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Effects of mycorrhizae, established from an existing intact hyphal network, on the growth response of capsicum (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.) to five rates of applied phosphorus

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Abstract The growth response of 2 vegetable crops to 5 rates of applied phosphorus (P) in the presence or absence of an existing network of extraradical mycorrhizal mycelium was determined in 2 greenhouse pot experiments (Expt 1, autumn–winter; Expt 2, summer–autumn) using a low-P growth medium (6 or 5 mg $NaHCO_3$-extractable P/kg for Expt 1 or 2, respectively). In both experiments, capsicum (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.) plants were grown at 0 (P_1), 9.2 (P_2), 27.5 (P_3), 82.5 (P_4), or 248 (P_5) mg P/kg oven-dry soil (spot-placed at sowing) within a nylon mesh (pore size 44 μm). The mesh excluded roots from the original sunflower (*Helianthus annuus* L.) host plants, to which either live (VAM+) or killed (VAM–) mycorrhizal [*Glomus etunicatum* Becker & Gerdemann and *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe] inoculum was added at sowing. The mesh did allow fungal hyphae to grow into the growth medium contained by the mesh.

Whereas VAM+ plants generally had higher P concentrations in index tissues than VAM– plants at low P rates, a concomitant increase in dry matter yield was restricted to the P_1 rate. At P_1 in Expt 2, the increase in the dry weight of whole plants as a result of VAM colonisation was as large as 91.7-fold and 17.9-fold for capsicum and tomato, respectively. Root starch analysis indicated that the lower dry matter yields of VAM+ plants than of VAM– plants at $\geq P_2$ could be attributed to insufficient photosynthate production by VAM+ plants to meet the carbon (C) demand of both host and endophytes within the relatively low-light environment of the greenhouse (average daily solar irradiance of 8.4 MJ/m^2 for Expt 1 and 13.4 MJ/m^2 for Expt 2).

The growth response of vegetable crops grown within the greenhouse from colonisation by an established mycorrhizal mycelium appears to depend on a critical balance of P and C supply; i.e. at P_1, P was more limiting than C, and the increased uptake of P as a result of colonisation of plant roots by VAM resulted in a growth response. At higher P rates, C was more limiting than P due to low light in the greenhouse, and the additional demand for photosynthate imposed by the endophytes on the host resulted in a growth depression relative to non-mycorrhizal plants.

Introduction

Few studies have investigated the effects of an existing VAM network on crop growth over a range of phosphorus (P) applications up to levels comparable with those used by commercial growers. In a greenhouse experiment (Fairchild and Miller 1990) and a field trial (McGonigle *et al*. 1990), the response of corn plants grown in undisturbed soil containing a vesicular-arbuscular mycorrhizal (VAM) network was compared with the response of corn plants grown in disturbed soil without a network. Neither of these studies employed a control treatment in which the corn plants were grown in soil devoid of living VAM propagules. Thus, the full effect of the VAM network on plant response could not be assessed.

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Most scientists who have studied the interaction between an existing VAM network and a crop species have selected broad-scale agricultural crops, such as field corn (e.g. Evans and Miller 1990; McGonigle and Miller 1993), or pasture plants, such as subterranean clover (e.g. Jasper *et al*. 1989), as the production (or bioassay) crop. There appear to be no studies which have investigated the role of mycorrhizae, established from an existing intact hyphal network, on intensively managed vegetable crop species such as capsicum and tomato, especially over a range of P supply from deficiency to adequacy.

In a previous study (Olsen *et al*. 1996), the growth response of capsicum, sweet corn, and tomato plants to addition of VAM inoculum was determined at 5 rates of applied P. In that study, the network of extraradical hyphae which eventually benefited the P nutrition of the plants grown without P was formed from carbon (C) invested by the host plants. Although there appear to be no published studies which have determined the C contribution by a host plant for development of the mycelial web, Tinker *et al*. (1994) reported that several studies have estimated that 6–10% of the total net C fixed by the host plant is transferred to the VAM roots. Without the C drain to form a network of extraradical hyphae, such as can be achieved by planting into undisturbed soil in which a VAM network was developed by a previous crop, a greater plant growth response to VAM may occur. The present greenhouse experiments assessed the effects of an existing network of extraradical mycorrhizal mycelium (measured against non-mycorrhizal control treatments) on the growth response of capsicum and tomato plants to 5 rates of applied P.

Materials and methods

Two pot experiments were conducted to assess the effects of an existing soil mycorrhizal network on the growth response of capsicum (*Capsicum annuum* L. cv. Target) and tomato (*Lycopersicon esculentum* Mill. cv. Floradade) plants over a range of P supply. Each experiment consisted of 2 phases. In the first (pre-conditioning) phase, sunflower (*Helianthus annuus* L. cv. Advance) was sown directly above either live (VAM+) or killed (VAM–) mycorrhizal inoculum to establish pots with or without mycelial networks. The 2 sunflower nurse plants were grown outside a centrally positioned nylon mesh (Nytal® Swiss screen with pore size 44 μm, BCNY-325-44-102) root exclusion cage (Fig. 1*a*), which was impervious to roots but not to mycorrhizal hyphae. The pre-conditioning phase ended when stems of the sunflower plants were severed at soil level and the tops were removed. In the second (production) phase, a capsicum or tomato plant was grown inside the cage (Fig. 1*b*). A schedule of operations for the pot experiments is shown in Table 1.

Inoculum description

The mycorrhizal inoculum used in the pot experiments (previously described in Olsen *et al*. 1996) contained *Glomus etunicatum* Becker &

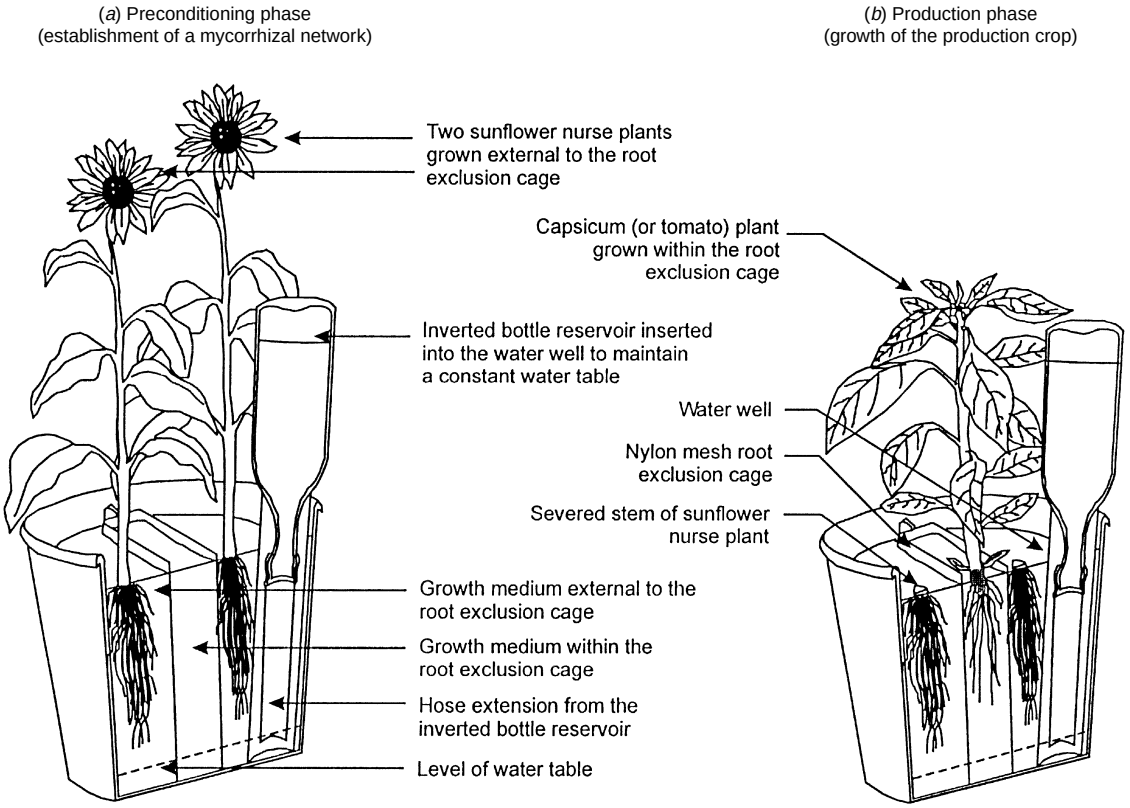

Fig. 1. Diagrammatic cross-section of the arrangement of the nylon mesh root exclusion cage, water well, the sunflower nurse plants grown in the pre-conditioning phase, and the capsicum (or tomato) plant grown in the production phase of Expts 1 and 2.

Table 1. A summary of the sequence of operations in each experiment

Task description	Expt 1	Expt 2
Pre-conditioning phase		
Sunflower nurse crop sown	2 Feb. 1994	14 Nov. 1994
Sunflower tops removed	28 Apr. 1994	30 Jan. 1995
Sowing–harvest (days)	85	77
Production phase		
Capsicum		
Germinated seeds sown	5 May 1994	31 Jan. 1995
Plants harvested	16 Aug. 1994	28 Mar. 1995
Sowing–harvest (days)	103	56
Tomato		
Germinated seeds sown	9 May 1994	2 Feb. 1995
Plants harvested	11 July 1994	13 Mar. 1995
Sowing–harvest (days)	63	39

Gerdemann and *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe. The inoculum has been lodged at INVAM (Morton *et al*. 1993) and was given the accession code 'AU401'.

