleon) and Y (Yagan). ** Significant at the 0.01 probability level.**

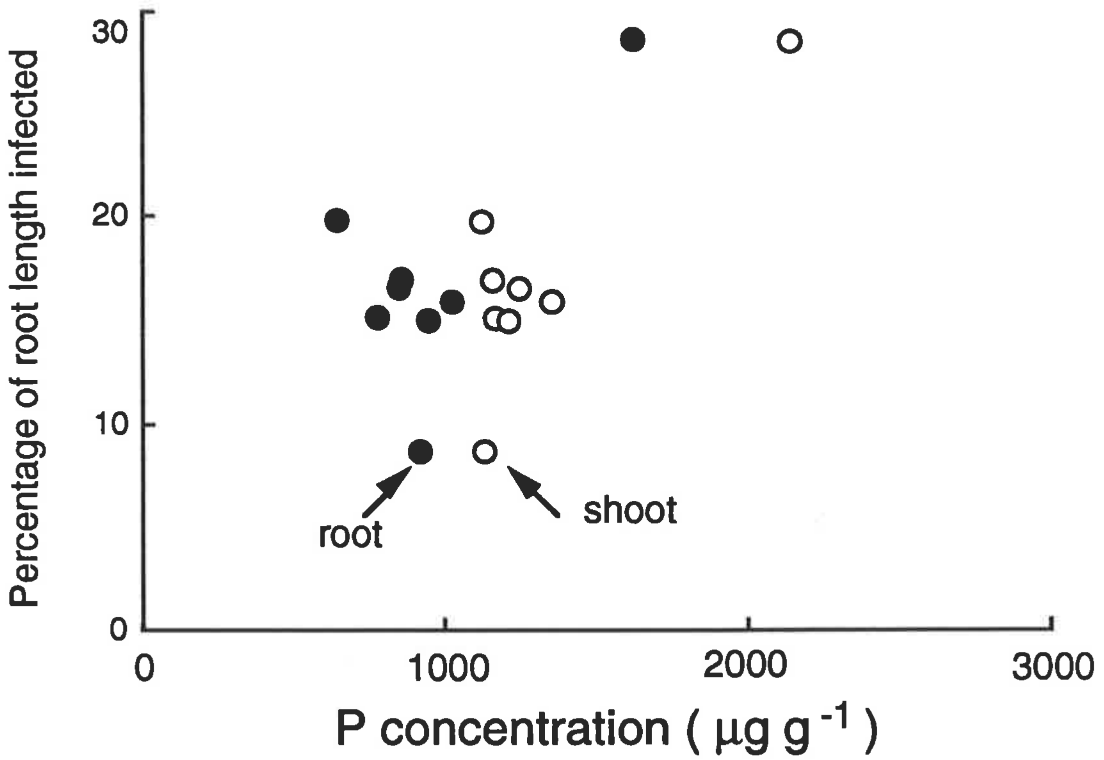


Figure 8.6. **Relationship between P concentration in barley cultivars, when neither P nor mycorrhizas were supplied, and percentage of root length colonized when inoculated with mycorrhizal inoculum.**

Mycorrhizal infection increased the uptake of P per unit weight of root (specific P uptake) by all cultivars except for Shannon (Fig. 8.3b).

Increasing the P supply to the barley plants reduced their dry matter yields per unit of P (P utilization efficiency). For cultivars other than Shannon and Skiff, P utilization efficiency of mycorrhizal plants was lower than in non-mycorrhizal plants, particularly in P deficient soil (Fig. 8.4). P utilization efficiency was negatively correlated ($r = -0.939$; $P = 0.01$) with mycorrhizal response.

For both phosphorus and mycorrhiza, there was a decrease in response as agronomic P efficiency (g dm with no added P and no mycorrhizal infection) of the cultivars increased (Fig. 8.5). The response to addition of 10 mg P kg^{-1} was higher than to mycorrhizal infection (Fig. 8.5).

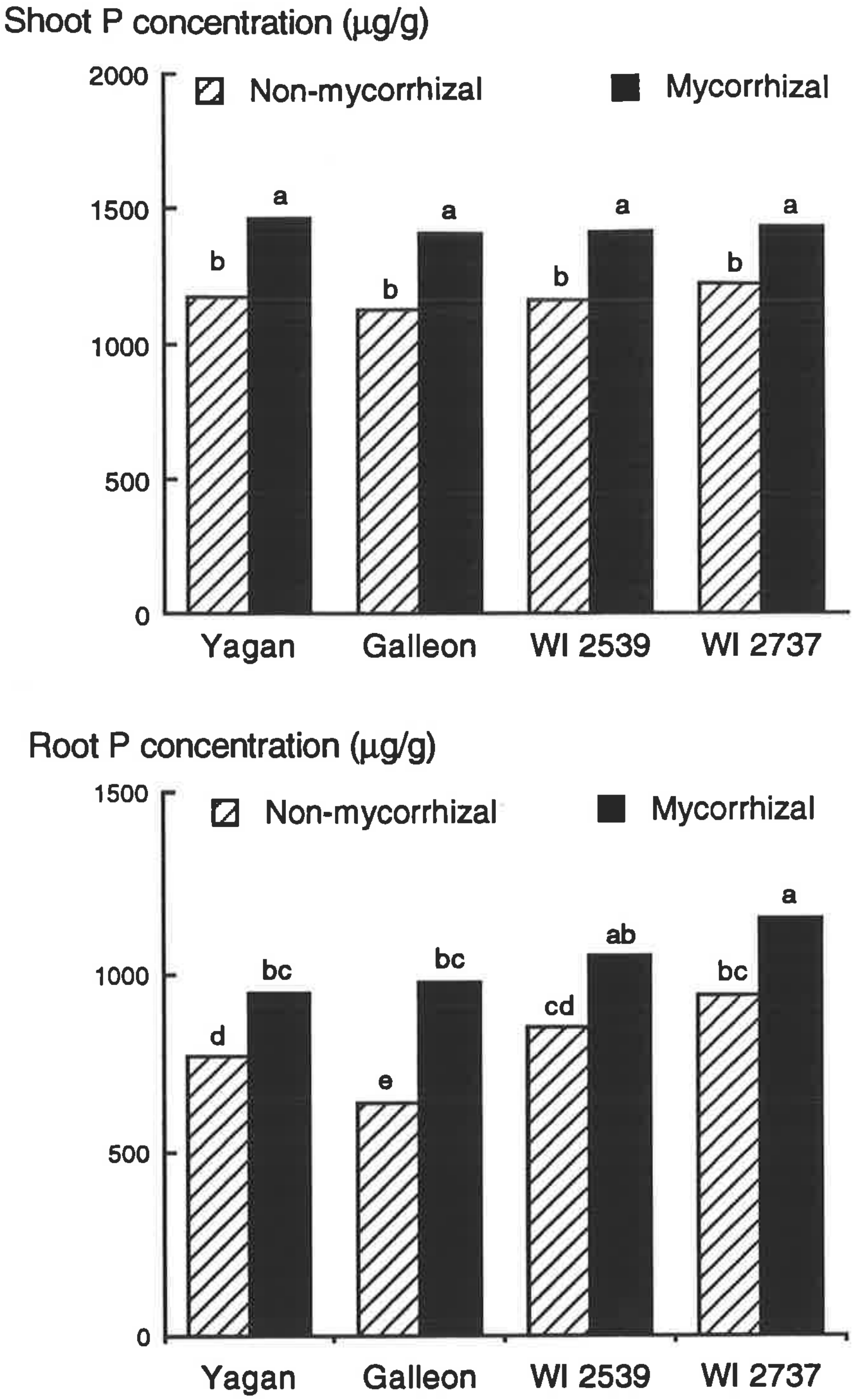
Among the cultivars, no statistically significant relationship was observed between the P concentration in the plant when neither P nor mycorrhiza were supplied, and the percentage of root length infected (Fig. 8.6).

8.3.2 P allocation in efficient and inefficient cultivars

Yagan and Galleon were chosen as efficient plants to study the allocation of P because they are very similar in their agronomic efficiency. The inefficent plants, WI 2737 and WI 2539 were chosen for the same reason. P concentration in both shoots and roots of mycorrhizal plants was higher than non-mycorrhizal plants in both agronomically P-efficient and inefficient cultivars (Fig. 8.7). There was no difference in shoot P concentration among the barley cultivars with the same treatment. However, differences in root P concentration among the barley cultivars were observed, with inefficient cultivars having higher root P concentrations than efficient cultivars in both mycorrhizal and non-mycorrhizal plants.

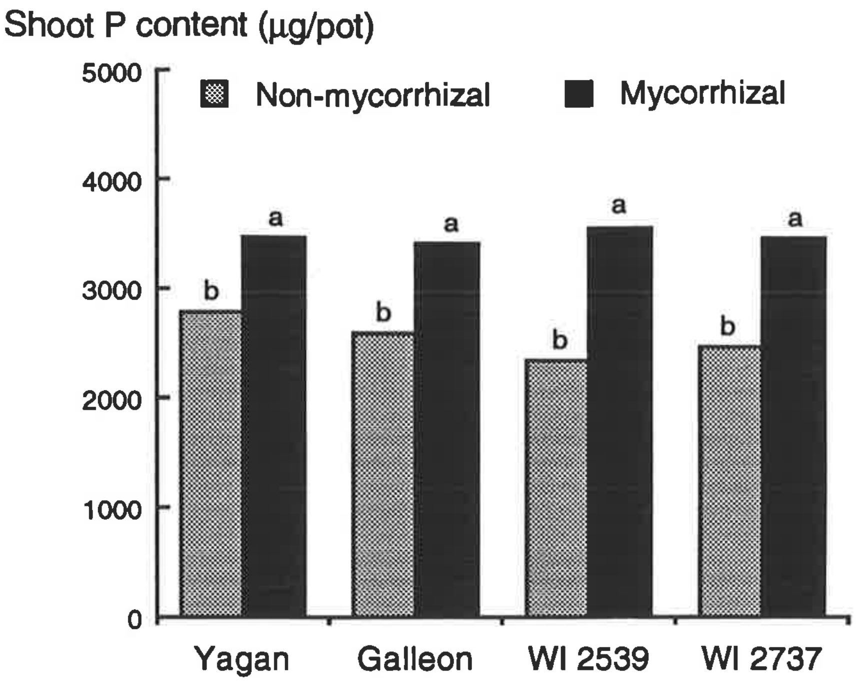
Shoot P content was significantly increased by the mycorrhizal infection in all four cultivars (Fig. 8.8). As with P concentration, shoot P content was not different among the barley cultivars whether or not they were inoculated. Mycorrhizal infection affected root P content only in Galleon. Root P content of inefficient cultivars was higher than that of efficient cultivars.

The proportion of total plant P in root systems of the inefficient cultivars was higher than that of the efficient ones if the plants were not mycorrhizal (Fig. 8.9). In P-inefficient cultivars, the effect of mycorrhiza in increasing allocation of P to the shoots was greater than in efficient cultivars.



% root length infected	14.7	19.8	16.7	14.9
	Efficient		Inefficient	

Figure 8.7 **Shoot and root P concentration and percentage of root length infected of efficient and inefficient barley cultivars. Bars followed by similar letter(s) are not siginificantly different at the 0.05 level of probability as determined by Duncan Multiple Range Test, n = 3.**



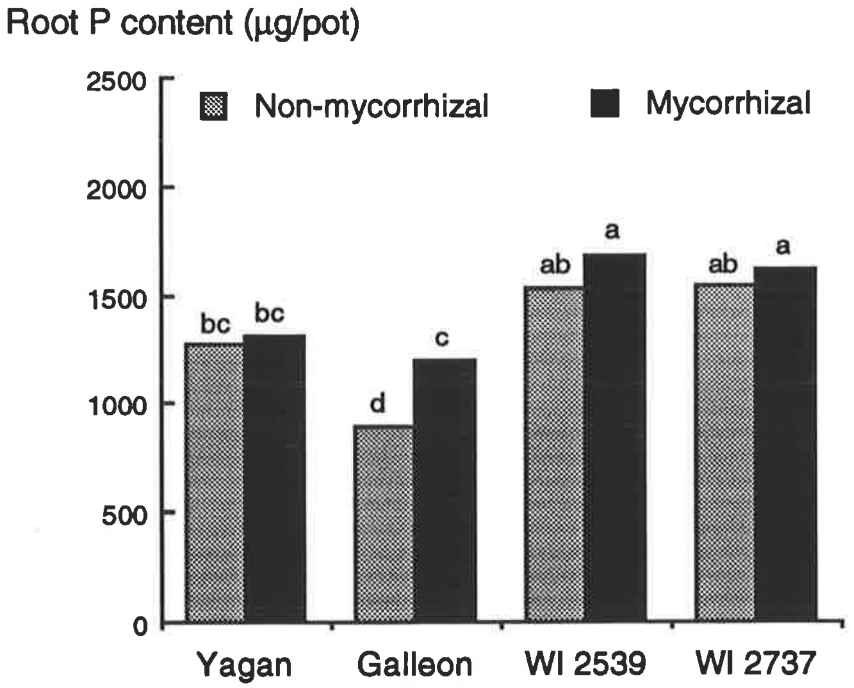


Figure 8.8 **Shoot and root P content in efficient and inefficient barley cultivars as affected by mycorrhizal infection. Bars followed by similar letter(s) are not significantly different at the 0.05 level of probability as determined by Duncan Multiple Range Test, n = 3.**

