Mycorrhiza (1992) 2:41-45

Mycorrhiza

Influence of vesicular-arbuscular mycorrhizal infection and P addition on growth and P nutrition of *Anthyllis cytisoides* L. and *Brachypodium retusum* (Pers.) Beauv.

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Abstract. The effect of P applications and mycorrhizal inoculation on the growth and P nutrition of *Anthyllis cytisoides* L. (Fabaceae) and *Brachypodium retusum* (Pers.) Beauv. (Poaceae) was studied. Both plants are widely distributed and well adapted to semi-arid habitats in southern Spain. In all treatments, even with high P doses, mycorrhizal plants showed a higher concentration of phosphorus in their tissues than non-mycorrhizal plants. Mycorrhizal inoculation enhanced the growth of the plants when no P was applied. At high P addition, non-mycorrhizal plants showed higher growth than mycorrhizal plants. The response of each plant type to P application was somewhat different.

Key words: Vesicular-arbuscular mycorrhizae - P-fertilization - *Anthyllis cytisoides* - *Brachypodium retusum*

Introduction

In nature, the mycorrhizal condition is the rule and the non-mycorrhizal condition the exception (Gerdemann 1971). The primary cause of growth and yield enhancement in vesicular-arbuscular mycorrhizal (VAM) plants is the improved phosphate uptake (Gianinazzi-Pearson and Gianinazzi 1983). This is particularly important in phosphate-deficient soils, where VAM-infected plants usually take up more phosphorus and grow better than non-mycorrhizal plants (Hayman and Mosse 1972).

Semi-arid southern Spanish soils are in general very poor and deficient in minerals. Environmental conditions in this area are very extreme. Long, dry summers, very irregular and heavy rainfall and other anthropological factors have contributed for a long time to the erosion and the desertification of extensive areas; revegetation programmes are needed. Fabaceae and Poaceae should be considered in revegetation programmes for the following reasons: (1) N-fixing of legume plants enriches the soils; (2) the high development of graminoid root systems helps to hold the soil.

Anthyllis cytisoides L. (Fabaceae) and *Brachypodium retusum* (Pers.) Beauv. (Poaceae) are widely distributed in southern Spain. They are Mediterranean plants well adapted to very dry and sunny habitats with semi-arid conditions. These two species are important in revegetation programmes in southern Spain for the above-mentioned reasons and because: (1) their dissemination and seed germination is feasible; (2) they are good alternatives as wild forage (high biomass and nutritive value); (3) they can grow where other plants usually used in reforestation (generally coniferous) cannot.

Furthermore, both species are colonized by mycorrhizal fungi in natural conditions: *A. cytisoides* 60–77% and *B. retusum* 58–72% VAM-colonized roots (López-Sánchez and Honrubia 1992).

Several papers have reported responses of mycorrhizal plants to phosphate addition. The magnitude of these responses varies greatly with the soil (Powell 1977; Hall 1980; Rangelay et al. 1982; Ikram et al. 1987; Sainz and Arines 1988) and the plant (Waterer and Coltman 1988; Plenchette et al. 1983). The purpose of the present study was to examine the response of *A. cytisoides* and *B. retusum* to phosphate addition in relation to their degree of mycorrhization.

Materials and methods

Soil and plant treatments

Samples of soil were collected from a pinewood near Cieza (Murcia, Spain). Chemical and physical properties of the unsterilized soil are: pH 7.45; 0.19 dS/m electrical conductivity; 2.3% organic matter; 51% $CaCO_3$ eq total; 0.23 mmol/kg P; 0.46 mmol/kg K; 0.11 cmol/kg Na; 0.13 cmol/kg SO_4; 46% sand; 26.6% silt and 26.5% clay. Soil was sieved through a 4-mm mesh sieve to remove stones and coarse plant residues. Soil and sand were steam-sterilized (100° C) without pressure for 1 h on 3 consecutive days.