Most probable number (MPN) tests (Porter 1979) were conducted on the VAM+ inoculum at the time of sowing the sunflower seeds. Inoculum was mixed with the fumigated growth medium used in Expt 1 (chemical properties shown in Table 2) in sequential 2-fold dilutions ranging from 1 : 125 to 1 : 64 000 for Expt 1 and from 1 : 250 to 1 : 128 000 for Expt 2. Prior to mixing, basal applications of 22.9 mg N/kg oven-dry soil [as $Ca(NO_3)_2.4H_2O$] and 2.3 mg P/kg oven-dry soil [as $Ca(H_2PO_4)_2.H_2O$] were applied to the growth medium; the remaining nutrients were applied as described in Table 3. Standard MPN statistics were used to calculate the number of propagules in each cell of undiluted inoculum (Daniels and Skipper 1982). The MPN tests revealed 56.7 (Expt 1) and 100.9 (Expt 2) non-dormant infective propagules/g air-dry inoculum at the commencement of the pre-conditioning phase, adequate to ensure high rates of colonisation (Haas and Krikun 1985; Olsen *et al*. 1996).

Preparation of pots

The growth medium placed in each pot (10-L bucket) consisted of 6 kg each of air-dry coarse sand and soil mixed together to give a loamy sand texture (McDonald *et al.* 1984). The soil component of the growth medium (excavated to a depth of 1 m) originated from Moolboolaman, south-eastern Queensland (25°02′S, 151°52′E), and is variously classified as a Mollic Ustifluvent (USDA 1975), a Basic Regolithic Orthic Tenosol (Isbell 1996), an Earthy Sand (Stace *et al.* 1972), and as Uc5.21 (Northcote 1979).

The coarse sand component of the growth medium used in both experiments was obtained from one uniformly mixed stockpile (4 mg $NaHCO_3$-extractable P/kg). However, the soil component was taken from 2 separate, uniformly mixed stockpiles (Expt 1 from one and Expt 2 from the other). For Expt 1, the growth medium consisted of 62% coarse sand, 29% fine sand, 5% silt, and 4% clay, whereas particle size analysis of the growth medium used in Expt 2 revealed 66% coarse sand, 26% fine sand, 3% silt, and 5% clay. Chemical analysis of the growth media showed an inadequate supply of NO_3-N, P, K, Ca, Cu, Zn, SO_4-S, and B (Table 2).

The 12 kg of growth medium placed in each pot was mixed thoroughly with basal nutrients and 3.0 g $Ca(OH)_2$ [which raised soil pH(1 : 5 soil : water) to approximately 6.5 after 3 weeks] (Table 3).

The nylon mesh was formed into cages by gluing all seams with solvent cement (Vinidex®) between 2 strips of polyvinyl chloride of dimensions 230 by 25 by 1 mm. Cages were made to fit tightly over a steel frame of external dimensions 200 by 150 by 50 mm welded from 8-mm-diameter solid steel rod to standardise the cage dimensions. The cages were positioned centrally within pots and filled with growth medium; following removal of the steel frame, each cage was set at a depth such that the top 10 mm of mesh extended above the surface of the

Table 2. Chemical analysis of the growth medium (1:1 coarse sand: Mollic Ustifluvent) used in the pot experiments

Property	Unit of measurement	Expt 1	Expt 2	Desirable range[A]	Method	Reference(s)
pH		6.20	6.40	6.0–7.0	1 : 5 soil : water	Loveday (1974)
Electrical conductivity	dS/m	0.082	0.118	<0.30	1 : 5 soil : water	Bower and Wilcox (1965)
Organic carbon	%	0.6	0.2	>2.0	$K_2Cr_2O_7 + H_2SO_4$	Walkley and Black (1934); Sims and Haby (1971)
NO_3-N	mg/kg	2	1	25–60	1 : 5 soil : water	Bremner (1965)
P	mg/kg	6	5	60–100	1 : 100 soil : 0.5 M $NaHCO_3$	Colwell (1963)
K	cmol(+)/kg	0.07	0.06	0.37–1.5	1 : 20 soil : 1 M NH_4Cl, pH 7.0	Tucker (1971); Loveday *et al.* (1972)
Ca	cmol(+)/kg	1.50	1.30	>3.0	1 : 20 soil : 1 M NH_4Cl, pH 7.0	Tucker (1971); Loveday *et al.* (1972)
Mg	cmol(+)/kg	0.86	0.76	>0.4	1 : 20 soil : 1 M NH_4Cl, pH 7.0	Tucker (1971); Loveday *et al.* (1972)
Na	cmol(+)/kg	0.35	0.48	<2.0	1 : 20 soil : 1 M NH_4Cl, pH 7.0	Tucker (1971); Loveday *et al.* (1972)
CEC[B]	cmol(+)/kg	2.78	2.60	>4.0	1 : 20 soil : 1 M NH_4Cl, pH 7.0	Tucker (1971); Loveday *et al.* (1972)
Fe	mg/kg	29	16	>2.0	1 : 2 soil : 0.005 M DTPA	Lindsay and Norvell (1978)
Cu	mg/kg	0.2	0.1	0.3–10	1 : 2 soil : 0.005 M DTPA	Lindsay and Norvell (1978)
Mn	mg/kg	9	4	4–45	1 : 2 soil : 0.005 M DTPA	Lindsay and Norvell (1978)
Zn	mg/kg	0.4	0.3	1–10	1 : 2 soil : 0.005 M DTPA	Lindsay and Norvell (1978)
Cl	mg/kg	108	132	<300	1 : 5 soil : water	Rayment and Higginson (1992)
SO_4-S	mg/kg	4	5	20–100	1 : 5 soil : 0.01 M $Ca(H_2PO_4)_2$	Fox *et al*. (1964); Barrow (1967); Beaton *et al*. (1968)
B	mg/kg	0.1	0.2	2–5	1 : 2 soil : hot 0.01 M $CaCl_2$	Cartwright *et al.* (1983)

[A] Incitec (1989).

[B] Cation exchange capacity estimated as the sum of Ca, Mg, Na, and K.

Table 3. Details of rates of nutrients applied to the pre-conditioning phase of the pot experiments
Total S application of 37.1 mg/kg oven-dry (OD) soil from addition of other nutrient salts

Element[A]	Rate of element application[B]	Nutrient salt	Rate of salt application	Application method
N	50 mg/L	$Ca(NO_3)_2.4H_2O$	422 mg/L	Irrigation solution
P[C]	4.6 mg/kg OD soil	$Ca(H_2PO_4)_2.H_2O$	0.22 g/pot	Dry mix
K	55.0 mg/kg OD soil	K_2SO_4	1.46 g/pot	Basal application of stock solution
Ca	136 mg/kg OD soil	$Ca(OH)_2$	3.0 g/pot	Dry mix
Mg	9.2 mg/kg OD soil	$MgSO_4.7H_2O$	1.11 g/pot	Basal application of stock solution
Zn	1.8 mg/kg OD soil	$ZnSO_4.7H_2O$	96 mg/pot	Basal application of stock solution
Cu	0.9 mg/kg OD soil	$CuSO_4.5H_2O$	43 mg/pot	Basal application of stock solution
Mn	1.8 mg/kg OD soil	$MnSO_4.4H_2O$	89 mg/pot	Basal application of stock solution
Mo	0.1 mg/kg OD soil	$Na_2 MoO_4.2H_2O$	2.8 mg/pot	Basal application of stock solution
B	0.2 mg/kg OD soil	H_3BO_3	16 mg/pot	Basal application of stock solution

[A] Two stock solutions were made and applied separately to minimise the possibility of precipitation within solution. Nutrient combinations were K, Mo, and B in one solution and Mg, Zn, Cu, and Mn in the other.
[B] Each pot was filled with 12.0 kg growth medium (1 : 1 coarse sand : Mollic Ustifluvent) which had <1% moisture.
[C] Half of the applied P (viz. 2.3 mg/kg oven-dry soil) was spot-placed at a depth of 50 mm on each side of, and external to, the root exclusion cage. The VAM+ or VAM– inoculum was placed immediately above this P, while 5 sunflower seeds were placed on top of the inoculum and covered with 10 mm of growth medium.

growth medium. A 240-mm length of Polydrain® (James Hardie Irrigation) corrugated drainage pipe (65 mm external diameter, class 400) was placed vertically along the pot wall. A 200-mm length of 25-mm-diameter garden hose was heated and pushed firmly over the neck of a bottle of capacity 750 mL to ensure a water-tight seal. The bottle and hose extension (total capacity approximately 860 mL) were inverted and placed into the corrugated drainage pipe. The shoulder of the bottle was supported by the top of the pipe so that the tip of the hose was suspended approximately 10 mm above the bottom of the pot. Provided water was in the bottle, this set-up maintained a constant watertable at the bottom of the pot (Hunter 1981).