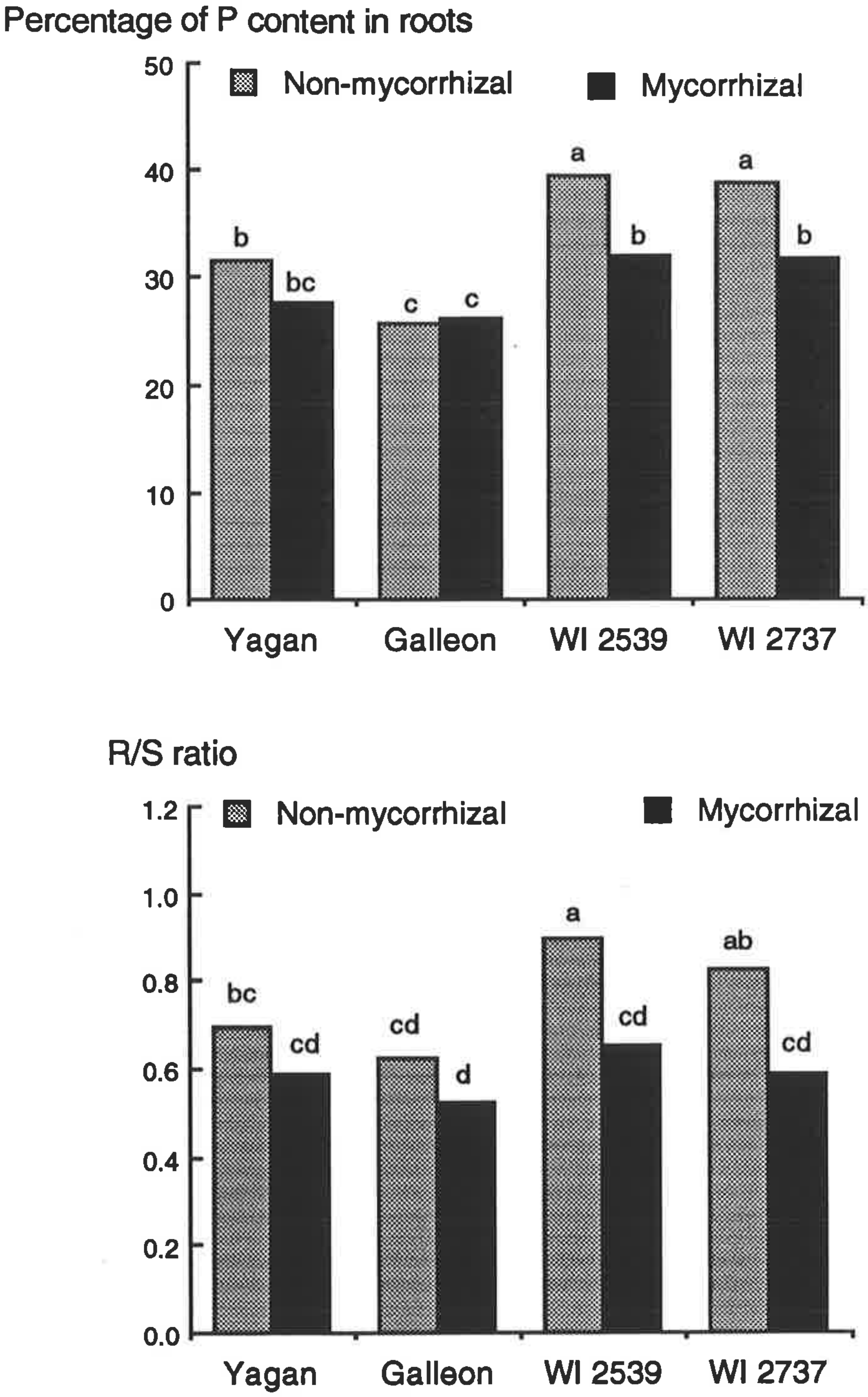


Figure 8.9 **Percentage of total P in plant which is present in roots and root:shoot dry weight ratios of efficient and inefficient barley cultivars as affected by mycorrhizal infection. Bars followed by similar letter(s) are not significantly different at the 0.05 level of probability as determined by Duncan Multiple Range Test, n = 3.**

In the absence of mycorrhiza, root:shoot dry weight ratio of inefficient cultivars was higher than in efficient cultivars. However, mycorrhizal infection significantly reduced root:shoot ratio of the inefficient cultivars but not of efficient cultivars.

8.4 Discussion

8.4.1 Responses of barley cultivars

This study clearly demonstrated that the yield response of the barley shoots to mycorrhizal infection depended upon the cultivar and that the response was negatively correlated with agronomic P efficiency. Thus, when grown with low P supply, a barley cultivar which is agronomically P efficient (*e.g.* Yagan) responds less to mycorrhizal infection than an inefficient cultivar (*e.g.* Shannon). Similar responses to P were noted, although application of P was more effective in increasing plant growth than was mycorrhizal infection. This observation is in agreement with the hypothesis of Lyness (1936) that the most P efficient cultivars in P deficient soil would not be the most effective with an optimal supply of P. Positive correlations between response to P application and response to mycorrhizal infection have also been reported by Ollivier *et al.* (1983) and Estaún *et al.* (1987) when they compared cultivars of cowpea and pea, respectively.

In contrast to findings in field selection studies (R D Wheeler pers. comm.), the present study showed that Galleon was P efficient and Kaniere inefficient with respect to agronomic P efficiency. These contradictory results may arise from differences in population and effectivity of VA mycorrhizal fungi, light intensity, soil temperature or soil P supply. It has been shown that differences in the extent of mycorrhizal infection of three cereals tested in different soils led to inconsistent results in the assessment of P efficiency (Chapter 4). Hence studies to select plants which are efficient in uptake or utilization of P should take into account the mycorrhizal status of the sites.

In general, the response to mycorrhizal infection by the barley cultivars in terms of shoot dry weight was small, even in soil with low P status, and similar results have been reported elsewhere (Jensen 1983). A significant response to infection was recorded only for Shannon. Barley with a fine-branched, extensive root system has an excellent capacity for uptake of immobile nutrients, such as P, and this may explain in part the low growth response to mycorrhizal infection. Nevertheless, significant responses to mycorrhizal infection in cereals have been reported by many workers (*e.g.* Thompson 1990). This could occur as a consequence of a higher percentage of root length infected and higher soil temperature in Thompson's (1990) study compared with the present experiment, although other factors (*e.g.* light intensity) could also contribute.

Mycorrhizal infection lowered the efficiency of utilization of P (higher P concentration in the whole plant) in most of the barley cultivars. Similar findings have been well documented and discussed (*e.g.* Smith 1980, Stribley *et al.* 1980a). The present results showed that barley cultivars efficient in utilization of P do not respond to mycorrhizal infection by increasing shoot dry weight. Shannon, inefficient in utilization of P, was highly responsive to mycorrhizal infection and shoot weight was nearly double that of non-mycorrhizal plants. In addition, this study has shown that agronomic P efficiency was positively correlated to efficiency in utilization of P and was negatively correlated to mycorrhizal response.

Mycorrhizal response appeared to be related primarily to P utilization efficiency. Results of this study are contradictory to the findings of Menge *et al.* (1978b) and Graham and Syvertsen (1985) who showed that sudangrass and citrus rootstocks, respectively, with low P concentration (high in P utilization efficiency) responded more to mycorrhizal infection than plants with high P concentration. This may reflect differences in mycorrhizal response of these crops, sudangrass and citrus being much more dependent on mycorrhizas than cereals. Baylis (1972) has hypothesized that plant species having fibrous root systems are generally considered to be less dependent on mycorrhizal infection than plants having coarse roots with few or no root hairs.

Response to mycorrhizal infection varies among the barley cultivars. Several factors, including root mass which contributes to the ability of the plants to absorb P from the soil, may control the response of the plants to mycorrhizal infection. In this study we found that some of the barley cultivars have a different percentage of root length infected in spite of having similar root mass. Variation in mycorrhizal infection may be due to an interaction between barley cultivars and the mycorrhizal fungus, *Glomus etunicatum*. Smith and Walker (1981) and Smith *et al.* (1992) also have pointed out that different levels of colonization between genotypes may arise from differences in the rates of growth of the fungus through the root cortex as well as the rates of root growth.

Differences in responses of plants to P nutrition are determined by the P required for the plant's metabolism, the allocation of P within plant, and the ability of the plant to take up P from the soil. Mycorrhizal infection increased P uptake by all barley cultivars from soil with low P supply. Specific P uptake was also increased by mycorrhizal infection, an effect which has been well documented (Smith *et al.* 1992). In the case of Shannon, the increase in total P uptake following mycorrhizal infection resulted in an increase in vegetative growth (including roots) which may explain the lack of difference in specific uptake between mycorrhizal and non-mycorrhizal plants of Shannon.

Data of this experiment indicate that P concentration in both roots and shoots of non-mycorrhizal barley plants when neither P nor mycorrhiza were supplied was not correlated with percentage of root length infected. This result contrasts with the results of Menge *et al.* (1978b), who found that high concentration of P in sudangrass tissues inhibited the infection of VA mycorrhizal fungi, and of Toth *et al.* (1984) who found that a positive correlation existed between P concentration in maize and percent mycorrhizal infection. Those findings suggest that besides P tissue concentration, genetic differences among the barley cultivars may control the extent of infection by a particular mycorrhizal fungus (Toth *et al.* 1984).

8.4.2 P allocation in efficient and inefficient cultivars

Agronomically P-inefficient barley cultivars responded to VA mycorrhizal infection by reducing the percentage of total plant P which was present in the roots, while efficient cultivars showed no significant response. This suggests that mycorrhizal infection increases the relative allocation of P to the shoots of inefficient cultivars. Increased supply of P to shoots resulted in better growth of above-ground parts, and reduction in root:shoot ratio in mycorrhizal plants compared with non-mycorrhizal plants. The results further showed that in the absence of mycorrhizal infection, inefficient cultivars had a higher proportion of total plant P allocated to the root systems than efficient ones. This effect is similar to results of Barrow (1975) and Schjørring and Jensen (1984) who found that plants responded to limited P supply by increasing the proportion of the total plant P allocated to the root systems. Comparing the present results with those of Barrow (1975) and Clarkson and Scattergood (1982), it appears that the response of inefficient cultivars is similar to that of clover and tomato, while the efficient ones behaved similarly to ryegrass and barley. Clarkson and Scattergood (1982) also demonstrated that P-stressed barley translocated less P to the shoot than non-stressed plants.

There was evidence in this study that agronomically P-inefficient barley cultivars actually had a higher capacity for uptake of P from the soil than efficient ones, especially when infected by mycorrhizal fungi. A high P uptake capacity might be expected in plants when grown at low P supply (Clarkson and Scattergood 1982, Cartwright 1972). As with agronomically P-inefficient cultivars, wild plants respond to P deficiency by increasing the absorption capacity for the limiting nutrient (Chapin, 1980).

In contrast, Azcón and Ocampo (1981) found that wheat cultivars that were less responsive to VA mycorrhizal infection had higher root:shoot ratios. The result differs from data presented here. In this study, although the inefficient cultivars were more responsive to both mycorrhizal infection and P addition, inefficient cultivars had higher root:shoot ratios than efficient cultivars. In their review, Smith and Gianinazzi-Pearson (1988) stated that root:shoot ratios of mycorrhizal plants are usually smaller than non-mycorrhizal plants. This present results confirmed their statement for inefficient cultivars, but not for P-efficient cultivars. The results also showed that inefficient barley cultivars which were non-mycorrhizal had higher root:shoot dry weight ratios than efficient cultivars. Increases in the proportion of the plant mass which is present as roots in response to limited P supply are well documented (Barrow 1975, Chapin and Bieleski 1982, Smith 1990a). However, mycorrhizal infection reduced the root:shoot ratio of the inefficient cultivars and produced in similar root:shoot ratios in all the barley cultivars. This result again showed that in the absence of mycorrhizas, inefficient cultivars were P-stressed where P supply was low and they allocated more biomass to their root systems.

Though the total amount of P present in the roots was not affected by mycorrhizal infection, the reduction (of about 17%) in allocation of P to the roots of inefficient plants following mycorrhizal infection, shows a greater plasticity in their response to nutrient supply than was exhibited by the efficient cultivars. They are able to increase root:shoot ratio and to survive, but they do not give high yields when P is inadequate. Greater plasticity permits greater response to P and mycorrhizal infection.

8.5 Conclusion

This study has demonstrated that agronomic P efficiency and P utilization efficiency of barley cultivars when neither P nor mycorrhiza were supplied affect the response of the plants to mycorrhizal infection. The mycorrhizal effect on these plant characteristics may have important implications for plant breeders in understanding the degree of mycorrhizal responses. P-inefficient cultivars allocated more P and biomass to root systems than efficient cultivars, in the absence of mycorrhiza. Response of efficient cultivars to mycorrhizal infection was smaller than that of inefficient ones. As efficiency in uptake can be affected by the presence of root hairs, the next study was to examine whether the different responses to mycorrhizal infection were associated with root hair characteristics.

