

CSIRO Publishing

Australian
Journal of
Agricultural
Research

VOLUME 52, 2001
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CSIRO Publishing
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Responses of sugarcane, maize, and soybean to phosphorus and vesicular-arbuscular mycorrhizal fungi

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Abstract. The presence of vesicular-arbuscular mycorrhizal (VAM) fungi in long-term cane-growing fields associated with yield decline led to the supposition that VAM fungi may be responsible for the poor yields. A glasshouse trial was established to test the effectiveness of a species of VAM fungi, *Glomus clarum*, extracted from one of these North Queensland fields on the growth of sugarcane (*Saccharum* interspecific hybrid), maize (*Zea mays*), and soybean (*Glycine max*) for 6 phosphorus (P) rates (0, 2.7, 8.2, 25, 74, 222 mg/kg). For maize and soybean plants that received VAM (+VAM), root colonisation was associated with enhanced P uptake, improved dry weight (DW) production, and higher index tissue-P concentrations than those without VAM (–VAM). By comparing DW responses of maize and soybean for different P rates, savings in fertiliser P of up to 160 and 213 kg/ha, respectively, were realised. Sugarcane plants were generally less responsive. Apart from a 30% DW increase with VAM when 2.7 mg P/kg was added, DW of +VAM plants was equivalent to, or worse than in the case of 222 mg P/kg, DW of –VAM plants. For all 3 host species, colonisation was least at the highest P application, presumably from excessive P within the plant tissue. Critical P concentrations for the 3 host species were below those reported elsewhere, and for soybean and sugarcane, the critical concentration for +VAM plants was lower than that of –VAM plants. There are 3 implications that arise from this study. First, VAM fungi present in cane-growing soils can promote the growth of maize and soybean, which are potential rotation crops, over a range of P levels. Second, the mycorrhizal strain taken from this site did not generally contribute to a yield decline in sugarcane plants. Third, application of P fertiliser is not necessary for sugarcane when acid-extractable P is <30 mg/kg if sufficient VAM propagules are present, or <47 mg/kg if a mycorrhizal response is not anticipated.

Additional keywords: mycorrhizal dependency, responsiveness, yield decline, critical P concentration.

Introduction

Sugarcane (*Saccharum* interspecific hybrid) cropping has taken place in Australia's northern tropics for around 100 years (Magarey 1996) and sugar is currently Australia's third-most valuable agricultural export commodity. In 1998–99, some 40 Mt of sugarcane was cut from around 414 000 ha, earning over AU\$1.2 billion (ABARE 2000).

Although the area of land planted to sugarcane has expanded consistently at 4% per annum, the yield of sugar has plateaued since the 1970s at 9–10 t/ha despite the introduction of new germplasm, improved agronomic methods, and increasingly efficient farming practices (SRDC 1995). Additionally, long-term sugarcane monoculture can lead to a reduced potential of cane-growing soils to produce high sugar yields; this phenomenon has been

referred to as yield decline (YD) (Magarey 1994). With limited land available for expansion, strategies to overcome this effect are critical. Losses in production associated with YD have been estimated to cost the industry in the order of \$300 million annually (Magarey and Croft 1995).

Soil fumigation, rotation, solarisation, steam-pasteurisation, and pesticide applications all appear to restore sugarcane production to varying extents, suggesting that soil-borne microbiological agents, such as nematodes, fungi, and bacteria, are responsible for the YD effect (Magarey 1996). The role of vesicular-arbuscular mycorrhizal (VAM) fungi on sugarcane nutrition has been questioned in relation to YD (Anon. 1992; Magarey 1996). The potentially damaging effect of excessive P levels, such as that found in many long-term cane fields, on VAM associations has also been posed (Bramley *et al.* 1996).

Ineffective strains of VAM may be selected preferentially when associations form between the mycorrhizal community and crops during monoculture, leading to the dominance of particular mycorrhizal species, many of which appear to be poor symbionts (Johnson and Pflieger 1992; An *et al.* 1993). For example, after a 5-year maize monoculture, spore numbers of the most prolific mycorrhizal strains were negatively correlated with dry matter yield, grain yield, and P concentration of maize plants (Johnson *et al.* 1992). In another study, strains of the mycorrhizal species *Glomus macrocarpum* that appeared to be preferentially selected during long-term monoculture actually decreased the dry weight of tobacco (*Nicotiana tabacum*) (Modjo and Hendrix 1986). Other forms of selection pressure, such as that created by long-term P fertilisation, could likewise ensure that highly infectious, but less effective, VAM strains proliferate in these mycorrhizal communities (Johnson 1993).

Observations have been made on mycorrhizal colonisation of sugarcane roots in Australia (Anon. 1992; Magarey 1996) and elsewhere (Pinchin 1986; Toro *et al.* 1987; Prasad and Bilgrami 1993). However, only one study linked root colonisation with plant growth and nutrition. In that glasshouse study, sugarcane plants were inoculated with *Glomus manihotis* and grown in a P-sorptive Hawaiian Ferrosol (11.3 mg P/kg; Olsen-P test) (Pinchin 1986). At 17 weeks, mycorrhizal plants that received no P fertiliser had 12% more dry matter in plant tops than those that received no inoculum, but there was no significant response to VAM when plants received 330 mg P/kg. Responses to the other 3 *Glomus* species used were not evident for any P treatment.