Each pot was surface-watered with 600 mL of water in order to moisten soil prior to fumigation. Prepared pots and one-half of the inoculum required for each experiment were placed within sealed plastic sheets and fumigated with a mixture of 98% CH_3Br and 2% CCl_3NO_2 at a rate of 680 g/m^3 soil. After 48 h, the plastic covers were removed and pots and sterilised inoculum vented for at least 72 h prior to sowing sunflower seed in the pre-conditioning phase.

Pre-conditioning phase

For each fumigated pot in Expts 1 and 2, 0.11 g $Ca(H_2PO_4)_2.H_2O$ (equivalent to 2.3 mg P/kg oven-dry soil) was spot-placed at a depth of 50 mm on each side of, and external to, the centrally positioned root exclusion cage; on each side of the cage, the P was positioned equidistant between the mesh wall and the edge of the bucket. Then, 50 g of either VAM– or VAM+ air-dry inoculum was placed immediately above the P; 5 sunflower seeds, which had been surface-sterilised by soaking in 0.03% calcium hypochlorite for 10 min, were placed immediately above the inoculum and covered with 10 mm of fumigated growth medium.

Following sowing (dates shown in Table 1), a layer (approximately 20 mm) of white polystyrene spheres (average diameter 9 mm) was placed on the surface of the growth medium to reduce surface evaporation and salt accumulation and the pot sides and tops were covered with reflective insulation to minimise temperature fluctuations. Pots were then placed on benches within a greenhouse. Approximately 1 week after seedling emergence, each bottle and hose extension was filled with an irrigation solution of 50 mg N/L (Table 3), quickly inverted, and placed in the pipe within each pot. This operation established a constant watertable at the base of each column of soil. Provided the bottle contained some solution, this system ensured that the water status throughout the soil mass remained static and was not affected by increasing plant weight (as occurs in the water-to-weight watering technique).

At about 2 weeks after emergence, all except the most vigorous single sunflower plant on each side of the root cage were removed from each pot by severing the stems at ground level. Within each experiment, pots designated for planting with capsicum or tomato in the production phase were randomised separately.

Each sunflower plant grown in Expt 2 was rated for P deficiency symptoms on 25 January 1995 by visually assessing the amount of necrosis of the blade of the third leaf below the youngest mature leaf.

At harvest (dates shown in Table 1), stems of the 2 sunflower plants in each pot were severed at soil level and the youngest mature blade (YMB) was removed from each plant and briefly rinsed in deionised water. These 2 YMBs and the remaining tops were placed separately into labelled paper bags, dried in a forced draught oven at 65°C for 72 h, and weighed. For pots designated to be planted with capsicum or tomato in the production phase of each experiment, YMBs were pooled across replicates/blocks into separate VAM– and VAM+ groups and ground through a 1-mm mesh in a stainless steel mill. Samples were dried again at 85°C for 48 h before chemical analysis. Total N was determined using Kjeldahl digestion followed by automated colorimetry (O'Neill and Webb 1970), whereas P and Mn were measured using HNO_3 digestion and inductively coupled plasma emission spectrometry (Zarcinas *et al.* 1987).

Production phase

Experimental design

Each production crop species was grown in 20 pots in Expt 1 and 40 pots in Expt 2 in a randomised factorial design, with the pots randomised separately within each crop species. For Expt 1, the factorial design comprised 5 P rates × 2 (+/–) VAM treatments with 2 replicates/blocks, whereas the factorial design of Expt 2 comprised 5 P rates × 2 (+/–) VAM treatments with 4 replicates/ blocks.

Greenhouse methodology

Six germinated capsicum or tomato seeds were sown within the centre of each root exclusion cage (dates shown in Table 1). Also within the cage, a tapered hole (approximately 100 mm deep, 20 mm diameter at the surface) was made on each side of the seeds, approximately equidistant between the seeds and the seamed edge of the cage. Into each of the 2 holes within the

root exclusion cage, half of the required amount of P was carefully placed at the bottom of the hole, minimising the amount adhering to the wall. Each hole was then back-filled using the soil located at the surface. One of 5 rates of P was applied to each pot using this technique: 0 (P_1), 9.2 (P_2), 27.5 (P_3), 82.5 (P_4), or 248 (P_5) mg/kg oven-dry soil as 0, 0.45, 1.34, 4.00, or 12.01 g $Ca(H_2PO_4)_2.H_2O$/pot, respectively.

Prior to seedling emergence, a 20-mL aliquot from each of 2 separate stock solutions of basal nutrients (K, Mo, and B in one and Mg, Zn, Cu, and Mn in the other; rates shown in Table 3) was applied to the bottom of the water well in each pot on consecutive days. Sown seeds were surface-watered daily with deionised water until emergence, after which the constant watertable method was established. The irrigation solution contained dissolved N at a concentration of 50 mg/L [422 mg $Ca(NO_3)_2.4H_2O$/L].

At about 2 weeks after emergence, all except the most vigorous single plant were removed from each pot by severing the stems at ground level. In order to ensure that sufficient plant material was available for diagnostic analysis of the index tissues, it was decided in Expt 2 not to thin plants growing in the VAM– P_1 pots to the most vigorous single plant until some growth response had occurred. It was assumed that such small plants would be neither light nor water limited. The capsicum and tomato plants growing in this treatment showed little or no growth following emergence and, consequently, were not thinned.

Three soil temperature probes were installed at a depth of 50 mm in randomly selected pots from 5 May to 23 August 1994 (Expt 1) and from 8 February to 28 March 1995 (Expt 2). Mean soil temperatures during these periods were 20.1ºC (range 9.0 – 32.4ºC) and 27.8ºC (range 20.7 – 37.8ºC), respectively. Air temperatures within the greenhouse from 6 June to 23 August 1994 ranged from 4.9 to 40.9ºC, with a mean of 19.0ºC; air temperatures within the greenhouse were not measured at any stage during the production phase of Expt 2.

The average daily solar irradiance outside the greenhouse from 5 May to 23 August 1994 (Expt 1) was 12.7 MJ/m^2, with daily irradiance values ranging from 4.8 to 15.9 MJ/m^2. Within the greenhouse, the daily average irradiance and range of values for the same period were estimated at 8.4 and 3.2–10.5 MJ/m^2, respectively, assuming a reduction in light transmission of 34%. For Expt 2, average daily solar irradiance within the greenhouse was 13.4 MJ/m^2 (with daily irradiance values ranging from 2.6 to 17.4 MJ/m^2) from 31 January to 28 March 1995.

Harvest methodology

At harvest, index leaves, fruit (if present), and remaining tops above soil level were separated for each pot. For each species, index leaf selection was based on those plant parts recommended by Piggott (1986) for diagnostic analysis and on recovery of sufficient material for analysis from the single plants grown in each pot (capsicum, 6 youngest mature leaf blades plus petioles, 6YMB+P; tomato, 3 youngest mature leaf blades plus petioles, 3YMB+P). Index leaves were washed in deionised water and all plant parts were placed separately into labelled paper bags which were placed in a forced-draught oven at 65ºC until the plant tissues were dry.

For capsicum, 2 vertical soil cores of diameter 22 mm were removed outside the root exclusion cage from the side of the external soil compartment not containing the water well (see Fig. 1*b*). Each core was centred 20 mm from the mesh wall and 50 mm to either the left or right of the severed stem of the sunflower plant. The left core was used for determination of the length of extraradical mycorrhizal hyphae and the right core for the quantification of soil moisture. Soil extracted from each core was mixed thoroughly and 10 g was subsampled from each. The hyphal length sample was stored in 70% ethanol and the soil moisture sample was placed in a forced-draught oven at 105ºC until dry and then weighed. Length of soil hyphae per g oven-dry soil was estimated using the filtration-gridline method (Sylvia 1992).

Root exclusion cages containing root systems of the production crop species were lifted from pots and the root systems were removed, washed, blotted dry with paper towels, cut into approximately 10-mm lengths, and weighed. Two weighed samples (approximately 1 g each) of randomly selected root pieces were taken from each root system for VAM and root length determination and placed in 70% ethanol until processing. For treatments where insufficient root material was available for 1 g each of VAM and root length samples, the total quantity of fresh root material sampled did not exceed 80% of the total fresh weight of roots in Expt 1 or 40% in Expt 2. The remaining roots were placed in labelled paper bags in a forced-draught oven at 65ºC until dry. Dry weight of each entire root system was then estimated using the moisture loss of the sample. After weighing, the dry roots were ground through a 1-mm mesh in a stainless steel mill and analysed for starch using an enzymic-colorimetric procedure (Rasmussen and Henry 1990).

Prior to cutting the entire capsicum root systems grown in the P_2 and P_3 treatments in Expt 2, 10 root tips (each approximately 10 mm long) were cut, weighed, surface-sterilised in sodium hypochlorite solution (0.1%) for 1 min, and transferred to culture plates of potato dextrose agar containing 50 mg/L of streptomycin sulfate (S/PDA), and penicillin-G (500 mg/L), polymixin B sulfate (50 mg/L), and pimaricin (0.4 mL/L) (3P agar). The culture plates were incubated at 27ºC for 7 days prior to assessing for the presence of fungal pathogens; none was detected for both VAM+ and VAM– pots. Inspection of the washed root systems of the capsicum and tomato plants grown in both experiments revealed no occurrence of *Meloidogyne* spp. galls in any root system.