Chapter Nine

GROWTH RESPONSE AND PHOSPHORUS UPTAKE OF RYE WITH LONG AND SHORT ROOT HAIRS INFECTED BY *GLOMUS INTRARADICES*

9.1 Introduction

Characteristics of root systems, both morphological or physiological, may affect the uptake of P from soil. Most plant species have root hairs, although there is considerable variation in density and length and some species have long root hairs or none. It seems particularly appropriate to recognize that in a paper published 56 years ago, Dittmer (1937) found that the area of root surface may be expanded nearly three times by root hairs. The presence of root hairs enables plants to explore more soil volume and it has been well documented that root hairs increase the uptake of P by plant roots (Lewis and Quirk 1967, Itoh and Barber 1983, Föhse *et al.* 1991). Bouldin (1961) estimated mathematically that the total flux of P across root hair surfaces may be 3 to 10 times greater than the flux across the surface of the central root cylinder (also see Nye 1966).

Mycorrhizal symbiosis, one of the root characteristics which is able to improve P nutrition of the plants, is still ignored by many workers. Although the roles of mycorrhizal fungi and root hairs have been well recognized in the uptake of relatively immobile nutrients, such as P, very little is known about the interactions between them in the P nutrition of plants. Assessment of P efficiency has been demonstrated to be affected both by root hairs (Föhse *et al.* 1991, Caradus 1981) and by mycorrhizal infection (Chapter 4). Results of Chapter 8 showed that plants which produced more shoot dry weight under low P conditions (*i.e.* agronomically P-efficient plants) were less responsive to mycorrhizal infection and P addition than less efficient plants. This suggests that a plant with long root hairs, which is agronomically more efficient (Caradus, 1981), may be less responsive to mycorrhizal infection. Thus, a hypothesis was proposed that growth of plants with short root hairs would be more responsive to mycorrhizal infection than plants with long root hairs. This is an extension of the "magnolioid root" hypothesis proposed by Baylis (1975), and examined by St John (1980) who compared the effects of root hair length in different species, on responsiveness to VA mycorrhizal infection.

The objective of this study was to investigate the influence of mycorrhizal infection and root hair length and their interaction on plant growth and P efficiency in a single species, using two selections of rye contrasting in root hair length.

9.2 Materials and Methods

9.2.1 Experiment 9.1

In this experiment, there were 16 treatments resulting from a factorial combination of two selections, two inoculations (mycorrhizal and non-mycorrhizal), two rates of P and two harvest times. The experiment had a randomized complete block design with four replicates per treatment.

The soil used in this study was Avon soil. After it was air dried and sievied, the soil was sterilized by steaming. Sterile air-dried soil (1.2 kg) was placed in each of 64 plastic pots and was supplied with nutrients as described in Chapter 3.

Two concentrations of applied P were established: 0 and 10 mg P kg^{-1} which was given as KH_2PO_4 and mixed with the soil. For the mycorrhizal treatment, half of the pots had 60 g inoculum pot^{-1} of *Glomus intraradices* added as pot culture material layered 5 cm below the soil surface. The other half of the pots, for non-mycorrhizal treatments, received 60 g pot^{-1} of non-mycorrhizal inoculum. Mycorrhizal inoculum consisted of soil and roots of *Trifolium subterraneum* L. colonized by *G. intraradices*, while in the non-mycorrhizal inoculum the roots were not infected by mycorrhizal fungi. Propagule density of the inoculum was determined by the most probable number method.

Seeds of rye (*Secale cereale* L. cv. Imperial) were obtained from Dr E R Sears, University of Missouri, USA. The original observation and selection of long (LRH) and short root hairs (SRH) were done by Mr Colin Rivers, Waite Agricultural Research Institute, Australia. The seeds were surface sterilized and germinated on moist filter paper at room temperature. Black paper was used to ease in distinguishing the two selections. Four seedlings of three-day old rye were transplanted into each pot and after establishment were thinned to two plants per pot.

Plants were grown in a growth chamber with a 14 h light and 10 h dark photoperiod and a 22°C day/17°C night thermoperiod with relative air humidity held at 60% day, 80% night. The photosynthetic photon flux density (measured at the soil surface) in the growth chamber was maintained at 380 µE m^{-2} s^{-1}. The plants were watered daily using deionized water to bring water content to field capacity (11%). After each watering the pots were rerandomized.

The plants were harvested 4 and 6 weeks after transplanting. Fresh and dry weight of shoots and roots were recorded. Samples of the root systems were taken for the assessment of mycorrhizal infection and root length measurement after staining. P content of shoots and roots were also determined.

One core (1.3 cm diameter) of soil was collected from the centre of each pot before the last harvest. The soil with roots was dispersed in 0.2% Na-pyrophosphate for 4 h. The root segments were placed onto a piece of sieve and carefully washed from soil in a bucket of water, rinsed and stained. Two 2-cm segments of undamaged roots per pot were examined under compound microscope for measurement of root hair length, root hair density and diameter of roots and root hairs.

Response (%) of plants to mycorrhizal infection or P application was calculated as described in Chapter 3. For all analysis, the F-test was used to test the significance of the main factors or analysis of variance using Genstat (Genstat 5 Committee 1987). Where appropriate, individual mean comparisons were determined by Duncan's Multiple Range Test.

9.2.2 *Modelling P uptake*

Calculation of predicted P uptake by the two rye selections used the nutrient uptake model described by Barber and Cushman (1981) and written in personal computer version by Oates and Barber (1987). The model combined equations which expressed both plant growth and nutrient availability and therefore it can calculate or predict the uptake of nutrients. When the values for soil and plant parameters are given, the model calculates nutrient uptake for a specified time. In this section the values for the parameters of soil nutrient supply (initial P concentration in soil solution, $C_{li} = 14 \times 10^{-4}$ mmol L^{-1}; effective diffusion coefficient, $D_e = 3.7 \times 10^{-10}$; buffer power, b = 1500) and root uptake kinetics (maximum net influx, $I_{max} = 17 \times 10^{-7}$ mmol m^{-2} s^{-1}; Michaelis-Menten constant, $K_m = 1300$ mmol L^{-1}; P concentration in soil solution when net influx equals zero, $C_{lmin} = 1 \times 10^{-4}$ mmol L^{-1}; water flux, $v_o = 2.7 \times 10^{-7}$ cm^2 s^{-1}) were taken from Föhse *et al.* (1991). The values of the parameters of root morphological characteristics (initial root length, L_o; rate of root growth, k; mean root radius, r_o; half distance between root axes, r_1) were obtained from the data of Experiment 9.1.

9.3 Results

No infection was observed in the plants inoculated with non-mycorrhizal inoculum. The most probable number of propagules of *G. intraradices* in the inoculum was calculated as 24.8 per g inoculum. Inoculation with this material (1500 propagules per pot) resulted in rapid infection in the roots of rye plants with mean of 21% for SRH and 10% for LRH plants, 4 weeks after transplanting (Fig. 9.1). The level of infection in both selections was reduced by the application of P to the soil. However, the total length of root infected did not differ between the selections or P treatment (Fig. 9.1), except for SRH plants grown at low P and harvested after 6 weeks, which had at least twice the infected length per pot of any other treatment. The mean length and diameter of root hairs after 6 weeks were 530 µm and 20 µm for SRH and 990 µm and 8 µm for LRH selections, respectively (Table 9.1). In the presence of mycorrhizal infection, total surface area of root of SRH was larger than LRH plants (Table 9.1). No significant interactions between mycorrhizal infection and the length of root hairs was found for any parameters measured. Similar result was obtained for M x S x P interaction, except for root length (Table 9.2).

Mycorrhizal infection did not significantly affect the shoot dry weight or root to shoot dry weight ratio of the plants (Tables 9.2 and 9.3), except LRH plants supplied with P. Generally, LRH plants had higher values for these parameters than SRH plants. With low P supply, non-mycorrhizal LRH plants had significantly greater root lengths than other treatments at 6 weeks.

Table 9.4 shows that the concentration of P in the shoots of LRH plants was consistently higher than that of SRH plants after 4 weeks, regardless of their mycorrhizal status. The only significant effect of mycorrhizas on P concentration was to increase values in LRH plants at 6 weeks.

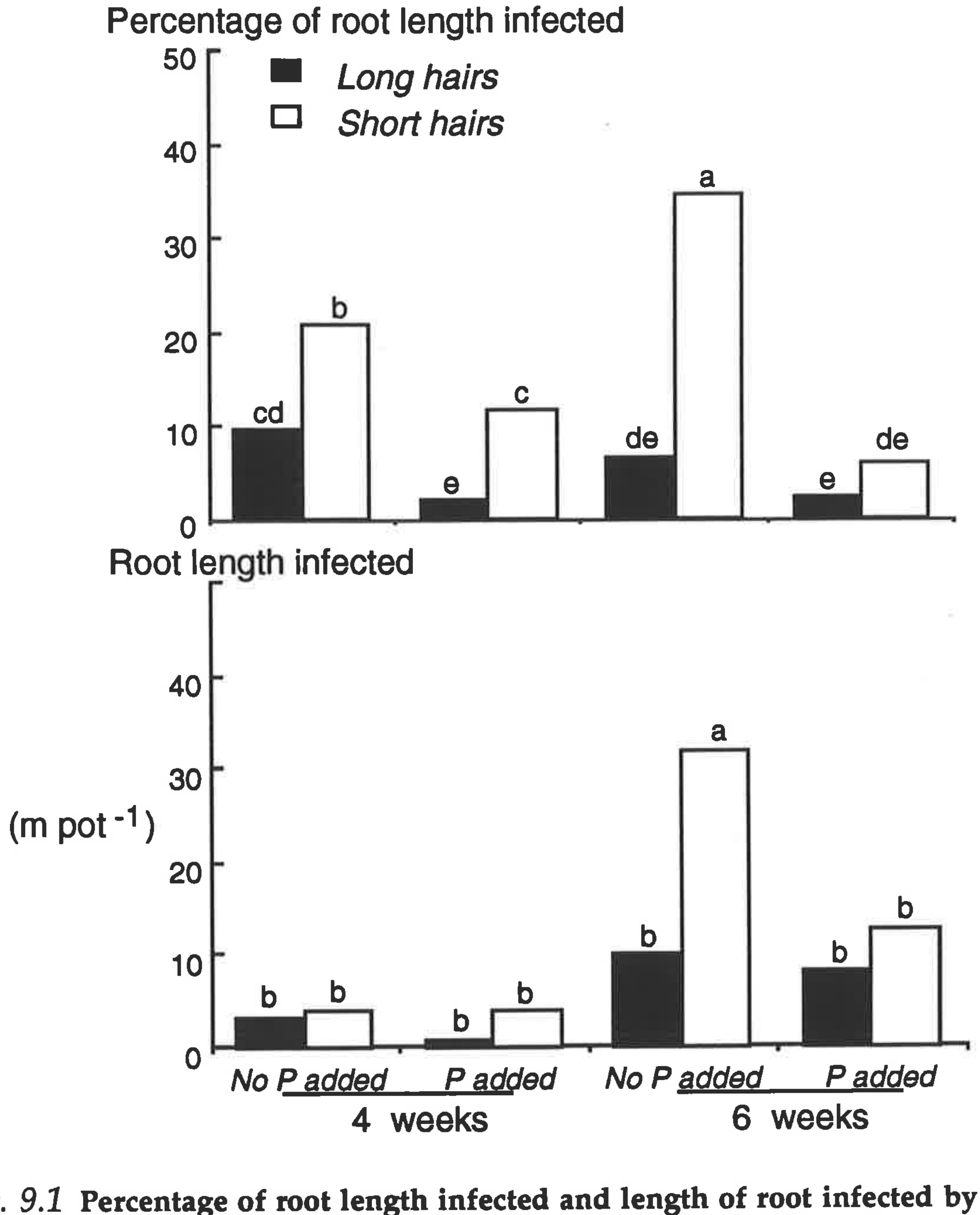


Fig. 9.1 **Percentage of root length infected and length of root infected by *G. intraradices* of two rye selections differing in their root hair length with and without application of P, 4 and 6 weeks after transplanting. Bars followed by similar letter(s) are not significantly different at the 0.05 level of probability as determined by Duncan Multiple Range Test, n = 4.**

Table. 9.1 **Root hair characteristics and total root surface per 100 m root of SRH (plant with short root hairs) and LRH (plant with long root hairs) plants as affected by mycorrhizal infection (M = mycorrhizal, NM = non-mycorrhizal) at low P supply 6 weeks after transplanting. Total surface area per pot given in the brackets. Values are means ± SE, n = 8.**

Plant	Root hair length (µm)	Root hair diameter (µm)	Root hair density (hairs mm^{-1})	Total surface area[a] NM (m^2)	Total surface area[b] M (m^2)
SRH	530	20	70	0.271 (0.198)	0.445 (0.396)
LRH	990	8	80	0.237 (0.768)	0.240 (0.252)

[a] Root diameter = 120 µm.

[b] Mean length of external hyphae was assumed to be 200 m m^{-1} infected root, based on the results of Jakobsen *et al.* (1992) and Pearson and Jakobsen (1993); hyphae diameter = 8 µm.

The relation between root length and total P uptake by the plants is presented in Fig. 9.2. For non-mycorrhizal plants the relationship tends to follow a logarithmic model, while for mycorrhizal plants a linear model provides the best fit to the data. Mycorrhizal plants from both LRH and SRH had higher total P uptake than non-mycorrhizal plants. Based on the area below the curves up to the same root length of 100 m per pot, it was calculated that mycorrhizal infection increased the uptake of P 2.1% for LRH plants and 19.4% for SRH plants. However, in the absence of mycorrhizas, the uptake for LRH was 86.6% higher than SRH plants.