The experiment consisted of three soil treatments (sterilized, sterilized + inoculated and unsterilized soils) and four levels of P. Mixtures of soil and sand 1:1 (v:v) were incubated at different P

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Table 1. Height, shoot and root fresh weight, and vesicular-arbuscular mycorrhizal (VAM) infection of *Anthyllis cytisoides* and *Brachypodium retusum* at different P addition doses and soil treatments. Values followed by the same letter in a column are not significantly different at the 5% level (Duncan's test)

		Anthyllis cytisoides				*Brachypodium retusum*			
Soil condition	P addition (mg/kg)	Plant height (mm)	Shoot fresh wt. (mg/plant)	Root fresh wt. (mg/plant)	VAM infection (%)	Plant height (mm)	Shoot fresh wt. (mg/plant)	Root fresh wt. (mg/plant)	VAM infection (%)
Sterilized	0	310 a	65 a	82 a	0 a	680 ab	37 a	69 a	0 a
	30	513 b	250 b	200 bc	0 a	1167 d	169 bcd	293 abc	0 a
	60	590 bc	351 bc	227 bc	0 a	1047 cd	159 abc	486 bc	0 a
	90	823 de	550 d	548 d	0 a	1202 d	316 d	557 c	0 a
Sterilized and inoculated	0	528 b	250 b	187 b	67 c	1297 d	242 cd	531 c	63 bc
	30	599 bc	247 b	160 b	40 b	890 bc	214 bcd	479 bc	68 bc
	60	525 b	267 bc	162 b	41 b	794 bc	187 bcd	275 abc	41 b
	90	746 d	348 bc	288 bc	63 c	825 bc	164 bcd	268 abc	71 c
Unsterilized	0	303 a	53 a	50 a	65 c	518 a	40 a	77 a	61 b
	30	630 c	259 b	152 b	60 bc	640 b	56 ab	166 ab	57 b
	60	840 de	460 cd	160 b	44 b	767 a	67 ab	88 a	50 b
	90	880 e	580 d	305 c	40 b	876 bc	125 abc	362 abc	40 b

concentrations (0, 30, 60, 90 mg P/kg soil) for 15 days. P was added as a KH_2PO_4 solution. The VAM fungal inoculum consisted of spores, mycelium and infected roots from a 1-year-old *Medicago sativa* pot culture of *Glomus fasciculatum* (Thaxter sensu Gerdemann) Gerdemann and Trappe (from Zaidin-Granada collections). A single 7 g layer was placed at a depth of 3 cm below the substrate surface in each pot (sterilized + inoculated treatment soil). Seeds of *A. cytisoides* and *B. retusum* were sown in pots containing 500 g of soil-sand. Plants were grown in the greenhouse under natural lighting and harvested after 95 days. Each treatment was composed of five replicates.

Measurements

Plant height and the fresh and dry (80° C for 16 h) weight of shoot and root were recorded. Shoot tissues were digested in nitric-perchloric acid 5:3 for 6 h and phosphorus contents determined colorimetrically with the malachite green reagent (Fernandez et al. 1985). Mycorrhizal infection was assessed by staining root samples, previously cleared in 10% KOH, in trypan blue (Phillips and Hayman 1970). The percentage of colonization was estimated by the gridline intersect method (Giovanneti and Mosse 1980). Data were subjected to one-way analysis of variance and significant differences determined by Duncan's test.

Results

Plant growth

Plant growth responses to mycorrhizal inoculation and P fertilization are shown in Table 1 and Figs. 1a, b and 2a, b. When no P was applied the growth of plants was markedly increased by mycorrhizal inoculation with *G. fasciculatum*. However, plants growing in sterilized and unsterilized soils showed no significant differences ($P < 0.05$) in growth.

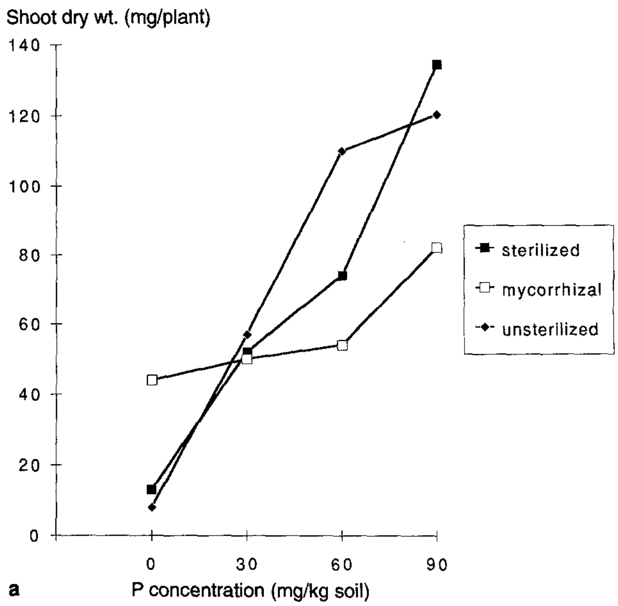
At the highest P doses, the growth (height, fresh and dry weight) of non-mycorrhizal *B. retusum* plants was higher than that of mycorrhizal plants (introduced and natural endophytes). However, at this P level the shoot weight and height of *A. cytisoides* growing in unsterilized and sterilized soils were not significantly different ($P < 0.05$). Increasing P addition enhanced plant growth in the two studied plants when they were grown in sterilized and unsterilized soils. In sterilized + inoculated soil, the growth of *A. cytisoides* remained practically constant and *B. retusum* growth slightly decreased with increasing P levels. P addition stimulated root growth of *A. cytisoides* in all soil treatments and also in sterilized and unsterilized soils in the case of *B. retusum*.