Sample root lengths were determined using a root length scanner (Comair®), and the root length of each entire root system was then estimated. Roots were cleared and stained using the methodology of Koske and Gemma (1989), although for capsicum, the methodology was slightly modified to improve differentiation between the root tissues and internal mycorrhizal structures. These modifications included: clearing roots in 2.5% KOH solution at 65ºC for 4 h; soaking roots at an initial temperature of 65ºC and allowing them to cool to room temperature and stand for a further 4 h; and destaining roots in 2.5% KOH solution at 65ºC for 5 min prior to rinsing with tap water.

The proportion of root colonisation by VAM (as a percentage of the total root length) was determined by the gridline intersect method (Ambler and Young 1977), observing 100 root intersections under a dissecting microscope ($\times$30) to obtain a standard error of ±4% (Giovannetti and Mosse 1980).

Relative mycorrhizal dependency (RMD) was calculated as:

$$\frac{100 \times (\text{DW mycorrhizal plants} - \text{DW non-mycorrhizal plants})}{\text{DW mycorrhizal plants}}$$

where DW is dry weight, as described by Plenchette *et al.* (1983).

Oven-dried index leaves were ground and analysed as previously described for sunflower.

Statistical analysis

For each crop species grown in the production phase of each experiment, a separate ANOVA was used to test the effects of treatments for each parameter. Means were compared using the protected l.s.d. procedure operating at $P = 0.05$. In the protected l.s.d. procedure, treatment effects are tested overall in the ANOVA F-test and pairwise differences among treatment means are t-tested only if the ANOVA F-test for treatments is significant (Steel and Torrie 1980).

Results

For the various plant parameters in this study, the VAM $\times$ P interaction was significant ($P < 0.05$) in most cases. Where this interaction was not significant, it was deemed to be biologically important and is presented in the Figures for completeness.

Pre-conditioning phase

Sunflower nurse plants were grown to flowering in the pre-conditioning phase of both experiments, at which stage it was deemed (based on previous work and a higher [$P < 0.05$ in Expt 2] incidence of P deficiency symptoms [described by

Hunter and Kochman 1985] in VAM– plants than in VAM+ plants) that a network of VAM hyphae had developed throughout each pot. In both experiments, the dry weights of tops of the sunflower plants grown with a VAM network were greater ($P < 0.05$) than those of plants grown without a network. For pots designated to be planted with capsicum or tomato in the production phase of each experiment, the concentration of P in the YMB of VAM+ plants (0.08–0.11%) was higher than that in the YMB of VAM– plants (0.06–0.08%), although a significance test of the differences was not possible since the YMBs were pooled across replicates/blocks for each of the VAM+ and VAM– treatments. Treatment with VAM had little effect on N concentration in the YMB.

Production phase

Total dry weight

The total dry weight response curves for VAM+ and VAM– plants grown in both Expts 1 and 2 tended to increase with applied P to approximately P_2–P_3 for capsicum (Figs 2*a* and 3*a*) and tomato (Figs 4*a* and 5*a*) plants. At higher P rates in Expt 2, the yield plateaux were maintained for VAM+ and VAM– tomato and VAM– capsicum. However, for VAM+ capsicum in Expt 2 and VAM+ and VAM– plants of both crop species grown in Expt 1, yields declined with increasing P supply above P_3.

At P_1, dry weights of VAM– plants were less ($P < 0.05$) than those of VAM+ plants for capsicum and tomato in Expt 2 (Figs

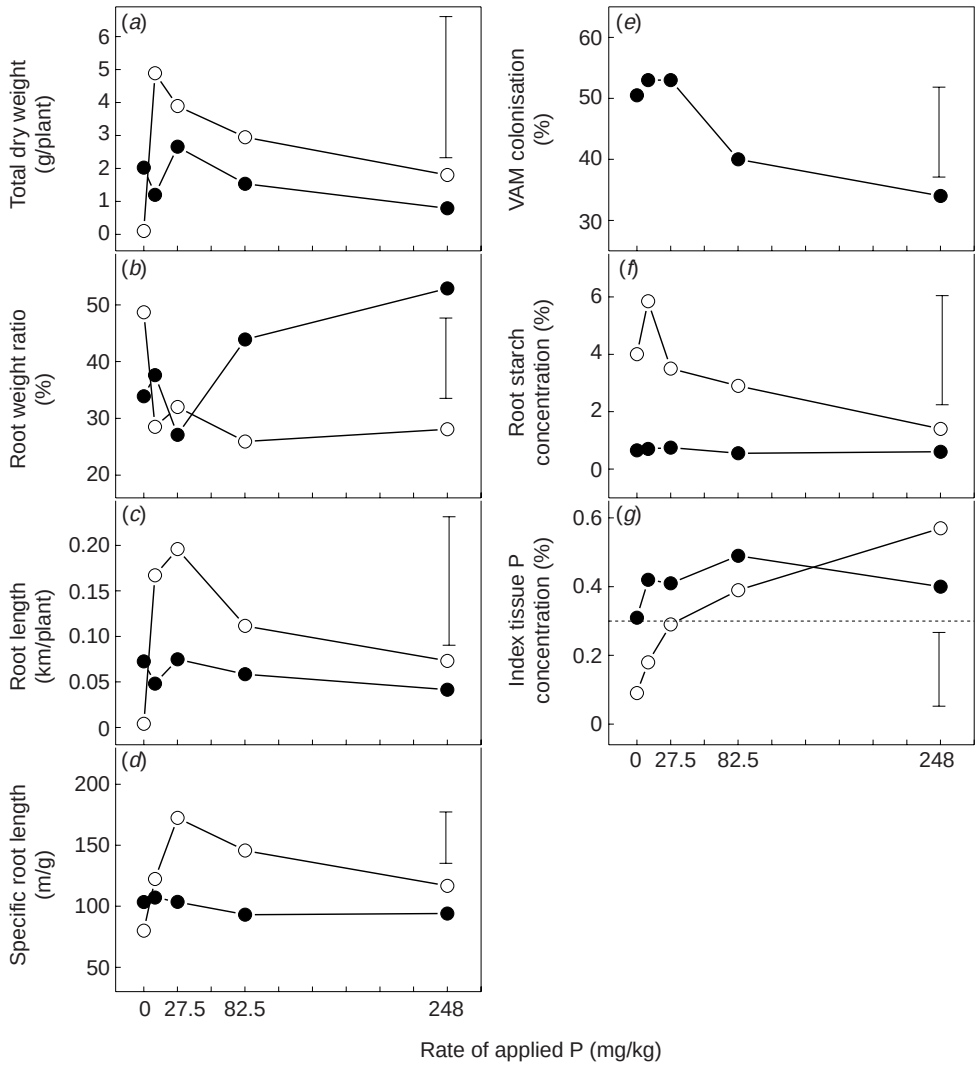

Fig. 2. Effect of applied P (mg/kg oven-dry soil) on (*a*) total dry weight, (*b*) root weight ratio, (*c*) root length, (*d*) specific root length, (*e*) vesicular-arbuscular mycorrhizal (VAM) colonisation, (*f*) root starch concentration, and (*g*) index tissue P concentration of 103-day-old capsicum plants grown in Expt 1 in the presence (VAM+, ●) or absence (VAM–, ○) of an existing extraradical mycorrhizal mycelium. Vertical bars, representing the l.s.d. at $P = 0.05$, are for the comparison of means (P × ±VAM means or the P means at VAM+ only, as appropriate). In (*a*), (*c*), (*f*), and (*g*), the ANOVA *F*-test for the VAM × P interaction effect was not significant ($P > 0.05$). The index tissue P concentration which is considered critical for deficiency appears as a horizontal dashed line in (*g*).

3*a* and 5*a*, respectively). For both experiments, VAM– plants grown at P_1 displayed P deficiency symptoms, including stunted growth and necrosis (and abscission) of old leaves (capsicum) or stunted growth and purple coloration of stems and old leaves (tomato). With the exception of capsicum plants grown at P_2 in Expt 2, dry weights of VAM+ plants were less than those of VAM– plants with the application of $\geq P_2$, although the differences were not significant in all cases. In Expt 2, application of P_5 produced VAM– plants with greater ($P < 0.05$) dry weights than VAM+ plants for both crop species, whereas no differences were measured at this P rate in Expt 1.

The dry matter yield of VAM+ plants as a percentage of VAM– plants was lower in Expt 1 than in Expt 2 at all P rates for capsicum (Figs 2*a* and 3*a*), and at all except P_5 for tomato (Figs 4*a* and 5*a*). At P_3 (deemed to correspond with maximal yield in most cases), relative dry matter yield of VAM+ plants as a percentage of VAM– plants in Expts 1 and 2 was 68.4 and 88.0%, respectively, for capsicum and 8.0 and 62.1%, respectively, for tomato.