Specific P uptake by all the plants decreased with time. Non-mycorrhizal plants of SRH plants had lower specific P uptake than LRH plants after 4 weeks, but had a higher value for that parameter after 6 weeks (Fig. 9.3). In the presence of mycorrhizas, there was no difference in specific P uptake between LRH and SRH plants. Mycorrhizal plants had lower P utilization efficiency than non-mycorrhizal ones, except LRH plants at 4 weeks. A greater percentage of total P in roots of mycorrhizal plants was observed only in SRH plants.

The mycorrhizal response of the plants was negatively correlated to agronomic P efficiency (Fig. 9.4). LRH plants, which were agronomically more P efficient, showed a lower response to mycorrhizal infection than SRH plants ($P \leq 0.05$).

A comparison of predicted and measured P uptake by the rye plants, as influenced by mycorrhizal infection, is presented in Fig. 9.5. The relationship showed that the model and/or the parameters of soil nutrient supply and root uptake kinetics used here underpredicted P uptake, especially for plants with long root hairs or infected by mycorrhizal fungi.

Table 9.2. **Probability of *F* for shoot dry weight, root length, root P concentration and specific P uptake.**

Source of variation	df	Shoot dry weight	Root length	Root P concentration	Specific P uptake
Selection (S)	1	*** [a]	**	N S	***
Mycorrhiza (M)	1	N S [b]	N S	**	*
Phosphorus (P)	1	***	***	**	***
Harvest (H)	1	***	***	***	***
S x M	1	N S	N S	N S	N S
S x P	1	N S	N S	*	**
S x H	1	*	*	*	**
M x P	1	N S	N S	N S	N S
M x H	1	N S	N S	N S	N S
P x H	1	***	**	N S	**
S x M x P	1	N S	*	N S	N S
S x M x H	1	N S	N S	N S	N S
S x P x H	1	N S	N S	N S	N S
M x P x H	1	*	N S	*	**
S x M x P x H	1	N S	*	N S	**

[a] *, **, *** significance at the 0.05, 0.01 and 0.001 probability level, respectively.

[b] N S = not significant at $p = 0.05$.

Table 9.3. **Shoot dry weight, root to shoot dry weight ratio and root length of rye with short and long root hairs as affected by infection by *G. intraradices* and P added when harvested 4 and 6 weeks after transplanting. Values are means ± standard errors, n = 4.**

Treatment	4 weeks after transplanting		6 weeks after transplanting	
	No P added	P added	No P added	P added
		Shoot dry weight (g pot^{-1})		
Short root hair:				
Non-mycorrhizal	0.11 c	0.41 b	0.37 d	1.51 b
Mycorrhizal	0.14 c	0.29 bc	0.41 d	1.52 b
Long root hair:				
Non-mycorrhizal	0.31 bc	0.71 a	1.29 bc	1.83 ab
Mycorrhizal	0.26 bc	0.41 b	0.69 cd	2.25 a
		Root/shoot ratio		
Short root hair:				
Non-mycorrhizal	0.48 d	0.62 ab	0.66 b	0.96 ab
Mycorrhizal	0.54 bcd	0.59 abc	0.92 b	0.88 b
Long root hair:				
Non-mycorrhizal	0.64 ab	0.65 ab	1.38 a	1.01 ab
Mycorrhizal	0.69 a	0.49 cd	1.16 ab	1.06 ab
		Root length (m pot^{-1})		
Short root hair:				
Non-mycorrhizal	14 b	45 ab	73 b	325 a
Mycorrhizal	18 b	33 ab	89 b	229 ab
Long root hair:				
Non-mycorrhizal	43 ab	67 a	324 a	342 a
Mycorrhizal	28 b	42 ab	105 b	415 a

Means within the same time of harvest followed by similar letter(s) are not significantly different at the 0.05 level of probability as determined by Duncan's Multiple Range Test, n = 4.

Table 9.4. **P concentration of shoots and roots of rye selections differing in root hair length as influenced by mycorrhizal infection in soil with low P supply. Values are means ± standard errors, n = 4.**

Treatment	P concentration ($\mu g\ g^{-1}$)			
	4 weeks after transplanting		6 weeks after transplanting	
	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
		Shoot		
Short root hair	1713 b	1824 b	1742 b	1999 ab
Long root hair	2837 a	2856 a	1622 b	2385 a
		Root		
Short root hair	1922 b	2229 a	1434 ab	1847 a
Long root hair	2038 ab	2166 ab	937 b	1652 a

Means within the same time of harvest followed by similar letter(s) are not significantly different at the 0.05 level of probability as determined by Duncan's Multiple Range Test, n =4.

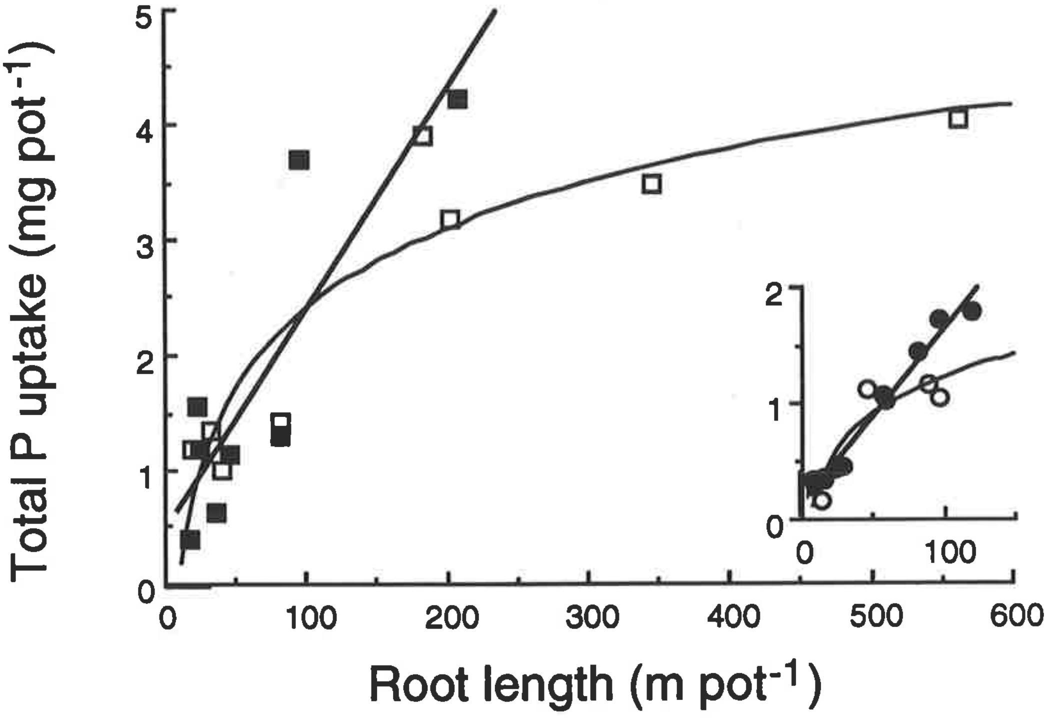


Fig. 9.2 **The relationship between root length and total P uptake of plants with long (□ ■) and short [(○ ●) inserted)] root hairs as affected by mycorrhizal (■ ●) and non-mycorrhizal (□ ○) treatments.**

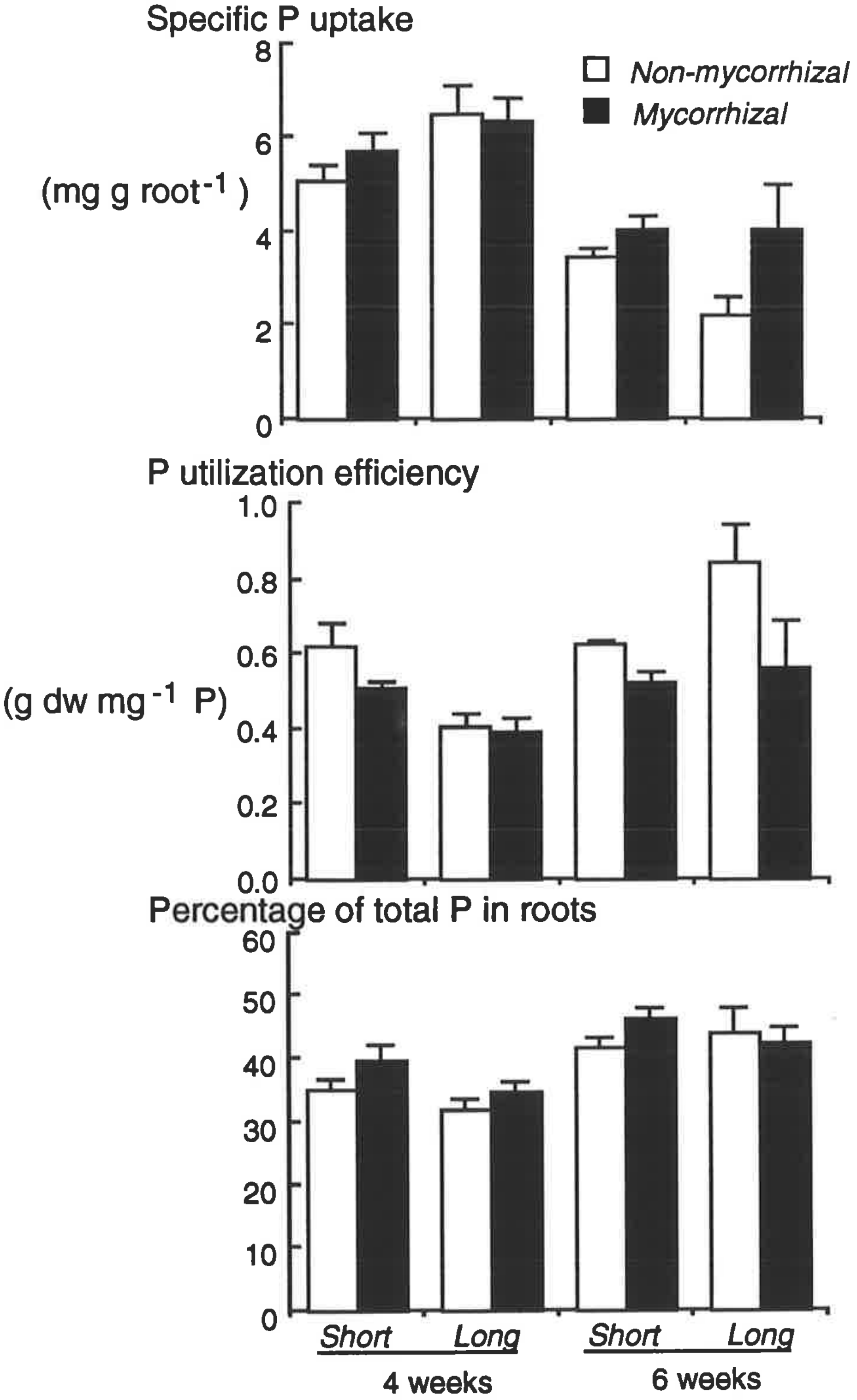


Fig. 9.3 **Specific P uptake, P utilization efficiency and percentage of total plant P present in roots of two rye selections with long and with short root hairs as affected by mycorrhizal infection. Vertical bars represent standard errors of the means, n = 4.**

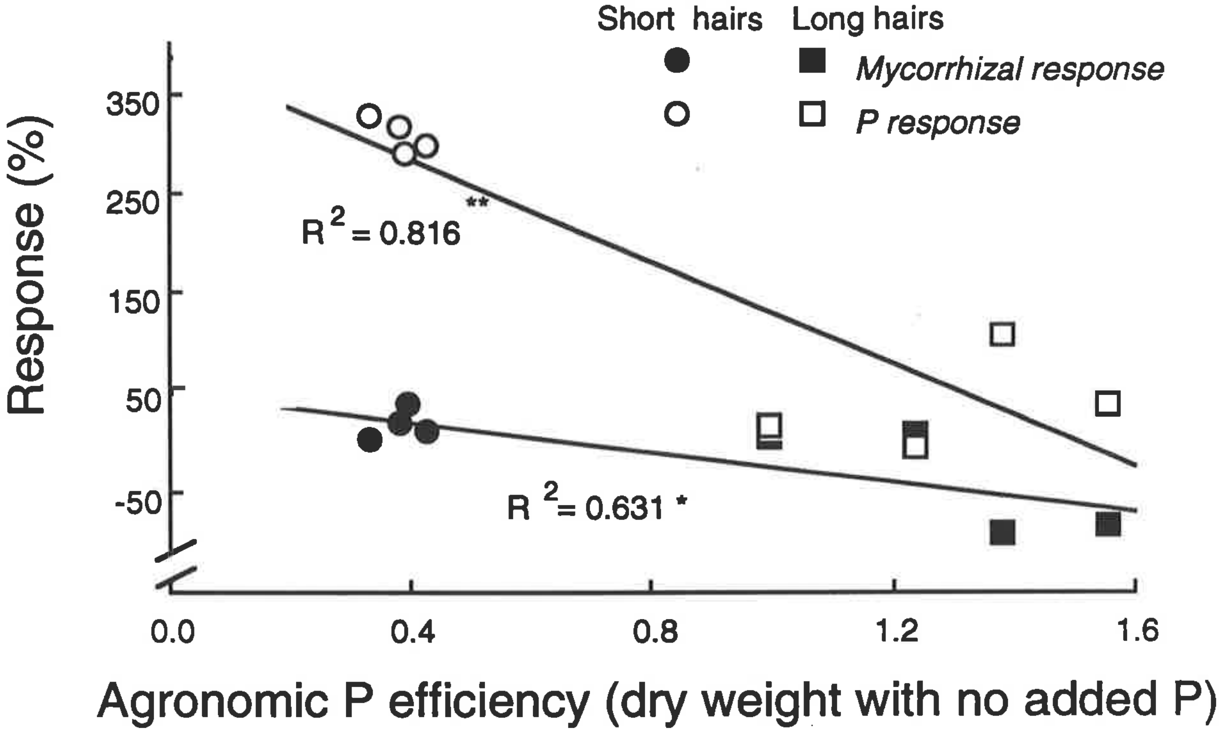


Fig. 9.4 **The relationship between agronomic P efficiency and responses of rye plants with long and short root hairs to mycorrhiza and P addition. *, ** significance at the 0.05 and 0.01 probability levels, respectively.**