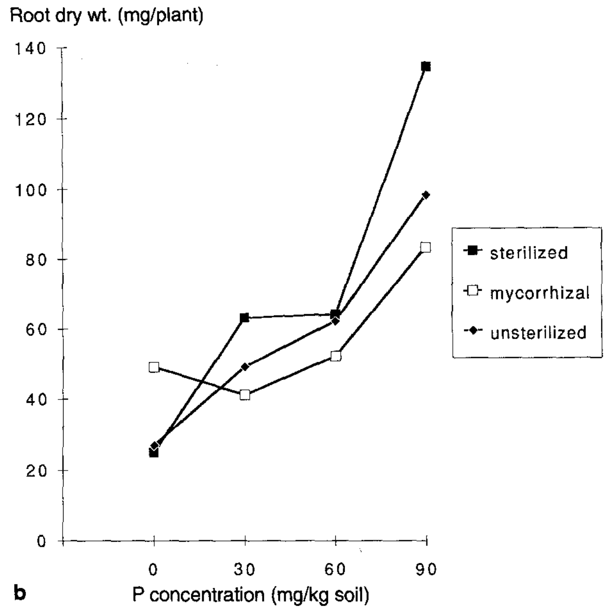
Phosphate nutrition

Phosphorus concentration in the tissues of studied plants increased with applied phosphate (Figs. 1c, 2c). Mycorrhizal plants had a higher P content than non-mycorrhizal plants in all treatments. The highest P concentration was recorded in plants grown in sterilized + inoculated soil when 90 mg P/kg soil was added. Plants grown in sterilized soil showed the lowest P concentration value when no P was applied. Phosphate fertilization tended to increase the P content of VAM-inoculated plants more markedly than those in sterilized and unsterilized soil.

Mycorrhizal infection

With phosphate fertilization, mycorrhizal infection of plants inoculated with *G. fasciculatum* was not affected, whilst the percentage of root colonization of plants grown in unsterilized soil (native endophytes) decreased slightly. The results were similar for *A. cytisoides* and *B. retusum* (Table 1).





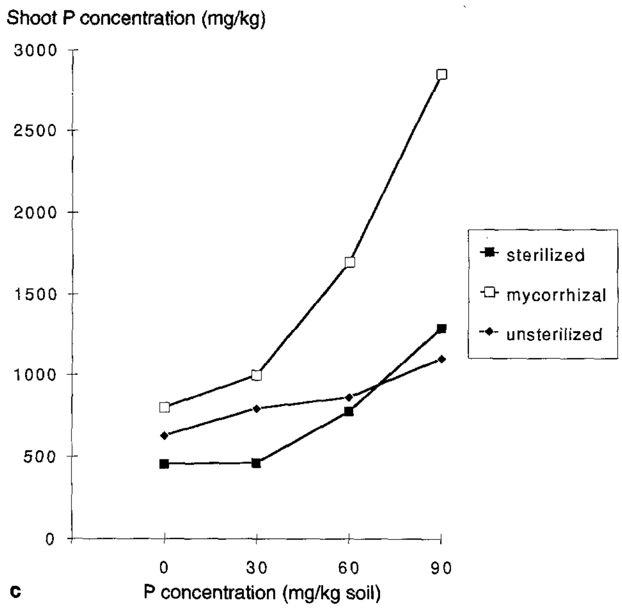


Fig. 1a–c. *Anthyllis cytisoides* responses to P fertilization. **a** Shoot dry weight (mg/plant); **b** root dry weight (mg/plant); **c** shoot P concentration (mg/kg)

Discussion

The results confirm the beneficial role of VAM symbiosis in the better utilization of the available P in soils by plants. Mycorrhizae can take up several times more phosphate than uninfected roots from soils (Mosse et al. 1973; Sanders and Tinker 1973; Smith and Gianinazzi-Pearson 1988).