Root weight ratio

For plants grown at P_1 in Expt 1, dry weight of roots expressed as a percentage of total dry weight (root weight ratio) was lower ($P < 0.05$) for VAM+ than for VAM– capsicum (Fig. 2*b*) and tomato (Fig. 4*b*); the root weight ratios of VAM+ plants were higher ($P < 0.05$) than those of VAM– plants for capsicum at P_4 and P_5 (Fig. 2*b*) and tomato at $\geq P_2$

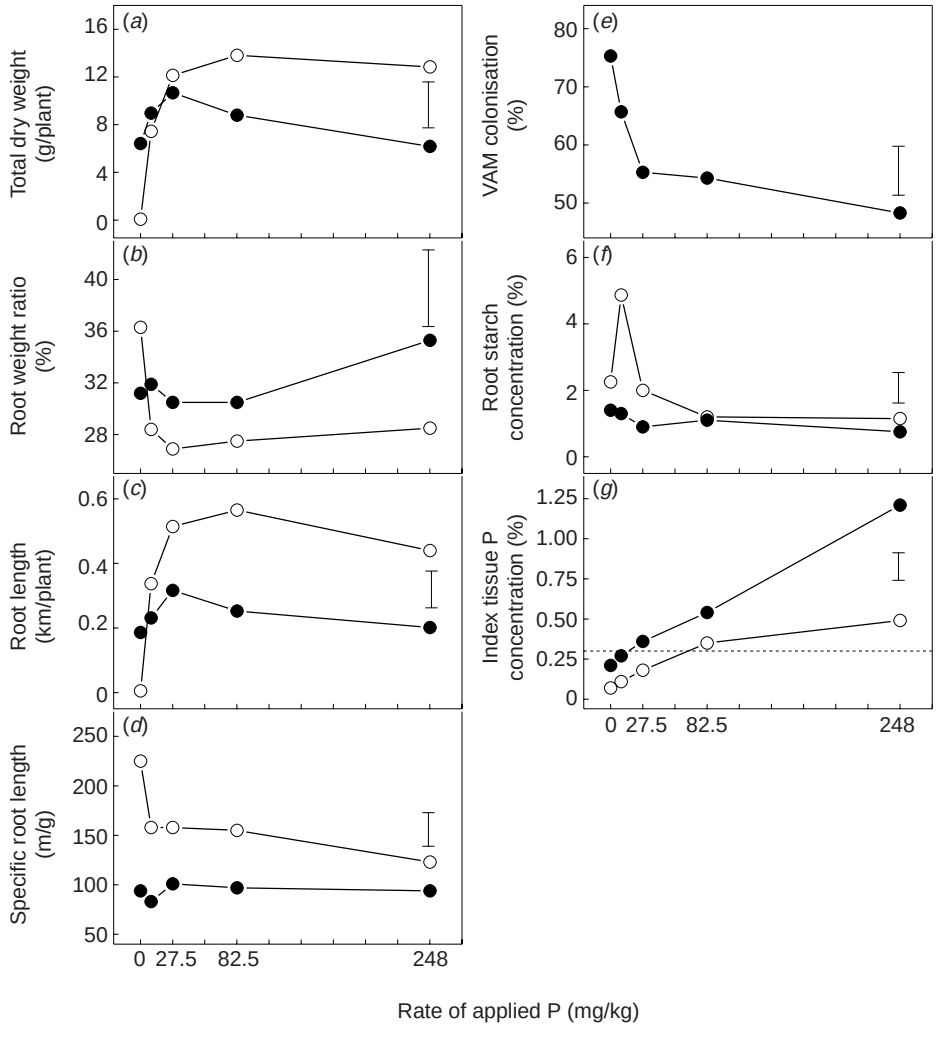

Fig. 3. The effect of applied P (mg/ kg oven-dry soil) on (*a*) total dry weight, (*b*) root weight ratio, (*c*) root length, (*d*) specific root length, (*e*) vesicular-arbuscular mycorrhizal (VAM) colonisation, (*f*) root starch concentration, and (*g*) index tissue P concentration of 56-day-old capsicum plants grown in Expt 2 in the presence (VAM+, ●) or absence (VAM–, ○) of an existing extraradical mycorrhizal mycelium. Vertical bars, representing the l.s.d. at $P = 0.05$, are for the comparison of means (P × ±VAM means or the P means at VAM+ only, as appropriate). In (*b*), the ANOVA *F*-test for the VAM × P interaction effect was not significant ($P > 0.05$). The index tissue P concentration which is considered critical for deficiency appears as a horizontal dashed line in (*g*).

(Fig. 4*b*). In Expt 2, there were no differences between root weight ratios of VAM+ and VAM– capsicum (Fig. 3*b*) and tomato (Fig. 5*b*) plants at any P rate.

For VAM+ plants of both production crop species grown at $\geq P_2$, root weight ratios were generally higher and total dry weights generally lower than for VAM– plants, although differences ($P < 0.05$) were only coincident in Expt 1 for tomato grown at P_2, P_3, and P_4 (Fig. 4).

Root length

At P_1, the root length of VAM+ plants was greater ($P < 0.05$) than that of VAM– plants for capsicum (Fig. 3*c*) and tomato (Fig. 5*c*) plants grown in Expt 2. Conversely, at higher rates of applied P, the root lengths of VAM– plants were generally higher than those of VAM+ plants; differences were significant ($P < 0.05$) at P_2 and P_3 for tomato grown in Expt 1 (Fig. 4*c*) and at P_3, P_4, and P_5 for both capsicum (Fig. 3*c*) and tomato (Fig. 5*c*) grown in Expt 2.

Specific root length

Root length divided by root dry weight (specific root length, SRL) gives a measure of root thickness, with high values representing thin roots. The SRL values for VAM– plants were greater ($P < 0.05$) than those for VAM+ plants at P_3 and P_4 for capsicum in Expt 1 (Fig. 2*d*), $\leq P_4$ for capsicum in Expt 2 (Fig. 3*d*), and at $\geq P_2$ for tomato in Expt 1 (Fig. 4*d*).

Relative mycorrhizal dependency

The RMD values for whole plants were positive at P_1 for both crop species grown in Expts 1 and 2 (Table 4). These positive RMD values at P_1 reflected the greater ($P < 0.05$ for both crop species in Expt 2) total dry weights of VAM+ plants than those of VAM– plants. For capsicum and tomato plants grown at P_1, the mean total dry weights of VAM+ plants were 19.3 and 13.2 times those of VAM– plants, respectively, in Expt 1, and 91.7 and 17.9 times those of VAM– plants, respectively, in Expt 2. With the exception of capsicum plants grown at P_2 in Expt 2, RMD values were negative at $\geq P_2$ for both crop species, reflecting the generally lower total dry weights of VAM+ plants than those of VAM– plants. The RMD values for P_1 were higher ($P < 0.05$) than those for $\geq P_3$ for capsicum in Expt 2 and $\geq P_2$ for tomato in Expt 2.

VAM colonisation of roots

Mycorrhizal colonisation was not detected in VAM– capsicum or tomato plants grown in either Expt 1 or 2, and there-

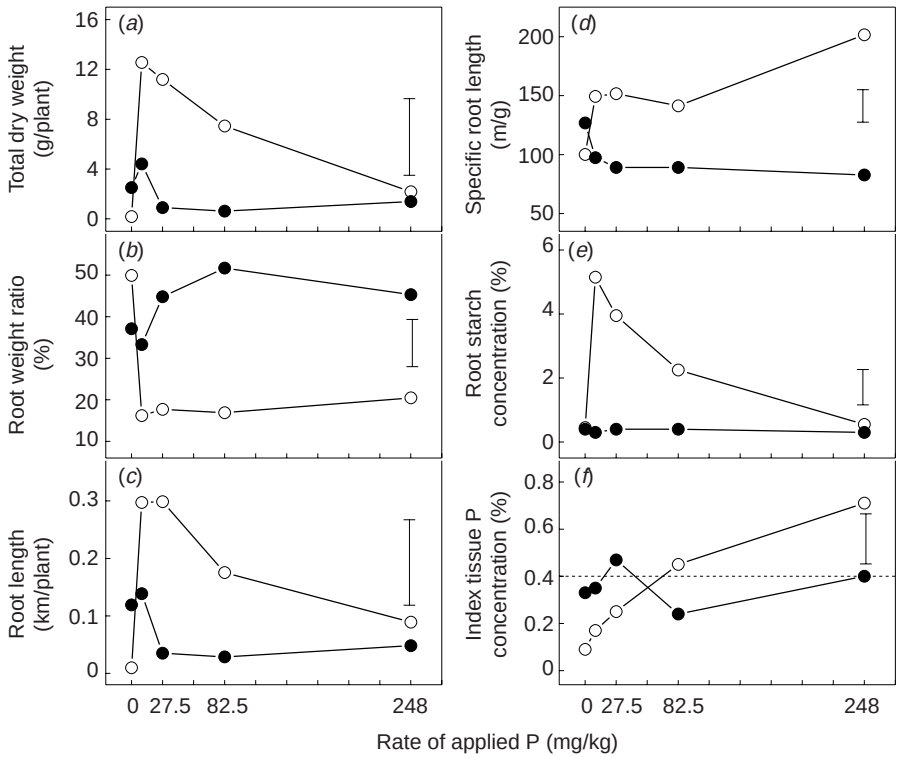

Fig. 4. Effect of applied P (mg/kg oven-dry soil) on (*a*) total dry weight, (*b*) root weight ratio, (*c*) root length, (*d*) specific root length, (*e*) root starch concentration, and (*f*) index tissue P concentration of 63-day-old tomato plants grown in Expt 1 in the presence (VAM+, ●) or absence (VAM–, ○) of an existing extraradical mycorrhizal mycelium. Vertical bars, representing the l.s.d. at $P = 0.05$, are for the comparison of means. The index tissue P concentration which is considered critical for deficiency appears as a horizontal dashed line in (*f*).