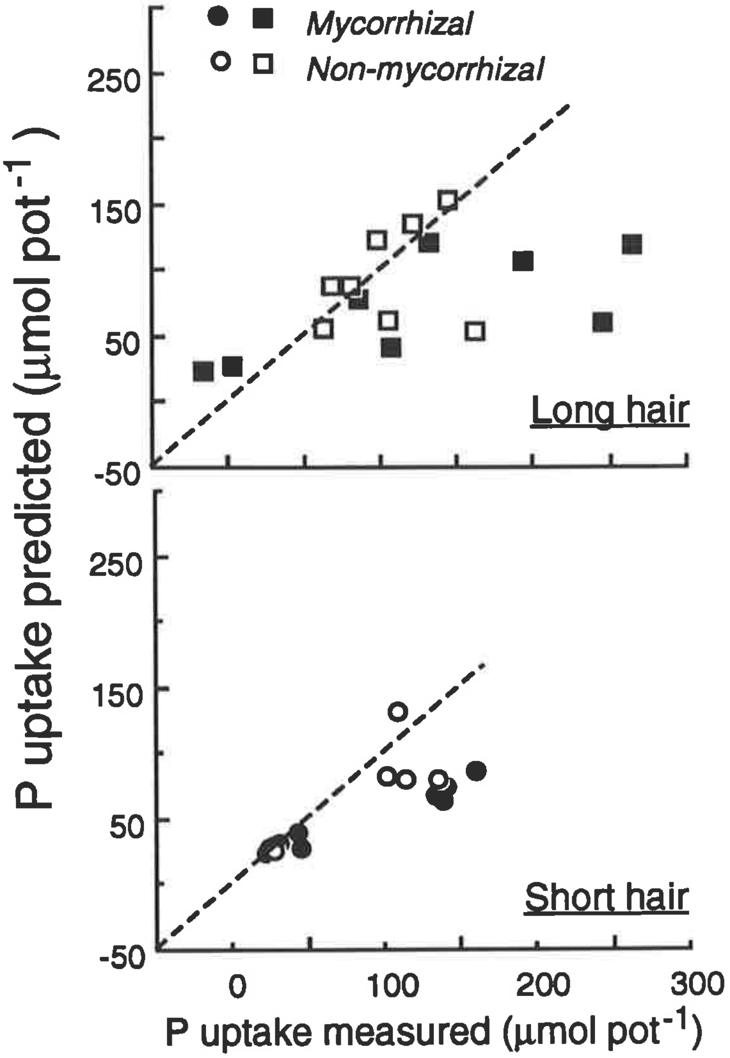


Fig. 9.5 **Relationship between measured P uptake by two rye selections and predicted P uptake by the Barber-Cushman model as affected by mycorrhizal infection. The dashed line is where predicted uptake equals measured uptake.**

9.4 Discussion

The main effects observed in this study were that LRH plants were less responsive to mycorrhizal infection than SRH ones and longer root hairs overrode mycorrhizal infection in their effects on the uptake of P from soil.

Considerable response to mycorrhizal infection by SRH plants supports the hypothesis put forward at the beginning of this chapter and confirms the previous finding with barley described in Chapter 8 that mycorrhizal response is negatively correlated with agronomic P efficiency. Similarly, comparisons by Baylis (1970) of seven plant families have also shown that plants with coarser roots are usually more responsive to mycorrhizal infection than plants with well developed root systems bearing long root hairs. In contrast to these results, Crush and Caradus (1980) found that root morphology and length of root hairs were relatively insignificant in affecting mycorrhizal response within the white clovers tested in their investigation.

As expected, this study showed that agronomic P efficiency (dry matter yield at no added P) of LRH plants was greater than SRH plants. Similar results have been reported for white clovers varying in root hair length (Crush and Caradus 1980, Caradus 1981). This suggests that plants with longer root hairs have a greater capacity to survive at low levels of P in soil than those with shorter root hairs. Caradus (1980) has shown that grasses were more tolerant to low-P soil than legumes, an effect possibly related to the high root length densities of the grasses, which were higher than for legumes.

Comparison of the uptake of nutrient by plants of the same species with and without root hairs was made for the first time by Barley and Rovira (1970). In the present investigation, two rye selections of the same cultivar were used. The results show that longer root hairs increased considerably the P uptake of both mycorrhizal and non-mycorrhizal plants, which is consistent with previous observations (Barley and Rovira 1970, Bole 1973, Caradus 1981, Itoh and Barber 1983, Föhse *et al.* 1991). Those studies, however, did not take into account the possible influence of mycorrhiza in the uptake of P by the plants. It has been suggested (Tinker 1975, Clarkson and Hanson 1980) and shown for white clover (Caradus 1982) that mycorrhizal effects might be greater than the effects of root hairs. However, the present results do not support the suggestion, as they clearly indicate that longer root hairs override mycorrhizal infection in their effects on the uptake of P from soil. In this study, the total surface area of roots per pot of non-mycorrhizal LRH plants was higher than either the mycorrhizal ones or SRH plants (see Table 9.1). Compared with their contribution to P uptake, this result suggests that the total root surfaces may primarily account for the extent of P uptake in non-mycorrhizal plants. This is possibly due to the greater root length of the LRH plants with their root hairs which had diameter similar to hyphae, thus enable the plant to explore large volume of soil including soil micropores. A similar explanation of the influence of root hairs may be applied to mycorrhizal LRH plants which had greater P uptake although their surface area were smaller than SRH plants. Nevertheless, the possibility that the selections of rye could differ in aspects other than root hair length should not be overlooked.

In the Barber-Cushman model, parameters of root hair characteristics are not taken into account. Underprediction in calculating the uptake of P by the plants, especially ones with long root hairs, confirms to the need to take into account the P uptake by root hairs in the models (Itoh and Barber 1983, Föhse *et al.* 1991). The presence of mycorrhizal infection also contributed to the underprediction of P uptake and inclusion of a factor to account for the extent of mycorrhizal infection in models is essential.

The higher allocation of P to the root systems of SRH than LRH plants was in agreement with the results of Caradus (1981). Similarly, inefficient barley cultivars were also found to accumulate more P in roots than efficient cultivars (Chapter 8). The latter also showed that mycorrhizal infection reduced the proportion of total P in roots of inefficient barley but not of efficient ones. However, the present results showed that non-mycorrhizal SRH plants had lower fraction of P in roots than mycorrhizal ones. The contradictory results were difficult to explain because root hair characteristics of those plants were not determined.

Increased P content in mycorrhizal plants, especially in low-P soils, has been associated with increased P uptake per unit length or weight of roots (Sanders and Tinker 1971, Smith 1982). Although similar results were obtained in this study, the increased specific P uptake was observed only at the second harvest for both selections. The decrease in P uptake per unit of root with time and the small difference in that parameter between mycorrhizal and non-mycorrhizal plants in the early stages of growth have been reported (Smith 1982). A higher specific P uptake and greater proportion of P in roots of SRH plants observed in this study support the results of Caradus (1981). Further, present results showed that mycorrhizal infection increased the specific P uptake of SRH and LRH plants in second harvest by 16% and 83%, respectively, which resulted in no difference in the parameter between the two selections. The higher specific P uptake by LRH plant is likely to be mediated by the small diameter of the root hairs which enabled the plant to explore a greater volume of soil.

In this study LRH plants also had higher density of root hairs and smaller root hair diameter than plants with short root hairs. Baylis (1970) observed a positive correlation between P uptake and length and frequency of root hairs. Nevertheless, Bole (1973) did not find any relation between root hair density and P uptake. Small diameter of root hairs was considered to be a beneficial morphology of the plant roots in taking up P from the soil (Bouldin 1961). However, a large number of root hairs may overlap each other in tapping soil P and depleting "available " P within root hair cylinder in a few days. This may result in lower amount of P accumulated in plants than might be anticipated and may also explain the curvilinear relationship described in Fig. 2 in which competition among root hairs of non-mycorrhizal plants occured (Itoh and Barber 1983). Lewis and Quirk (1967) demonstrated autoradiographically that soil P is depleted in the roor hair zone. These results showed that those plants maintained their differences in the length of root hairs suggesting that the volume of depletion zone per unit length of root for LRH plants was greater than in SRH ones.

That a greater percentage of root length was infected by the VA mycorrhizal fungus in LRH plants is in agreement with work on white clovers (Crush and Caradus 1980). However, comparison of the root length infected revealed that in low-P soil, the growth rate of *G. intraradices* within the root of the LRH plants was smaller compared with SRH plants. Factors that control the development of the mycorrhizal fungus within the LRH plants remain unclear. Nevertheless, higher concentration of P in LRH plants (Table 3) may explain the low extent of fungal growth, as suggested by Menge *et al.* (1978b). The other possible explanation was that the degree of mycorrhizal infection was under genetic control (Mercy *et al.* 1990, Chapter 8).

This study has shown that there was no significant interaction between mycorrhizal infection and root hair length in rye. Similar result was obtained in the work of Crush and Caradus (1980), and based on this they suggested that there was no need to take into account mycorrhizal effects in selecting P efficient plants. Their view was supported by the result on dry matter yields. However, P efficiency in plants can also be due to their ability to take up a high amount of P from soil or a great ability in utilization of P by the plants.

9.5 Conclusion

Rye plants with short root hairs, which were agronomically less efficient, were more responsive to either mycorrhizal infection or P addition than those with long root hairs. However, mycorrhizal infection reduced P utilization efficiency of plants with short root hairs but not of ones with long root hairs. No interaction was observed between mycorrhizal infection and root hair length.

Chapter Ten

GENERAL DISCUSSION AND CONCLUSIONS

10.1 Introduction

The findings of this study clearly show the importance of mycorrhizal symbiosis in the assessment and selection of P-efficient cereals. In this final chapter, the findings presented in Chapters 4 to 9 of the thesis are integrated, compared and discussed in the light of the hypothesis and questions raised in Chapter 1. The discussions cover four main parts: 1) P efficiency of cereal plants as affected by mycorrhizal infection, 2) characterization of P efficiency of plants in relation to responses to mycorrhizas, 3) relative allocation of P in efficient and inefficient plants as influenced by mycorrhizal infection and 4) factors affecting the contribution of mycorrhizas to assessment of P efficiency. Finally, this chapter draws some general conclusions and suggests future research needed.

10.2 Influence of mycorrhiza on P efficiency assessment

Considerable previous research has focused on the development and selection of cereal species or cultivars which are able to give high yields in soil with a low concentration of P. However, those studies paid scant attention to the importance of mycorrhizas despite the fact that inoculation with VA mycorrhizal fungi of soils with low P-supply has been shown to increase shoot and grain yields of cereals (Khan 1975, Thompson 1990, Jakobsen 1983). It is evident from the results reviewed in Chapters 1 and 2 that mycorrhizal infection is likely to affect the assessment of P efficiency whether it be agronomic efficiency, efficiency in uptake or efficiency in utilization.

The results presented clearly demonstrate the significance of mycorrhizas in the assessment of P efficiency. The present findings are important in that they emphasize the extent to which VA mycorrhizal infection may change the relative performance of cereal species and cultivars in terms of several definitions of P efficiency. P efficiency of wheat, barley and rye as influenced by mycorrhizal infection was investigated (Chapter 4). Though mycorrhizal infection by indigenous fungi was shown to increase efficiency in uptake of P of barley, the infection had no effect on this aspect of efficiency in wheat or rye. In contrast, the efficiency of utilization of P of wheat and barley was decreased by mycorrhizal infection, but again there was no effect on rye.

The effects of mycorrhizal infection on P efficiency were further examined in three cultivars of barley (Chapter 5). It was shown that VA mycorrhizal infection was able not only to increase but also to decrease agronomic P efficiency of the barley, depending on the environmental conditions. In general, specific P uptake and concentration of P of the barley cultivars was higher in mycorrhizal than in non-mycorrhizal plants. This increase in P concentration was greater in a moderately efficient cultivar (Clipper) than in an inefficient one (Galleon), implying that an agronomically inefficient cultivar was more efficient in utilization of P when the plants were mycorrhizal.

An investigation to verify this hypothesis using 8 barley cultivars (Chapter 8) showed that mycorrhizal infection by *G. etunicatum* reduced the efficiency of utilization of P, but increased agronomic P efficiency, and that the extent of these effects varied among the cultivars. Increase in agronomic P efficiency due to mycorrhizal infection was generally small, even in soil with low P status and similar results were obtained in experiments described in other chapters.