In this study, mycorrhizal plants had higher internal P concentrations than non-mycorrhizal ones, but the concentrations did not correlate with increased growth. This agrees with Sainz and Arines (1988) and Waterer and Coltman (1988). With the lowest P level in soil, inoculation increased height and fresh and dry weight of the two species studied, as occurs with the majority of plants (Stribley et al. 1980; Fredeen and Terry 1987). The higher shoot dry matter of inoculated plants compared to non-mycorrhizal ones, expressed as a percentage of the dry matter of the mycorrhizal plants, was 70% for *A. cytisoides* and 87% for *B. retusum*. With the highest P addition, VAM inoculation resulted in a depression of growth by both plants.

Decreased growth following mycorrhizal infection may be explained by the carbohydrate source of the VAM symbiosis (Buwalda and Goh 1982). Parasitic effects of VAM infection have been reported during initial stages of VAM formation (Cooper 1975) at high P availability (Bethlenfalvay et al. 1983) and under conditions when photosynthesis is limited (Daft and El Giahmi 1978). Competition between roots and VAM fungi for host photosynthate could explain growth depression in mycorrhizal plants at high P (Thomson et al. 1986). Snellgrove et al. (1982) reported higher translocation of ^{14}C to belowground parts in mycorrhizal plants than in non-mycorrhizal plants, associated both with increased below-ground respiration and with increased loss of organic matter (possibly mycelium) to the soil. We observed a massive production of mycelium and spores of *G. fasciculatum* with high P addition. This could be another reason for the decrease in growth of mycorrhizal plants. In general, the higher the level of P in the soil, the greater the nodulation in roots of *A. cytisoides*, which was positively affected by the presence of VA infection.

The decrease in VAM root infection on adding P fertilizer has been widely reported in mycorrhizal studies (Gianinazzi-Pearson 1985). Some authors have also re-