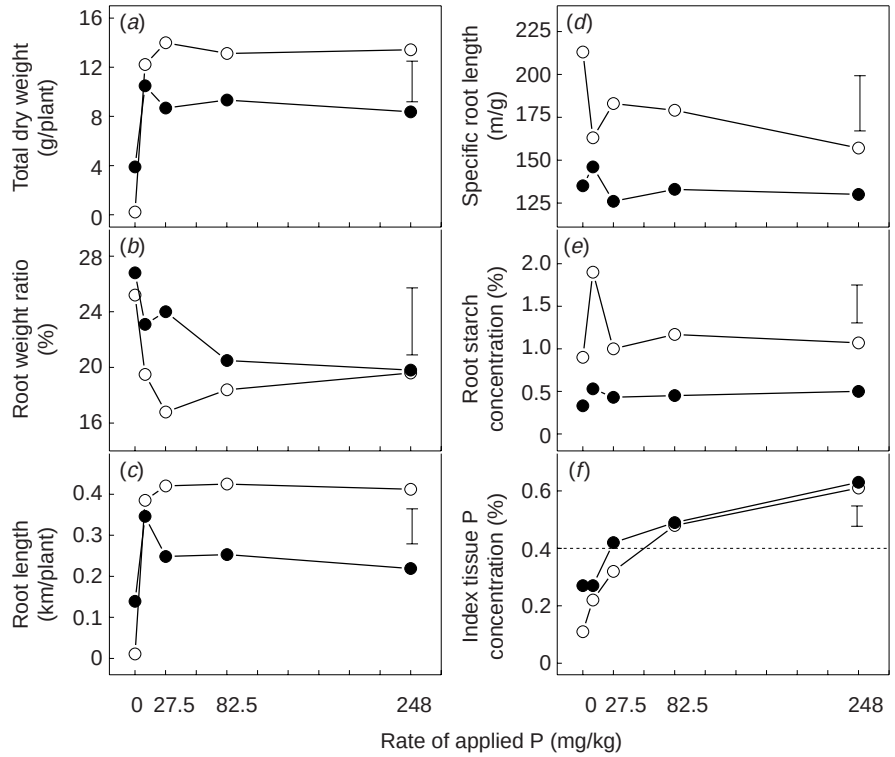

Fig. 5. Effect of applied P (mg/kg oven-dry soil) on (*a*) total dry weight, (*b*) root weight ratio, (*c*) root length, (*d*) specific root length, (*e*) root starch concentration, and (*f*) index tissue P concentration of 39-day-old tomato plants grown in Expt 2 in the presence (VAM+, ●) or absence (VAM–, ○) of an existing extraradical mycorrhizal mycelium. Vertical bars, representing the l.s.d. at $P = 0.05$, are for the comparison of means. In (*b*), (*d*), and (*e*), the ANOVA F-test for the VAM × P interaction effect was not significant ($P > 0.05$). The index tissue P concentration which is considered critical for deficiency appears as a horizontal dashed line in (*f*).

Table 4. Relative mycorrhizal dependency (RMD) of two crop species grown in the pot experiments at five rates of applied P

For a given rate of applied P, RMD was calculated as 100 × (DW mycorrhizal plant – DW non-mycorrhizal plant)/DW mycorrhizal plant (Plenchette *et al*. 1983)

For any crop species within a column, means followed by the same letters are not significantly different at $P = 0.05$ (*F*-test for the main effect of P addition)

Rate of applied P (mg/kg OD[A] soil)	Expt 1	Expt 2
	Capsicum	
0	94	99c
9.2	–510	12bc
27.5	–43	–44ab
82.5	–332	–76ab
248	–207	–116a
l.s.d. ($P = 0.05$)	1166	101
	Tomato	
0	92	94b
9.2	–183	–20a
27.5	–1292	–81a
82.5	–1106	–41a
248	–266	–72a
l.s.d. ($P = 0.05$)	3013	79

[A]Oven-dry.

fore, only colonisation data pertaining to VAM+ plants are presented. Colonisation of capsicum roots by VAM at P_1 was greater ($P < 0.05$) than at P_5 in Expt 1 (Fig. 2*e*) and at all higher P rates in Expt 2 (Fig. 3*e*). The overall colonisation of the roots of tomato plants grown in Expts 1 and 2 was 48.6 and 42.2%, respectively; the individual treatment means did not differ since the ANOVA *F*-test for P addition was not significant.

Length of root colonised by VAM

In Expt 2, the actual length of root per tomato plant colonised by VAM was greater ($P < 0.05$) at P_2 (157 m/plant) than at the other P rates (72–96 m/plant). The overall mean values of the actual length of root per plant colonised by VAM were 36.6 m for tomato in Expt 1, and 27.0 or 141 m for capsicum in Expt 1 or 2, respectively. Since the overall effect of P addition on this parameter was not significant for these crop species × experiment combinations, pairwise differences among treatment means were not *t*-tested, in accordance with the protected l.s.d. procedure.

Length of external hyphae

The length of VAM hyphae within soil located externally to the root exclusion cage did not differ ($P > 0.05$) among P

Table 5. Concentrations of Mn (mg/kg) in various tissues of capsicum and tomato plants grown in the presence (VAM+) or absence (VAM–) of an extraradical mycorrhizal mycelium in Expts 1 and 2

Index tissues were analysed in Expt 2: the 6 youngest mature leaf blades plus petioles (6YMB+P) for capsicum and the 3 youngest mature leaf blades plus petioles (3YMB+P) for tomato. The whole tops less these index tissues were analysed in Expt 1

For any crop species within an experiment, means followed by the same letters are not significantly different at $P = 0.05$ (F-test in the ANOVA for the VAM × P interaction effect)

Rate of applied P (mg/kg OD[A] soil)	Expt 1		Expt 2	
	VAM–	VAM+	VAM–	VAM+
		Capsicum		
0	190	116	163	122
9.2	198	137	124	148
27.5	321	141	179	197
82.5	232	161	264	252
248	316	201	237	301
l.s.d. ($P = 0.05$)	213		109	
		Tomato		
0	117	107	329g	72a
9.2	113	90	174f	86ab
27.5	112	198	147ef	129cde
82.5	111	117	112bcd	106bc
248	190	135	135de	137de
l.s.d. ($P = 0.05$)	131		29	

[A]Oven-dry.

treatments applied within the root exclusion cage for capsicum plants grown in either Expt 1 or 2; the overall mean values were 1.4 or 1.0 m/g oven-dried soil, respectively.

Root starch concentration

The starch concentration in the roots of VAM+ plants was lower ($P < 0.05$) than that in the roots of VAM– plants at P_1, P_2, and P_3 for capsicum grown in Expt 2 (Fig. 3*f*) and at P_2, P_3, and P_4 for tomato grown in Expt 1 (Fig. 4*e*). Although starch concentrations in the roots of VAM+ plants were lower than those in the roots of VAM– plants at each rate of applied P for capsicum in Expt 1 (Fig. 2*f*) and tomato in Expt 2 (Fig. 5*e*), the VAM × P interaction effect was not significant ($P > 0.05$), so pairwise comparisons could not be made. The ratio of the concentration of starch in the roots of VAM+ plants relative to VAM– plants in Expt 1 was less than half the value determined in Expt 2 for both capsicum (Figs 2*f* and 3*f*) and tomato (Figs 4*e* and 5*e*) grown at each of P_2, P_3, and P_4.

Total starch in roots

The total starch content in the roots of VAM+ and VAM– plants closely followed the trends established for root starch concentration. The range in values (mg/ plant) for VAM+ and VAM– plants is given as follows: capsicum in Expts 1 (2–5 and 2–106, respectively) and 2 (16–36 and 1–98, respectively); tomato in Expts 1 (1–4 and 1–103, respectively) and 2 (3–13 and 1–45, respectively).

Index tissue P concentration

Concentrations of P which were considered critical for deficiency (Piggott 1986) or at the lower end of the 'normal' range (Weir and Cresswell 1993) for similar index tissues sampled at approximately the same phenological stage as in the present study may be summarised thus: 0.30% capsicum, 0.40% tomato. These concentrations are displayed in the relevant Figures as a dashed horizontal line.

For both production crop species grown in both experiments, P concentrations in index tissues of VAM+ plants grown at P_1, P_2, and P_3 were higher than those in the VAM– plants grown at the same P rates, although differences were only significant ($P < 0.05$) for capsicum grown at P_3 in Expt 2 (Fig. 3*g*) and tomato grown at P_1 and P_3 in both Expt 1 (Fig. 4*f*) and Expt 2 (Fig. 5*f*).