To examine whether the differences among cultivars were related to root hair length, an experiment was conducted using two selections of one cultivar of rye. As expected, agronomic P efficiency of rye plants with long root hairs was greater than plants with short root hairs. Mycorrhizal infection increased the agronomic efficency of both selections, but the increase for the plants with long root hairs was lower than for plants with short root hairs (Chapter 9). Longer root hairs increased considerably the efficiency in uptake of P by both mycorrhizal and non-mycorrhizal plants. Furthermore, long root hairs had a much greater effect on the total uptake of P from the soil than mycorrhizal infection in this experiment. In non-mycorrhizal plants specific P uptake was greater by roots with short than long root hairs in this study. However, mycorrhizal infection increased the specific P uptake of both selections in such a way that no difference in this parameter between the two selections was found for mycorrhizal plants.

The difference between cultivars or selections may be not only in their root hairs. Although the effect of long root hairs on P uptake overrode the effect of mycorrhiza, other aspects of P efficiency of the two selections were still affected by mycorrhizal infection. This means that the ability of mycorrhizal fungi both in exploring P sources in soil and transfer of P from fungal tissues to host plant tissues which is not found in root-root hair system should be considered in developing P efficient plants.

The results suggest that plant breeders should consider the contribution of mycorrhizal infection in selection of P efficient plants. The number of mycorrhizal propagules in soils can be modified easily by agricultural management (*e.g.* application of P fertilizers and pesticides, choice of crop species and cultivars and tillage system). Mycorrhizal populations can vary with time at the same site and differ between sites at the same time. This means that the performance and ranking of species or cultivars for their P efficiency may be altered when the investigations are carried out at different sites or in different seasons.

Inoculation of cereals with mycorrhizal fungi was not a big issue in this study. Nevertheless, the main outcome of this study reveals that the extent of mycorrhizal infection influences aspects of uptake and utilization P efficiency in cereals.

Mycorrhizal infection did not increase the dry matter yield of the cereal plants, but it may not be unimportant in the production of cereals. I realize that in this study, plant yield in terms of grains was not measured. It would be interesting to investigate P efficiency in terms of grain production, particularly in relation to "luxury accumulation" of P by mycorrhizal plants during the vegetative growth stage,.

Although above explanations highlight some problems which could be faced in selection of P efficient plants in sites different in mycorrhizal status, it should be emphasized that low levels of infection are able to increase P concentration and uptake in plants. The implications of this is that extra care should be taken in the methods of eliminating VA mycorrhizal fungi from the soil. Partial sterilization, for example, may leave small amount of mycorrhizal propagules which later may infect the plants.

10.3 Mycorrhizal responses of efficient and inefficient plants

Chapters 5, 8, and 9 revealed significant differences in the extent to which cereal cultivars respond to mycorrhizal infection. In general, mycorrhizal infection increased P concentration in all the cultivars and the increase was smaller in the agronomically inefficient than in the moderately efficient cultivars (Chapter 5).

Yield response of the barley shoots to mycorrhiza was negatively correlated with agronomic efficiency of the cultivars (Chapter 8). A similar response to application of P was noted, although application of P was more effective in increasing plant growth than was mycorrhizal infection. The positive correlations between response to P application and response to mycorrhizal infection support the view that a better supply of P is a major contribution of mycorrhizal infection to the nutrition of the plants.

In the present study, it was demonstrated that rye plants with long root hairs were less responsive to mycorrhizal infection than the agronomically less efficient plants with short root hairs. This result supports the hypothesis put forward at the beginning of Chapter 9, and confirms the previous finding for barley reported in Chapter 8, that mycorrhizal response among cultivars was negatively correlated with agronomic P efficiency.

Mycorrhizal response appeared to be related primarily to P utilization efficiency. This study has shown that agronomic P efficiency was positively correlated to efficiency in utilization of P (Chapter 5), but was negatively correlated to mycorrhizal response. It has been proposed that plants can be placed in four groups with respect to efficiency and response to P, *i.e.* efficient responder, efficient non-responder, inefficient responder and inefficient non-responder (Gerloff 1977, Whiteaker *et al.* 1977). However, a positive relationship between P efficiency and response was established in this study, implying that there are only two groups of plants: inefficient responders and efficient responders. This finding suggests that a characteristic of inefficient plants is that they respond to mycorrhizal infection and to P addition, whereas efficient plants do not. It is possible that the other two groups do exist, but the range of cultivars tested in this study was not broad enough to cover them. Further study is needed to clarify the existence of the groups. The availability of efficient responder cultivars is highly demanded by the agricultural communities.

In previous studies, the focus of discussion has often been on mycorrhizal dependency (the ratio of the dry weights of mycorrhizal and non-mycorrhizal plants) rather than alteration in efficiency. The definition of mycorrhizal dependency is nearly similar to mycorrhizal response defined in this study. It is important to place more emphasize on efficiency rather than dependency because mycorrhizal dependency considers only the dry matter yield, whereas efficiency includes yield, uptake and utilization of P by plants. It is evident that mycorrhizal infection can increase the uptake and utilization of P in plants without increasing dry matter yield.

10.4 P allocation in plants as affected by mycorrhizal infection

The lack of response of efficient cultivars to mycorrhizal infection and P addition may be related to the allocation of P from roots to shoots. Although the uptake of P by mycorrhizal plants was high, these plants also had higher concentrations of P in their roots than non-mycorrhizal plants (Chapter 5). It is possible that the fungal tissues in roots accumulate high amounts of P (Cox and Tinker 1978). Moreover, it was shown that an inefficient cultivar, accumulated more P in its roots than an efficient cultivar. Similarly, plants with short root hairs were also found to accumulate more P in roots than those with long root hairs (Chapter 9). This suggests that poor translocation of P from root to shoot may explain the agronomic inefficiency of a cereal cultivar. However, in the presence of mycorrhizal infection the percentage of total P content that was present in the roots of inefficient cultivar was declined. It appears that in the absence of mycorrhizas, an agronomically inefficient cultivar has lower P utilization efficiency in both shoots and roots than an efficient cultivar.

Transfer between the fungal symbiont and the plant host may be controlled by processes that lead to the mobilization of polyphosphate reserves in the fine arbuscule branches (Smith and Gianinazzi-Pearson 1988). The present study reveals that agronomically P-inefficient barley cultivars responded to VA mycorrhizal infection by reducing the percentage of total plant P which was present in the roots, while efficient cultivars showed no significant response. This suggests that mycorrhizal infection increases the relative allocation of P to the shoots of inefficient cultivars, though the total amount of P present in the roots was not affected by mycorrhizal infection.

10.5 The effects of soil temperature and light on the contribution of mycorrhizas to P efficiency

It appears that the response of the cereals at low P supply to infection by mycorrhizal fungi depends on the soil temperature (Chapter 4). Lack of response to mycorrhiza in cereals at a soil temperature of 10°C may be attributed to restricted fungal infection (Hetrick *et al.* 1984) and host plant metabolism.

The results of the experiments clearly showed that barley plants infected with *G. intraradices,* a coarse endophyte, were able not only to increase (Experiment 5.1) but also to decrease (Experiment 5.2) growth compared with non-mycorrhizal plants. The different effects in the two experiments were clearly related to differences in growing conditions: at low soil temperature (15°C), the dry weight of mycorrhizal plants was lower than non-mycorrhizal plants (Experiment 5.2), while it was greater when the soil temperature was high (20°C) (Experiment 5.1).

Low soil temperatures affected both the growth of barley roots and the mycorrhizal symbiosis. The results in Chapter 6 show that P efficiency and the responses of both inoculated and non-inoculated barley plants were affected by soil temperature. In inoculated barley plants, growth at low soil temperature decreased agronomic P efficiency. However, the increase in specific P uptake in inoculated plants at higher soil temperatures was largely due to the effect of mycorrhizal infection, rather than to the effect of temperature.

Although soil temperature had no effect on specific P uptake by mycorrhizal plants, these results (Chapter 6) showed that mycorrhizal plants had a higher specific P uptake than non-mycorrhizal plants. Compared with non-mycorrhizal plants, P uptake efficiency of mycorrhizal plants was more sensitive to the decrease in soil temperature. Although specific P uptake of mycorrhizal plants was not affected by low soil temperature, total uptake of P in shoots and roots was reduced, which may be due to the differential allocation of biomass to these tissues at different soil temperatures.

Although barley plants grown at a soil temperature of 20°C had been shown to benefit from mycorrhizal infection, the total dry matter produced was still smaller than for plants grown at a soil temperature of 15°C. A possible explanation for the observation is that the light intensity of the Experiment 5.1 was nearly half of that used for the Experiment 5.2 (soil temperature of 15°C). However, a positive response to mycorrhizal infection in term of total dry weight was observed, although it was not significant at $P \leq 0.05$. This means that dry matter yield was controlled mainly by light intensity, whereas mycorrhizal benefit was controlled mainly by soil temperature.

When comparing soil temperatures of 15 and 20°C, this study (Chapter 6) also shows that low soil temperature restricted shoot growth more than root growth of non-mycorrhizal plants. However, in mycorrhizal plants growth of both shoots and roots was reduced at low soil temperatures. Such a growth depression is probably in large part be associated with the low light intensity encountered in this experiment, which was conducted in winter time. The competition for assimilates due to mycorrhizal infection appears to be reflected in lower biomass partitioning to roots in mycorrhizal plants compared with non-mycorrhizal plants. If mycorrhizal symbiosis increases nutrient absorption, the plant may limit energy loss associated with extra root maintenance by decreasing root growth (Hetrick 1989).

10.6 General conclusions

1) The assessment of P efficiency in cereal cultivars is influenced by the presence of mycorrhizal infection. The extent of mycorrhizal infection varies with plant species, propagule density and P supply. There is complex pattern of interaction among those factors.

2) Breeding programs should take into account the contribution made by mycorrhizal fungi in assessing and selecting cereals on the basis of P efficiency under field conditions, and the possible variations in both infection and response in different cultivars.

3) Responses of plants to mycorrhizal infection are determined by their agronomic P efficiency and P utilization efficiency. Cultivars which are inefficient in these attributes respond more to mycorrhizal infection than those which are efficient.

4) Responses of plants to mycorrhizal infection are influenced by the length of their root hairs. Plants with short root hairs, which are agronomically less efficient, are shown to be more responsive to mycorrhizal infection and to P addition.

5) Mycorrhizal infection affects P utilization efficiency of plants. A plant species or cultivar tends to become less efficient in utilization of P when mycorrhizal.

6) Soil temperature and light intensity interact in determining P efficiency in cereals. Soil temperature seems to affect mycorrhizal infection and its contribution to P uptake, whereas the main effect of light intensity appears to be on growth.

7) Plants infected by mycorrhizal fungi are more sensitive to low soil temperature than non-mycorrhizal plants but maintain higher specific P uptake.

8) The percentage of root length infected may or may not be influenced by concentration of P in tissues of the barley cultivars.

10.7 Future research

1) There is a need for further work to determine the carbon cost for maintaining the mycorrhizal symbiosis, and the carbon allocation between root and shoot in cultivars contrasting in P efficiency.

2) It would be interesting to investigate the existence of two groups of plants in Gerloff's model, *i.e.* inefficient non-responder and efficient responder, and why they were not found in the cultivars tested. Cereal cultivars which are efficient responders are highly demanded by agricultural communities.

3) Future studies need to investigate the interaction between P efficient cultivars and efficient mycorrhizal fungi in the determination of P efficiency and in the allocation of P between shoot and root.

4) The significance of numerous of interacting factors such as nutrients in soil, root characteristics, soil temperature, light intensity and other soil microorganisms, require further investigation.

5) It is suggested that factors which affect the growth of mycorrhizal fungi inside the roots of plants with long and short root hairs should be examined.

6) Further work is needed to include mycorrhizal infection as a parameter in development of models to estimate the uptake of P from soil.

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APPENDIX

(Reprints of journal papers)

1. Baon J B, Smith S E, Alston A M and Wheeler R D 1992 Phosphorus efficiency of three cereals as related to indigenous mycorrhizal infection. *Aust. J. Agric. Res.* **43**, 479-491.

2. Baon J B, Smith S E and Alston A M 1993 Mycorrhizal responses of barley cultivars differing in P efficiency. *Plant Soil* **157**, 97-105.

Baon, J. B., Smith, S. E., Alston, A. M. & Wheeler, R. D. (1992). Phosphorus efficiency of three cereals as related to indigenous mycorrhizal infection. *Australian Journal of Agricultural Research, 43*(3), 479-491.

Plant and Soil 157, 97–105 ~~000: 000–000~~, 1993.