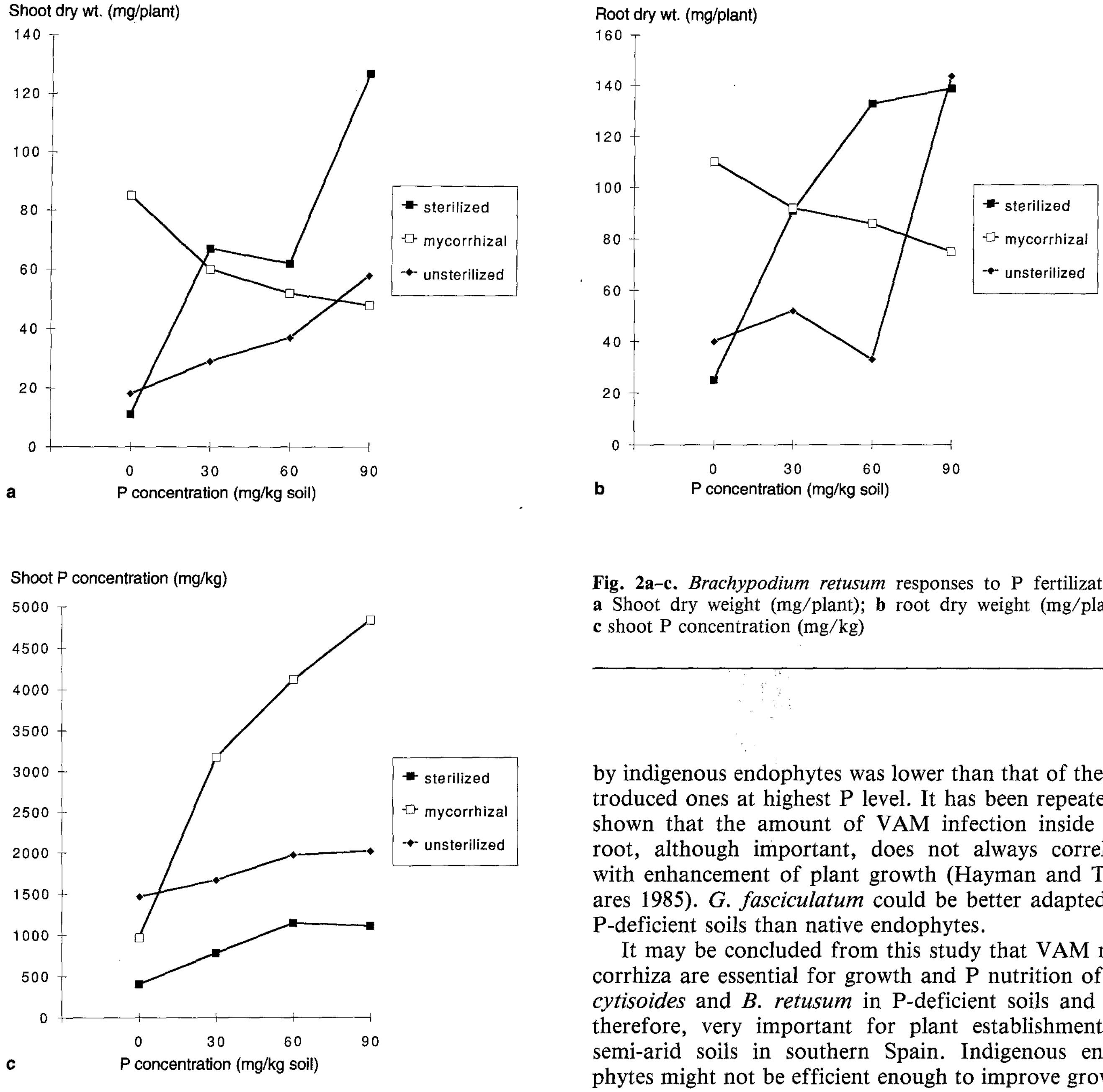


Fig. 2a–c. *Brachypodium retusum* responses to P fertilization. **a** Shoot dry weight (mg/plant); **b** root dry weight (mg/plant); **c** shoot P concentration (mg/kg)

ported that small additions of P to the soil increase VAM root infection when the supply of P is extremely deficient for plant growth. In this study there was no clear trend in percentage infection with increasing applied P. VAM colonization was maintained in sterilized + inoculated soils (introduced endophytes) and decreased slightly in unsterilized soils (indigenous endophytes). Clarke and Mosse (1981) reported that infection by indigenous endophytes was clearly more depressed by added phosphate than infection by introduced endophytes.

Indigenous endophytes were less efficient than inoculated *G. fasciculatum* in promoting plant growth of studied plants with low P concentration. However, they were more efficient in plant growth improvement when phosphate was added, although mycorrhizal infection by indigenous endophytes was lower than that of the introduced ones at highest P level. It has been repeatedly shown that the amount of VAM infection inside the root, although important, does not always correlate with enhancement of plant growth (Hayman and Tavares 1985). *G. fasciculatum* could be better adapted to P-deficient soils than native endophytes.

It may be concluded from this study that VAM mycorrhiza are essential for growth and P nutrition of *A. cytisoides* and *B. retusum* in P-deficient soils and are therefore, very important for plant establishment in semi-arid soils in southern Spain. Indigenous endophytes might not be efficient enough to improve growth in these soils and the selection of introduced endophytes more effective than native ones is an aspect of great interest for practical application.

References

Bethlenfalvay GJ, Bayne HG, Pacovsky RS (1983) Parasitic and mutualistic association between a mycorrhizal fungus and soybean: the effect of phosphorus on host plant endophyte interaction. Physiol Plant 57:543–548

Buwalda JG, Goh KM (1982) Host fungus competition for carbon as a cause of growth depressions in vesicular-arbuscular mycorrhizal ryegrass. Soil Biol Biochem 14:103–106

Clarke C, Mosse B (1981) Responses of VA mycorrhizas. XII. Field inoculation responses of barley at two soil P levels. New Phytol 85:696–703

Cooper KM (1975) Growth responses to the formation of endotrophic mycorrhizas in *Solanum*, *Leptospermun*, and New Zealand ferns. In: Sanders F, Mosse B, Tinker PB (eds) Endomycorrhizas. Academic Press, London New York, pp 391–407