At P_5, P concentrations in index tissues of VAM+ plants were greater than those in VAM– plants for capsicum in Expt 2 (Fig. 3*g*). Conversely, for tomato grown at P_5 in Expt 1, P concentrations were higher in index tissues of VAM– than of VAM+ plants (Fig. 4*f*). In Expt 2, the mean P concentration in the 6YMB+P of VAM+ capsicum plants grown at P_5 (1.21%) was high, and possibly in the toxic range, and plants were stunted in the same way that symptoms of P excess were described for tomato by Bingham (1966).

Concentrations of P within the 6YMB+P of VAM+ capsicum plants were in excess of the critical concentration for deficiency in comparable index tissue at all P rates in Expt 1 (Fig. 2*g*) and at $\geq P_3$ in Expt 2 (Fig. 3*g*). For VAM– capsicum plants, index tissue P concentrations were above the critical concentration for deficiency only at $\geq P_4$ in both experiments. Concentrations of P within the 3YMB+P of tomato were in excess of, or equal to, the critical concentrations for deficiency at lower P rates for VAM+ than for VAM– plants in both Expt 1 (P_3 and P_5 VAM+, $\geq P_4$ VAM–; Fig. 4*f*) and Expt 2 ($\geq P_3$ VAM+, $\geq P_4$ VAM–; Fig. 5*f*).

Concentrations of Mn in plant tissues

For tomato grown in Expt 2, the concentrations of Mn in the index tissues were higher ($P < 0.05$) for VAM– plants grown at P_1 than for plants grown in any other VAM × P treatment combination (Table 5).

Discussion

The capsicum and tomato plants responded positively to added P up to a rate of approximately 9.2–27.5 mg P/kg oven-dry soil (P_2–P_3). This positive growth response indicated that conditions were appropriate for the evaluation of the efficiency of VAM in influencing yield response to P nutrition in these 2 crop species.

Role of the root exclusion cage in determining plant response

The root exclusion cage may have confounded the response of the production crop plants to the VAM network; however, an unpublished experiment (J. K. Olsen, 1998) identical in

design to Expt 1 but without root exclusion cages gave similar results, indicating that the use of the root exclusion cage in Expt 1 did not modify the response of the production crop plants to the VAM network.

Growth depression due to VAM

The P toxicity hypothesis

For VAM+ capsicum plants grown at P_5 in Expt 2, the high (and possibly toxic) P concentration of the 6YMB+P (1.21%, Fig. 3*g*) was associated with a lower ($P < 0.05$) total dry weight than that obtained at P_3 (Fig. 3*a*). Weir and Cresswell (1993) stated that a concentration of >0.6% P in the upper young mature leaf (including the petiole) is high for capsicum at the early fruiting stage, whereas Mills and Jones (1996) indicated that P concentrations >1.0% in the most recent fully developed leaves of capsicum plants are in excess of the upper limit of the sufficiency range from first bloom to fruit fill. However, Abbott and Robson (1984) stated that, although P toxicity has been suggested as an explanation for certain growth depressions in mycorrhizal plants, characteristic symptoms of P toxicity in crop and pasture species have not generally been observed where growth has been depressed by inoculation with mycorrhizal fungi. Furthermore, growth has been depressed when P concentrations within shoots and roots are much lower than those likely to be associated with P toxicity. With the possible exception of VAM+ capsicum plants grown at P_5 in Expt 2, there was little evidence to suggest that P toxicity was responsible for yield depressions in VAM+ plants relative to VAM– plants. The possibility of P toxicity arising from VAM colonisation without the high inoculum potential of a VAM network would seem remote, given the self-regulatory effect of tissue P concentration on colonisation (Menge *et al*. 1978) and the implication by Abbott and Robson (1984) that some other phenomenon is responsible for the majority of growth depressions observed in mycorrhizal plants.

The carbon drain hypothesis

Abbott and Robson (1984) suggested that concentrations of soluble carbohydrates within the roots of mycorrhizal plants may be less than those of non-mycorrhizal plants because the latter are more P-deficient, and, hence, have fewer sinks for C. In the present study, starch concentrations within the roots of VAM+ capsicum and tomato plants tended to be less than those of VAM– plants at all rates of applied P, suggesting that P deficiency or sufficiency was not related to root starch concentration. Indeed, for tomato plants grown at P_4 in Expt 1 (Fig. 4), the starch concentration in roots and the P concentration in the 3YMB+P of VAM– plants were higher ($P < 0.05$) than those in VAM+ plants. Therefore, the most plausible explanation for lower starch concentrations in VAM+ than in VAM– roots is the additional demand for photosynthate imposed by the mycorrhizal association with the host plant.

Mycorrhizal root systems are estimated to require 1.5–20% more photosynthate than non-mycorrhizal roots (Douds *et al*. 1988; Jakobsen and Rosendahl 1990; Eissenstat *et al*. 1993; Pearson and Jakobsen 1993). At P_1, the generally higher total dry weights and lower root starch concentrations of VAM+ plants than of VAM– plants ($P < 0.05$ for both parameters for capsicum plants grown in Expt 2; Fig. 3*a* and *f*, respectively) suggest that, although the endophytes were a C-drain to the host plants, there was a net gain in C accumulated as a result of mycorrhizal colonisation of roots. This effect at P_1 may be attributed to the improved P nutrition of mycorrhizal compared with non-mycorrhizal plants in the P-deficient growth medium (5–6 mg $NaHCO_3$-extractable P/kg), as demonstrated by a higher P concentration in the index tissue of VAM+ than of VAM– plants. The benefit of VAM colonisation (due to increased P availability) diminished with the application of $\geq P_2$, and the likely cause for the growth depression of VAM+ relative to VAM– plants at these P rates was the high demand for photosynthate (or C-drain) imposed upon the host plant by the endophytes. At $\geq P_2$, the lower starch concentrations in the roots of VAM+ plants than in VAM– plants [$P < 0.05$ at P_2 and P_3 for capsicum in Expt 2 (Fig. 3*f*) and at P_2, P_3, and P_4 for tomato in Expt 1 (Fig. 4*e*)] are consistent with the premise that a C-drain by the endophytes limited growth of the VAM+ plants.

The relatively low light levels within the greenhouse (light transparency of the roof was 66%) would have exacerbated the C-drain by the fungi by limiting the production and availability of photosynthate for optimal plant growth. This conclusion agrees with the C use efficiency model developed by Tinker *et al*. (1994), who defined efficiency in terms of the C gained by the plant via the growth response to VAM colonisation and the C loss by the plant to support the fungus. In low light conditions, they stated that the plant would be expected to be in a C-source limited condition, and any C-drain would result in a growth reduction. The finding that the growth of the VAM+ plants was depressed relative to that of the VAM– plants under the relatively low light levels of the greenhouse experiments is also consistent with the statement by Johnson *et al*. (1997) that the allocation to the fungal associate of the limited supply of C under low light might potentially reduce plant allocation to functions related to its fitness. Therefore, under conditions of suboptimal light, the relative costs of a mycorrhizal association increase, whereas the benefits might remain constant. However, it is possible that under field conditions, where light levels may be appreciably higher than in the greenhouse, the benefits of a VAM network may not be abated by the C-drain of the endophytes. This hypothesis was investigated by Olsen *et al*. (1999).

For both total dry weight and starch concentration of roots, the ratio of VAM+ relative to VAM– plants was generally lower in Expt 1 than in Expt 2. This result corresponded with lower average daily solar irradiance values within the greenhouse in the former experiment (estimated as 8.4 MJ/m^2) than

in the latter experiment (13.4 MJ/m^2). Therefore, in the lower light conditions of Expt 1, it is plausible that a reduced availability of photosynthetically derived C in association with the C-drain imposed by the endophytes was a major limitation to growth of the VAM+ plants. Mohd Razi and Zainab (1994) identified a mean daily irradiance of 14.7 MJ/m^2 (full sun) for optimal fruit production of tomato cv. Fireglow plants grown in a greenhouse, whereas no fruit was produced at an irradiance of ≤3.3 MJ/m^2.

The lower mean soil temperature during the production phase in Expt 1 (20.1°C) than in Expt 2 (27.8°C) may have also had a major effect upon the larger growth depression of VAM+ relative to VAM– plants in Expt 1 than in Expt 2. The metabolic rate (and demand for C) of the endophytes may have been relatively constant within the temperature range encountered in the experiments, whereas the ability of the host plant to fix C may have been considerably reduced at lower temperatures. The net effect may have been diminished availability of photosynthetically derived C for plant growth at the lower temperatures encountered in Expt 1 than at the higher temperatures in Expt 2. In general support of this hypothesis, Borges and Chaney (1989) found reduced growth enhancement of VAM+ (both *Glomus fasciculatum* and *Glomus macrocarpum*) relative to VAM– green ash (*Fraxinus pennsylvanica* Marsh.) seedlings (16 weeks after transplanting) when root temperatures were decreased from 25°C to 15°C.