PLSO 3126

Mycorrhizal responses of barley cultivars differing in P efficiency

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Received 23 March 1993. Accepted in revised form 5 July 1993

Key words: barley cultivars, efficiency, *Glomus etunicatum*, *Hordeum vulgare*, low temperature, phosphorus, response, VA mycorrhiza

Abstract

The purpose of this study was to investigate how barley cultivars which are different in dry matter yield at low phosphorus (P) supply (i.e. they differ in agronomic P efficiency) respond to mycorrhizal infection. In a preliminary experiment, six mycorrhizal fungi were tested for their ability to colonize barley (*Hordeum vulgare* L.) roots at a soil temperature of 15 °C. *Glomus etunicatum* was the most effective species and was used in the main experiment. The main experiment was conducted under glasshouse conditions in which soil temperature was maintained at 15 °C. Treatments consisted of a factorial arrangement of 8 barley cultivars, 2 mycorrhiza (inoculated and non-inoculated), and 3 rates of P (0, 10 and 20 mg kg^{-1}). P utilization efficiency (dry matter yield per unit of P taken up) and agronomic P efficiency among the barley cultivars was significantly negatively correlated with mycorrhizal responses. However, the response to mycorrhizal infection was positively correlated with response to P application. Poor correlation was observed between P concentration when neither mycorrhiza nor P were supplied and the percentage of root length infected. The extent of mycorrhizal infection among the barley cultivars in soil without P amendment varied from 8.6 to 28.6%. Significant interactions between cultivar and P addition, and between mycorrhiza and P addition were observed for shoot dry weight but not root dry weight.

Introduction

There is an increasing interest in breeding for nutritional traits to improve the productivity of crops on soils with low concentrations of available phosphorus (P) and increase the efficiency of fertilizer use (Graham, 1984). The physiological and morphological factors responsible for the differences in P efficiency, and the prospects of selecting plants with an improved P efficiency have been reviewed (Clark, 1990). Föhse et al. (1991) have shown that properties of root systems, such as root hairs, influenced the efficiency of P uptake. Differences in uptake of P have been reported for some barley cultivars and have been attributed to variations in root growth (Schjørring and Nielsen, 1987).

While substantial progress has been made in elucidating factors affecting efficiency of P (uptake and utilization) in plants, less attention has been devoted towards the role of mycorrhiza in influencing assessment of P efficiency, particularly for cereal crops, which are widely believed to have a low dependency on mycorrhizas. Recently, Baon et al. (1992) addressed the interaction between mycorrhizal infection and P efficiency in three cereals. P nutrition of barley (cv. Galleon) was found to be more dependent on and influenced by mycorrhizal infection from field inoculum than that of wheat (cv. Spear) or rye (cv. S.A. Commercial). The objectives of the present study were to develop the work of Baon et al. (1992) and to investigate how barley cultivars which are different in P efficiency respond to mycorrhizal infection.

Few studies of mycorrhizas have been done at soil temperatures lower than 20 °C, even though the temperatures in field soils of a large part of the region where barley is grown are below this value (Bowen, 1991; Fabig et al., 1989; Jensen, 1983). Power et al. (1963) considered that 15 °C is the optimum for barley. There is some evidence that growth responses of both the plant and the fungal symbiont are different at low soil temperatures and this suggests that barley cultivars may associate with certain VA mycorrhizal fungi which can grow and develop at low soil temperatures (Baon et al., 1992; Grey, 1991; Hetrick et al., 1984; Volkmar and Woodbury, 1989). This study was set up at soil temperature of 15 °C.

Materials and methods

Soil

The experiments used soil from 0–20 cm depth of a solonized brown soil (Stace et al., 1968) or Xeralf (Soil Survey Staff, 1975) collected from Avon, South Australia. The soil was air-dried, sieved to <2 mm and steam-sterilized (100 °C) for one hour to eliminate indigenous mycorrhizal fungi. Chemical properties of the soil are as follows: pH(H_2O) 8.6, organic C content 7.1 g kg^{-1}, total N 0.53 g kg^{-1} and $NaHCO_3$ extractable (Colwell, 1963) P content 4 mg kg^{-1}.

Nutrients

The following nutrient salts were added to the soil (all per kg air-dry soil) and mixed thoroughly to ensure that nutrients other than P did not limit plant growth: 133 mg NH_4NO_3, 25 mg $FeSO_4$ $7H_2O$, 145 mg K_2SO_4, 24.5 mg $CaCl_2$ $2H_2O$, 22.5 mg $MgSO_4$ $7H_2O$, 14.7 mg $MnSO_4$ $4H_2O$, 2.3 mg $ZnSO_4$ $7H_2O$, 1.33 mg $CuSO_4$ $5H_2O$, 0.83 mg H_3BO_3, 0.42 mg $Co(NO_3)_2$ $6H_2O$, 0.20 mg Na_2MoO_4 $2H_2O$.

Experiment 1

The aim of this experiment was to select a VA mycorrhizal fungus which was able to colonize roots at a soil temperature of 15 °C. The six mycorrhizal fungi used in the experiment were: *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe, *Glomus fasciculatus* (Thaxter *sensu* Gerdemann) Gerdemann & Trappe, *Glomus versiforme* (Karsten) Berch, *Glomus intraradices* Schenck & Smith, *Glomus etuni~~f~~catum* c/ Becker & Gerdemann, and *Glomus* spp. 'City Beach'. The inocula were taken from pot cultures of the mycorrhizal fungi grown with subterranean clover as the host plant and were composed of a mixture of spores, hyphae, infected roots and soil. Seventy g of each inoculum was layered 5 cm below the soil surface in the experimental pots.

Seeds of barley (*Hordeum vulgare* L.) cv. Galleon were surface sterilized with 0.5% NaOCl twice for two minutes followed by two rinses in sterile distilled water and germinated on moist filter paper at room temperature. Two 2-day-old seedlings were transplanted into pots containing 1.4 kg of soil. All the pots with plants were set in a thermostatically controlled water tank at 15 °C. The plants were grown in the glasshouse under natural daylight in winter (June to August) for 42 days before harvest. Minimum and maximum air temperatures inside the glasshouse ranged from 10 to 23 °C (night) and 17 to 26 °C (day), respectively. Mean solar radiation in the glasshouse ranged from 118 $\mu E\ m^{-2}\ s^{-1}$ in June to 199 $\mu E\ m^{-2}$ ~~d~~$^{-1}$ in August. /s At harvest samples of the root systems were cleared in 10% KOH and stained using trypan blue (Phillips and Hayman, 1970) and the percentage of root length infected was determined by grid-line intersect method (Giovannetti and Mosse, 1980). The fungus with the most infected roots (*G. etunicatum*) was selected for Experiment 2.

Experiment 2

The objective of this experiment was to investigate P efficiency of eight barley cultivars in relation to mycorrhizal response. The barley cultivars were chosen to provide a range of dry matter yields at no added P (i.e. a range of agronomic P efficiencies). Four cultivars (Yagan, WI 2737, O'Connor and Kaniere) had been shown in field to be efficient and four cultivars (Galleon, Shanon, WI 2539 and Skiff) inefficient (R D Wheeler, pers. comm.). Galleon has been used in previous studies (Baon et al., 1992). The experiment had a randomized complete block design with three replicates. The treatments were 8 cultivars, 2 mycorrhiza (inoculated and non-inoculated) and 3 rates of P application in factorial combination. The seeds of barley were surface-sterilized and germinated as described for the previous experiment. Phosphorus as KH_2PO_4 was added at 0, 10 or 20 mg kg^{-1} soil. For mycorrhizal treatments, barley plants were inoculated with 70 g inoculum of *G. etunicatum*. The inoculum which consist of roots, hyphae, spores was layered 5 cm below the surface.

Two 2-day-old seedlings of barley prepared as in Experiment 1, were transplanted into pots containing 1.4 kg of soil. The pots were placed in a glasshouse in three thermostatically controlled water tanks which were set at 15 °C. The water content of the soil was kept at field capacity (11%) by adding deionized water daily.

The experiment was conducted during October to November. Air temperature ranged from 25–31/13–24 °C (day/night). Mean solar radiation in the glasshouse ranged from 449 μE m^{-2} s^{-1} in October to 534 μE m^{-2} s^{-1} in November. The plants were harvested 42 days after transplanting. At harvest, the fresh and dry weights of the plants were recorded. Samples of the root systems were stained and assessed as described previously for the percentage of root length colonized by the fungus. Dry plant materials were digested in a mixture of nitric and perchloric acids, and the P content of the digests was determined with modification of phospho-vanado-molybdate method (Hanson, 1950).

Plant response (%) to mycorrhizas was expressed as follows:

$$\frac{\text{Shoot dry weight }(+M) - \text{Shoot dry weight }(-M)}{\text{Shoot dry weight }(-M)} \times 100$$

where +M and −M stand for mycorrhizal inoculated and non-inoculated plants, respectively. A similar expression was used to calculate plant response to P.

Analyses of variance was performed with GENSTAT 5 (Genstat 5 Commitee, 1987). Data with non-homogenous variances were subjected to logarithmic or to angular transformation before analyses. The data have been retransformed for clarity of presentation in the tables and figures.

Results

Experiment 1

Colonization of the barley plants by the mycorrhizal fungi tested was relatively low (Table 1). Only three VA mycorrhizal fungi viz. *G. intraradices*, *G. etunicatum* and *Glomus* spp. 'City Beach' colonized more than 10% of the root length of barley roots. *Glomus etunicatum* was more tolerant of a soil temperature of 15 °C than the other mycorrhizal fungi tested and was selected for use in Experiment 2.

Experimental 2

There were large differences among the barley cultivars in the percentage of root length infected (Fig. 1). This parameter varied from 8.6% for Kaniere to 28.6% for Shannon in soil without added P. No infection was observed in the roots of non-inoculated plants. In general, the extent of mycorrhizal colonization was lower following the application of P to the soil and the extent of this effect ~~c~~arried among the cultivars. /v

Figure 2 shows that mycorrhizal infection had little effect on shoot dry weight and that all cultivars, except Shannon, had similar dry weights. With most cultivars application of P increased shoot dry weight. The cultivar ×

Table 1. Galleon barley inoculated with six VA mycorrhizal fungi: percentage of root length infected (mean ± standard error of the mean). Values derived from three replicates

Mycorrhizal fungi	Percentage of root length infected
Glomus versiforme	1.2 ± 0
Glomus etunicatum	20.5 ± 4.8
Glomus intraradices	9.8 ± 0.9
Glomus fasciculatus	2.6 ± 0.8
Glomus mosseae	0.17 ± 0.03
Glomus sp. 'City Beach'	14.5 ± 3.6

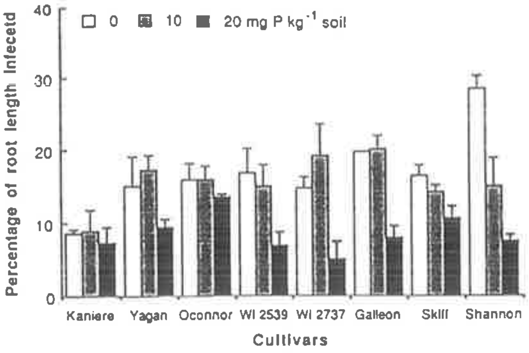

Fig. 1. Percentage of root length of barley cultivars colonized by *G. etunicatum* as influenced by P application. Vertical bars represent standard errors of the means.

phosphorus and the mycorrhiza × phosphorus interactions were significant for shoot dry weight but this was not the case for root dry weight (Table 2). Mycorrhizal treatments significantly affected only root dry weight, while phosphorus significantly affected only shoot dry weight. Except Kaniere and Shannon, mycorrhizal infection reduced root dry weights of the barley cultivars (Table 3).

□ Total P uptake of mycorrhizal plants was higher than that of non-mycorrhizal plants where no P was added to the soil (Fig. 3A). (NP)

Mycorrhizal colonization increased the uptake of P per unit weight of root (specific P uptake) by all cultivars except for Shannon (Fig. 3B).

Table 2. Mean squares and significance of the effects of barley cultivars, mycorrhizal infection and phosphorus application on some parameters of P status and plant growth. PUE (P utilization efficiency, total dry matter yield per total P uptake) and SPU (specific P uptake, amount of P taken up by per unit weight of root)

Source	df	Shoot DW	Root DW	Total P uptake	Infection	PUE	Spec P upt
Cultivar (C)	7	9.462***	2.493***	26.87**	23.069***	0.2772***	1.2258***
Mycorrhiza (M)	1	0.000	0.655**	204.7***	16301***	0.3825***	2.0264***
Phosphorus (P)	2	24.021***	0.166	3249***	195.57***	1.2302***	8.4635***
C × M	7	0.054	0.042	2.411	23.112***	0.0035	0.0133
C × P	14	0.439***	0.053	10.11	14.620**	0.0051*	0.0332
M × P	2	1.163***	0.061	33.37*	195.757***	0.0437***	0.0830*
C × M × P	14	0.068	0.077	2.958	14.677**	0.0040	0.0501*
Error	93	0.096	0.067	7.982	5.721	0.0022	0.0249

*, **, *** Significant at 0.05, 0.01, 0.001 levels of probability, respectively.

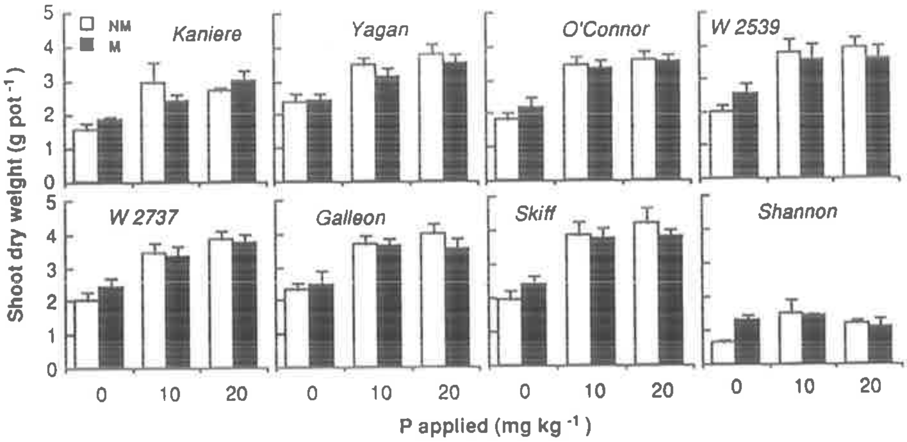

Fig. 2. Shoot dry weight of barley cultivars as affected by addition of P and mycorrhizal inoculation (M = mycorrhizal, NM = non-mycorrhizal). Vertical bars represent standard errors of the means.