Daft MJ, El-Giahmi AA (1978) Effects of *Endogone* mycorrhiza on plant growth. VIII. Effects of defoliation and light on selected hosts. New Phytol 73:743–749

Fernandez JA, Niell FX, Lucena J (1985) A rapid and sensitive automated determination of phosphate in natural waters. Limnol Oceanogr 30:227–230

Fredeen AL, Terry N (1987) Influence of vesicular-arbuscular mycorrhizal infection and soil phosphorus level on growth and carbon metabolism of soybean. Can J Bot 66:2311–2316

Gerdemann JW (1971) Fungi that form the vesicular-arbuscular type of endomycorrhiza. In: Hacskaylo E (ed) Mycorrhizae. United States Department of Agriculture, pp 1189, 9–18

Gianinazzi-Pearson V (1985) Mycorrhizal effectiveness in phosphate nutrition: how, when and where? In: Molina R (ed) Proceedings of the 6th North American Conference on Mycorrhizae, Bend, Ore. Forest Research Laboratory, Corvallis, Ore, pp 150–154

Gianinazzi-Pearson V, Gianinazzi S (1983) The physiology of vesicular-arbuscular mycorrhizal roots. Plant Soil 71:197–209

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–499

Hall IR (1980) Growth of *Lotus penduculatus* Cav. in an eroded soil containing pellets infected with endomycorrhizal fungi. NZ J Agric Res 23:103–105

Hayman DS, Mosse B (1972) The role of vesicular-arbuscular mycorrhiza in the removal of phosphorus from soil by plant roots. Rev Ecol Biol Sol 9:463–470

Hayman DS, Tavares M (1985) Plant growth responses to vesicular-arbuscular mycorrhiza. XV. Influence of soil pH on the symbiotic efficiency of different endophytes. New Phytol 100:367–377

Ikram A, Mahmud AW, Napi D (1987) Effects of P-fertilization and inoculation by two vesicular-arbuscular mycorrhizal fungi on growth and nodulation of *Calopogonium caeruleum*. Plant Soil 104:104–207

López-Sánchez ME, Honrubia M (1992) Seasonal variation of vesicular-arbuscular mycorrhizae in eroded soils from southern Spain. Mycorrhiza 2:33–39

Mosse B, Hayman DS, Arnold DJ (1973) Plant growth responses to vesicular-arbuscular mycorrhizas. V. Phosphate uptake by three plant species from P-deficient soils labelled with ^{32}P. New Phytol 72:809–815

Phillips JM, Hayman DS (1970) Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Plenchette C, Fortin JA, Furlan V (1983) Responses of endomycorrhizal plants grown in a calcined montmorillonite clay to different levels of soluble phosphorus. I. Effect on growth and mycorrhizal development. Can J Bot 61:1377–1383

Powell CL (1977) Mycorrhizas in hill-country soils. II. Effect of several mycorrhizal fungi on clover growth in sterilized soils. NZ J Agric Res 20:59–62

Rangelay A, Daft MJ, Newbould A (1982) The inoculation of white clover with mycorrhizal fungi in unsterile hill soils. New Phytol 92:89–102

Sainz MJ, Arines J (1988) Effects of native vesicular-arbuscular mycorrhizal fungi and phosphate fertilizer on red clover growth in acid soils. J Agric Sci Camb 111:67–73

Sanders EF, Tinker PB (1973) Phosphate flow into mycorrhizal roots. Pestic Sci 4:385–389

Smith SE, Gianinazzi-Pearson V (1988) Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. Annu Rev Plant Physiol Plant Mol Biol 39:221–224

Snellgrove RC, Splittsoesser WE, Stribley DP, Tinker PB (1982) The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. New Phytol 92:75–87

Stribley DP, Tinker PB, Rayner JH (1980) Relation of internal phosphorus concentration and plant weight in plants infected by vesicular-arbuscular mycorrhizas. New Phytol 86:261–266

Thomson BD, Robson AD, Abbot LK (1986) Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to carbohydrates. New Phytol 103:751–765

Waterer DR, Coltman RR (1988) Phosphorus concentration and application interval influence growth and mycorrhizal infection of tomato and onion transplants. J Am Soc Hortic Sci 113:704–708