General yield decline with increasing P application in Expt 1

For the 2 production crop species grown in Expt 1, the generally lower dry weights of whole VAM– plants grown at P_4 and P_5 than at P_3 (Figs 2*a* and 4*a*) were associated with index tissue P concentrations (Figs 2*g* and 4*f*) which lay within the range recommended by Piggott (1986) for adequate growth: viz. 0.3–0.7% for capsicum and 0.4–0.8% for tomato. Similarly, the dry weight of whole VAM+ plants grown in Expt 1 also tended to decline with increased P application rate, and index tissue P concentrations at P_4 and P_5 were either within (capsicum) or close to (tomato) the range for adequate growth provided by Piggott (1986). Therefore, the yield decline observed at the higher P rates in Expt 1 was not likely to be the result of toxic P concentrations within plant tissues. In an unpublished experiment (J. K. Olsen 1998), which was identical in design to Expt 1 except that root exclusion cages were not used (experiments run concurrently), there was a smaller yield decline at high rates of P for both VAM+ andVAM–. It is therefore possible that use of root exclusion cages in Expt 1 limited the extent to which roots of the production crop plants could grow away from the zone of highly acidified soil which probably existed around each band of $Ca(H_2PO_4)_2.H_2O$ placed within each cage. Root growth within these zones may have been reduced due to adverse osmotic effects of the concentrated fertiliser solution and the development of H, Mn, and/or Al toxicity as a consequence of the low pH (Moody *et al.* 1995). The greater volume of soil available to roots of production crop plants grown in the unpublished experiment than in Expt 1 may have minimised the overall adverse effects of the highly acidic soil around the fertiliser bands at P_4 and P_5 and produced plants with top dry weights similar to those at P_3.

The yield decline with high rates of applied P in Expt 1, which was generally not observed in Expt 2 (except for VAM+ capsicum plants grown at P_5, Fig. 3*a*), may be attributed to reduced root growth at P_5 in the former experiment through a combination of osmotic effects, and H, Mn, and/or Al toxicity as a consequence of the low pH in proximity to the 2 bands of $Ca(H_2PO_4)_2.H_2O$ within each cage. The general absence of a similar yield decline with applied P in Expt 2 may be associated with less extensive diffusion of saturated $Ca(H_2PO_4)_2.H_2O$ from each band than in Expt 1 due to the shorter duration over which the production crop species were grown (Table 1). The reduced dry matter yield of VAM+ capsicum plants grown at P_5 than at P_3 in Expt 2 may have been due to P toxicity (1.21% P in the 6YMB+P, Fig. 3*g*) resulting from the activity of the mycorrhizal network. The better growth conditions encountered in Expt 2 than in Expt 1 (viz. higher temperature and light) may have resulted in greater exploration of a larger volume of the soil in the root exclusion cage in the former than in the latter experiment, before diffusion of $Ca(H_2PO_4)_2.H_2O$ led to the development of unsatisfactory conditions for the growth of plant roots.

For plants grown at P_5 in Expt 1, mean concentrations of Mn in the tops (less index tissue) of capsicum (316 mg/kg, VAM–; 201 mg/kg, VAM+) and tomato (190 mg/kg, VAM–; 135 mg/kg, VAM+) were not excessive and were similar to the mean concentrations of Mn in the index tissues of plants grown at P_5 in Expt 2 for capsicum (237 mg/kg, VAM–; 301 mg/kg, VAM+) and tomato (135 mg/kg, VAM–; 137 mg/kg, VAM+) (Table 5). The Mn concentrations were within, or close to, the range in the YMB+P deemed by Piggott (1986) as adequate for plant growth for capsicum (26–300 mg/kg) and tomato (50–500 mg/kg) and below the values cited by Weir and Cresswell (1993) as being high for these crop species (>400 mg/kg in the YMB+P of capsicum; >700 mg/kg in the YMB of tomato). Based on these reference concentrations, growth of capsicum and tomato plants was probably not limited by Mn toxicity at P_5 in either Expt 1 or Expt 2. Therefore, although Mn concentrations in plant tissues were not toxic for plants grown at P_5 in Expt 1, reduced growth at high P in this experiment may have been due to the relatively small volume of non-acidified soil available within the root exclusion cage to accommodate normal root growth and function.

Root weight ratio

In the absence of added P (viz. P_1), the root weight ratio of VAM+ plants tended to be lower than that of VAM– plants, indicating that the better P nutrition of VAM+ plants was associated with the production of lower root dry weights as a

proportion of the whole plant dry weights. This assumption is supported by Marschner (1995) who stated that shoot growth is more enhanced than root growth in response to higher nutrient supply, leading to a decrease in the root/shoot dry weight ratio. Similarly, Abbott and Robson (1984) cited several studies which showed that mycorrhizal plants had a lower root weight ratio than non-mycorrhizal plants at the same level of nutrient application. At higher P rates ($\geq P_2$), however, root weight ratios of VAM+ plants tended to be higher than those of VAM– plants. Higher root weight ratios of VAM+ than of VAM– sweet corn (Khalil *et al*. 1994), sour orange (Eissenstat *et al*. 1993), and Volkamer lemon (Peng *et al*. 1993) have been similarly reported; these authors generally attributed this outcome to the relatively greater partitioning of photosynthate to the roots than to the shoots of mycorrhizal than of non-mycorrhizal plants. The hypothesis that VAM colonisation may partition photosynthate more to the roots than to the shoots of plants does not concur with the lower starch concentrations measured in the roots of VAM+ than of VAM– capsicum and tomato plants in the present study, although due to the potential loss of C to the endophytes, this parameter probably does not accurately reflect the degree to which photosynthate is partitioned to roots as opposed to shoots. The generally higher root weight ratios of VAM+ than of VAM– plants grown at $\geq P_2$ in the present study reflected a lowered C-source capacity to supply the additional photosynthate required by the fungus and the colonised host-root tissue, resulting in the endophytes inhibiting host growth.

Effect of a VAM network on P nutrition

The benefit of a VAM network in increasing the P concentration in index tissues of capsicum and tomato plants, especially at $\leq P_3$, was established from this study, although a concomitant increase in dry matter yield was usually restricted to the P_1 rate only. These findings agree with the assertion by Creighton Miller *et al*. (1986) that plant growth responses to VAM colonisation are due primarily to improved uptake of P. At P rates in excess of P_3, generally higher index tissue P concentrations in VAM+ plants than in VAM– plants may have been associated more with higher dry matter yields of VAM– plants and, hence, a dilution effect, than with any benefit in P nutrition imparted by VAM.

Relative mycorrhizal dependency

For both crop species, RMD values were usually negative at $\geq P_2$, reflecting the depressive effect of VAM on dry matter yields. However, at P_1, RMD values were generally positive, highlighting positive growth responses to VAM. The consistently high RMD values for capsicum and tomato plants grown at P_1 in Expts 1 and 2 (≥92, Table 4) were greater than the range in values reported by Khalil *et al*. (1994) for 6 corn cultivars (7 for cv. Reid Yellow Dent to 81 for cv. Argentine Pop) grown in a low P soil (6.2 mg Bray I-extractable P/kg).

The change in RMD from positive at low P supply to negative at higher P supply levels has been reported previously. For example, in a pot experiment using a pasteurised low-P Quilichao soil (1.8 mg Bray I-extractable P/kg) as the growth medium, Howeler *et al*. (1987) compared the growth of mycorrhizal (inoculated with *Glomus manihotis* and *Entrophospora colombiana*) and non-mycorrhizal (not inoculated) corn plants at 3 rates of P application (0, 100, or 500 kg/ha); corresponding RMD values were 75.4, 74.8, and –10.6, respectively.

VAM parameters

The general lack of variation with added P of the actual length of root per plant colonised by VAM indicated that both root length and VAM colonisation remained relatively constant as P rate increased. The comparatively high level of VAM colonisation of roots of the 2 production crop species, even at P_5, may suggest that the VAM network was a highly effective inoculum source, even when index tissue P concentrations were adequate to high. The high inoculum potential of an undisturbed network of VAM hyphae, even at high P rates, was also reported in a greenhouse experiment conducted by Fairchild and Miller (1990). They found that arbuscular intensity and hyphal intensity of roots (viz. the proportion [range from 0 to 1] of cortex colonised with visible VAM structures) of corn plants grown for 3 weeks in undisturbed soil supplied with 480 mg P/kg soil (0.175 and 0.213, respectively) were similar to those measured in roots of corn plants grown for 3 weeks in disturbed soil to which no P was added (0.181 and 0.174, respectively), even though the shoot P concentration of plants grown in the former treatment (0.38%) was more than twice the P concentration of shoots of plants grown in the latter treatment (0.17%).

The length of VAM hyphae located in the external soil compartment did not change with the application of P within the root exclusion cage for capsicum in Expts 1 and 2, indicating that new hyphae were not formed to an appreciable extent in the production phase, or, if the development of new hyphae occurred in this phase, the amount of hyphae produced was independent of the amount of P applied in the cage. The lengths of extraradical mycorrhizal mycelium (m/g soil) reported in other studies are lower (0.64, Schubert *et al*. 1987) or higher (3.4–6.7, Kothari *et al* 1991; 3.3–8.1, Schreiner *et al*. 1997) than the mean values reported for capsicum in Expts 1 (1.4) and 2 (1.0). These differences may be attributed to variation in the plant or mycorrhizal species selected, the edaphic environment, and/or the length of the growth cycle.

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