Table 3. Root dry weight of barley cultivars as affected by mycorrhizal inoculation and P application (10 mg kg^{-1}). Values are the means of three replicates with standard errors of the means in parentheses

Cultivar	Root dry weight (g pot^{-1})			
	No P added		P added	
	7N −M^a	+M 07	7N −M	+M 07
Kaniere	1.85 (0.14)	2.03 (0.05)	2.12 (0.21)	2.29 (0.07)
Yagan	1.67 (0.16)	1.40 (0.09)	1.94 (0.15)	1.37 (0.27)
O'Connor	1.38 (0.20)	1.18 (0.11)	1.61 (0.07)	1.35 (0.12)
W 2539	1.80 (0.18)	1.60 (0.05)	1.76 (0.13)	1.51 (0.12)
W 2737	1.67 (0.19)	1.43 (0.09)	1.51 (0.09)	1.38 (0.18)
Galleon	1.41 (0.10)	1.24 (0.08)	1.62 (0.06)	1.37 (0.08)
Skiff	1.50 (0.08)	1.31 (0.08)	1.44 (0.03)	1.38 (0.02)
Shannon	0.46 (0.02)	0.95 (0.16)	1.13 (0.38)	0.76 (0.14)

^{a} +M (Mycorrhizal) and −M (Non-mycorrhizal).

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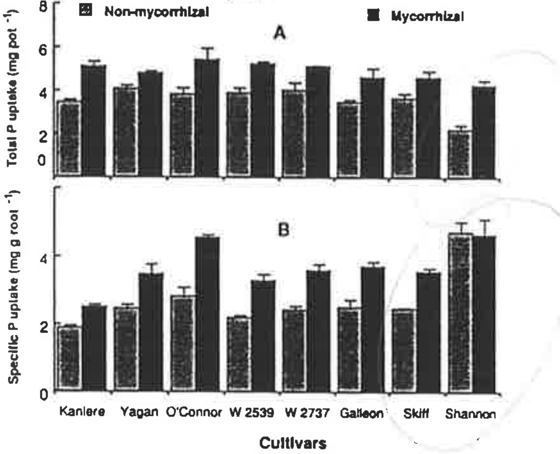

Fig. 3. Total P uptake (A) and specific P uptake (B) of the barley cultivars as affected by mycorrhizal inoculation (M = mycorrhizal, NM = non-mycorrhizal) where no P was added. Vertical bars represent standard errors of the means.

Increasing the P supply to the barley plants reduced their dry matter yields per unit of P (P utilization efficiency). For cultivars other than Shannon and Skiff, P utilization efficiency of mycorrhizal plants was lower than in non-mycorrhizal plants, particularly in P deficient soil (Fig. 4). P utilization efficiency was negatively correlated ($r = -0.939$; $p = 0.01$) with mycorrhizal response.

For both phosphorus and mycorrhiza, there was a decrease in response as agronomic P efficiency (g dm with no added P) of the cultivars increased (Fig. 5). The response to addition of 10 mg kg^{-1} was higher than to mycorrhizal infection (Fig. 5).

Among the cultivars, no statistically significant relationship was observed between the P concentration in the plant, when neither P nor mycorr-

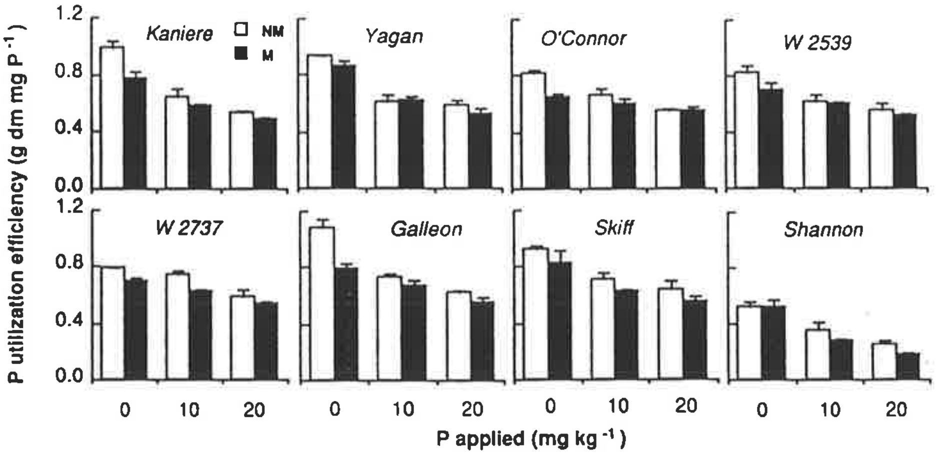

Fig. 4. P utilization of the barley cultivars as affected by mycorrhizal colonization (M = mycorrhizal, NM = non-mycorrhizal) and P addition. Vertical bars represent standard errors of the means.

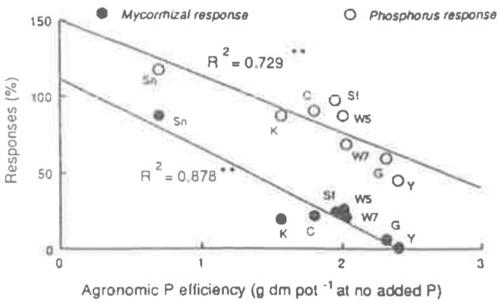

Fig. 5. Relationship between agronomic P efficiency of barley cultivars and their responses to both mycorrhizal colonization and phosphorus addition (10 mg kg^{-1}). Sn (Shannon), K (Kaniere), C (O'Connor), Sf (Skiff), W5 (WI 2539), W7 (WI 2737), G (Galleon) and Y (Yagan). ** Significant at the 0.01 probability level.

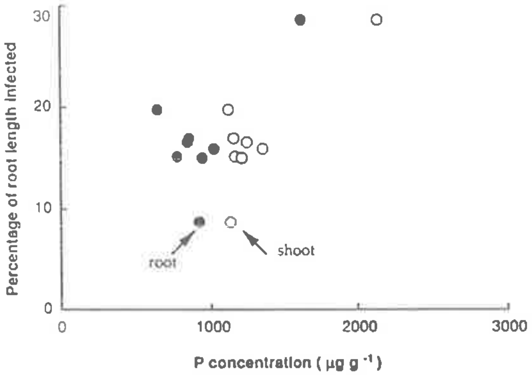

Fig. 6. Relationship between P concentration in barley cultivars, when neither P nor mycorrhizas were supplied, and percentage of root length colonized when inoculated with mycorrhizal inoculum.

hiza were supplied, and the percentage of root length infected (Fig. 6).

Discussion

In the present study, the ability of six VA mycorrhizal fungi to infect and colonize barley plants at a soil temperature of 15 °C was examined. The results showed that *Glomus etunicatum* was more responsive at 15 °C than the other mycorrhizal fungi tested and infected more than 20% of the barley roots. However, we recognize that differences in colonization were evaluated in this experiment on the basis of weight of pot-culture inoculum used per pot. This does not take into account possible differences between the pot-culture of different fungi in terms of numbers of infective propagules per g inoculum which was not measured.

This study clearly demonstrated that the yield response of the barley shoot to mycorrhizal infection depended upon the cultivar and that the response was negatively correlated with agronomic P efficiency. Thus, when grown at low P supply, a barley cultivar which is agronomically P efficient (e.g. Yagan) responds less to mycorrhizal infection than an inefficient cultivar (e.g. Shannon). Similar responses to P were noted, although application of P was more effective in increasing plant growth than mycorrhizal infection. This observation is in agreement with the hypothesis of Lyness (1936) that the most P efficient cultivars in P deficient soil would not be the most effective with an optimal supply of P. Positive correlations between response to P application and response to mycorrhizal infection has also been reported by Ollivier et al. (1983) and Estaún et al. (1987) when they compared cultivars of cowpea and pea, respectively.

In contrast to findings in field selection studies (R D Wheeler, pers. comm.), the present study showed that Galleon was P efficient and Kaniere inefficient with respect to agronomic P efficiency. These contradictory results may arise from differences in population and effectivity of VA mycorrhizal fungi, light intensity, soil temperature or soil P supply. It has been shown that differences in the extent of mycorrhizal infection of three cereals tested at different soils led to inconsistent results in the assessment of P efficiency (Baon et al., 1992). Hence studies to select plants which are efficient in uptake or utilization of P should take into account the mycorrhizal status of the sites.

In general, the response to mycorrhizal infection by the barley cultivars in terms of shoot dry weight was small, even at low P treatment and similar results have been reported elsewhere (Jensen, 1983). A significant response was recorded only for Shannon. Barley with a fine-branched, extensive root system has an excellent capacity for uptake of immobile nutrients, such as P, and this may explain in part the low response to mycorrhizal infection. Nevertheless, significant responses to mycorrhizal infection in cereals have been reported by many workers (e.g. Thompson, 1990). This could occur as consequence of higher percentage of root length infected and soil temperature in Thompson's (1990) study compared to ours, although other factors (e.g. light intensity) could also contribute (Pearson et al., 1991).

Mycorrhizal colonization lowered the efficiency of utilization of P (higher P concentration in the whole plant) in most of the barley cultivars. Similar findings have been well documented and discussed (e.g. Smith, 1980; Stribley et al., 1980). Our present results showed that barley cultivars efficient in utilization of P do not respond to mycorrhizal infection by increasing shoot dry weight. Shannon, inefficient in utilization of P, was highly responsive to mycorrhizal infection and shoot weight was nearly double that of non-mycorrhizal plants. In addition, our study has shown that agronomic P efficiency was positively correlated to efficiency in utilization of P and was negatively correlated to mycorrhizal response.

Mycorrhizal response appeared to primarily be related to P utilization efficiency. Results of this study are contradictory to the findings of Menge et al. (1978), and Graham and Syvertsen (1985) who showed that sudangrass and citrus rootstocks, respectively, with low P concentration (high in P utilization efficiency) respon~~s~~ed more to mycorrhizal infection than plants with high P concentration. This may reflect differences in mycorrhizal response of these crops, sudangrass and citrus being much more dependent on mycorrhizas than cereals. Baylis (1972) has hypothesized that plant species having fibrous root systems are generally considered to be less dependent on mycorrhizal infection than plants having coarse roots with few or no root hairs.

Response to mycorrhizal infection varies among the barley cultivars. Several factors, including root mass which contributes to the ability of the plants to absorb P from the soil, may control the response of the plants to mycorrhizal infection. In this study we found that some of the barley cultivars have a different percentage of root length infected in spite of having similar root mass. Variation in mycorrhizal colonization may be due to an interaction between barley cultivars and the mycorrhizal fungus, *Glomus etunicatum*. Smith and Walker (1981) and Smith et al. (1992) also have pointed out that different levels of colonization between genotypes may arise from differences in the rates of growth of the fungus through the root cortex as well as the rates of root growth.

Differences in responses of plants to P nutrition are determined by the P required for the plant's metabolism, the allocation of P within plant, and the ability of the plant to take up P from the soil. Mycorrhizal infection increased P uptake by all barley cultivars from soil with low P supply. Specific P uptake was also increased by mycorrhizal infection, an effect which has been well documented (Smith et al., 1992). In the case of Shannon, total P uptake increase following mycorrhizal infection, resulted in the increase in vegetative growth (including roots) which may explain the lack of difference in specific uptake between mycorrhizal and non-mycorrhizal plants of Shannon.

Our data indicate that P concentration in both roots and shoots of non-mycorrhizal barley plants, when neither P nor mycorrhiza were supplied, was not correlated with percentage of root length colonized. This result contrasts with the results of both Menge et al. (1978), who found that high concentration of P in sudangrass tissues inhibited the colonization of VA mycorrhizal fungi, and Toth et al. (1984) who found that, in maize, a positive correlation existed between P concentration and percent mycorrhizal colonization. Those findings suggest that besides P tissue concentration, genetic differences among the barley cultivars may control the extent of colonization by a particular mycorrhizal fungus (Toth et al. 1984).

In conclusion, this study has demonstrated that agronomic P efficiency and P utilization efficiency of barley cultivars when neither P nor mycorrhiza were supplied affect the response of the plants to mycorrhizal infection. Mycorrhizal effect on these plant characteristics may have important implications for plant breeders in understanding the degree of mycorrhizal responses, and for selecting cultivars for nutritional traits, such as P efficiency.

Acknowledgements

Funding was provided by the Australian International Development Assistance Bureau (AIDAB). The authors thank Colin Rivers and David Hein for technical assistance, Lynne Giles and Ian Nevison for assistance in statistical analysis and Dr P A McGee for supplying cultures of *G. etunicatum*. J B Baon thanks the Research Institute for Estate Crops, Jember, Indonesia, for study leave.

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Section editor: G D Bowen

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