Given the potential selection of poor VAM strains through long-term monoculture and fertilisation and the scant information on local effects of VAM on sugarcane, it was important for the sugarcane industry in Australia to understand whether VAM was a causal agent in the YD phenomenon.

We measured the response of sugarcane to a *Glomus clarum* isolate extracted from a North Queensland cane-growing field associated with YD, for a range of P fertiliser rates. Responses were also measured on 2 mycorrhiza-responsive crops, maize (*Zea mays*) and soybean (*Glycine max*), considered likely to be part of a sugarcane rotation.

Materials and methods

Inoculum source and preparation

Mycorrhizal spores were extracted from a long-term cane-growing field at Hewitt, near Tully, North Queensland (17°58'S, 145°56'E), where YD had been previously noted. The spores were multiplied from single-spore accessions in sand cultures at the BSES, Tully, and in soil cultures at the University of Queensland, Brisbane, using sugarcane as the host plant. Spores from 4 accessions, all identified as *Glomus clarum* (S. Bentivenga, pers. comm.) (synonymous with *Glomus manihotis*; INVAM 2001), were extracted from both sources by wet dispersion of the medium with water in a cement mixer, and then decanting the suspension through a nest of sieves, trapping the fungal

spores on a 125- μ m mesh sieve. This material was further clarified by centrifuging in water twice (700G for 5 min) to remove suspended organic matter (Tommerup 1992). Spores were surface-sterilised by being placed in an aqueous Tween-20 solution (0.05%) for 1 min, then briskly washed in sterile distilled water (SDW), soaked in a chloramine-T solution (2%), rinsed again in SDW, and stored at 4°C in a filter-sterilised streptomycin solution (0.2%) (Fiske and Thompson 1988). The concentration of spores in the suspension was determined by counting the spores present in replicate 1-mL aliquots taken when the suspension was stirred at $c.10\pi$ rad/s.

Most probable number study

To confirm the actual rate of propagule addition, the number of viable propagules present in the inoculum was quantified using the most probable number (MPN) test (Tommerup 1992). Using the prepared suspension, sufficient spores were evenly mixed through 2.5 kg of previously autoclaved (121°C for 1 h on 2 consecutive days) field soil to provide an estimated 4 spores/g soil (oven-dry basis). This inoculated soil was then diluted with autoclaved soil in 12 sequential 2-fold dilutions. Each dilution consisted of 5 replicate tubes containing 200 g oven-dry (OD) equivalent soil. Maize seeds (cv. Pioneer 3906) were surface-sterilised by rinsing in 70% ethanol for 1 min, soaking in 0.05% sodium hypochlorite for 20 min, then rinsing twice in SDW. The seeds were planted into growth tubes, and placed in a growth cabinet with 29°/25°C day/night temperature and a 12-h day length. Contamination from adjoining growth tubes was minimised by grouping tubes with the same dilution, and by adequately spacing the tubes, and using a fine droplet spray for watering. After 6 weeks, roots were cleared and stained as described below, then examined for the presence or absence of VAM. Standard MPN tables were used to determine the concentration of spores within the suspension (Fisher and Yates 1963).

Preparation of soil and pots

The soil prepared for use in the pot experiment was a silty-clay loam taken to a depth of 15 cm from the Syndicate district near Tully. The soil was classified as a Dermosol (Isbell 1996) within the Tully-Coom series (Cannon *et al.* 1992). A composite sample was passed through a 2.5-cm mesh sieve, then steam-pasteurised at 70°C for 30 min, a treatment previously shown to eliminate indigenous VAM fungi (Thompson 1990). Chemical analyses of this soil prior to steam-pasteurisation indicated that there was an inadequate supply of P, potassium (K), calcium (Ca), magnesium (Mg), and zinc (Zn) for sugarcane (Calcino 1994); the soil also exhibited a high P-sorption capacity (Table 1).

The steamed soil (3.6 kg per pot) was added into polythene-lined 20-cm-diameter pots after mixing thoroughly with basal nutrients and 1 of 6 rates of P. Phosphorus rates were 0, 2.7, 8.2, 25, 74, or 222 mg P/kg applied as powdered $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ (monocalcium phosphate). The basal nutrients, nitrogen (N), K, Mg, iron (Fe), and sulfur (S), were added in solution form as NH_4NO_3 , K_2SO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Fe}(\text{II})\text{SO}_4 \cdot 7\text{H}_2\text{O}$, and as the various SO_4^{2-} salts to provide 120, 200, 18, 9, and 113 kg/ha equivalent, respectively. Calcium was added in powder form as CaCO_3 to provide 382 kg/ha equivalent. Pots were incubated for 49 days prior to spore addition to maximise nutrient equilibration.

Spore inoculum was added to inoculated pots at an estimated rate of 4 spores/g soil (OD basis), sufficient to produce a field-equivalent infection level in roots of sugarcane (Kelly *et al.* 1997). The suspension was dispensed throughout the soil using a stubbed Gilson pipette, which provided a maximum volume of 40 mL. Pots were watered, as determined by a pressure plate apparatus, to 90% field capacity (or 36% OD soil).

Table 1. Chemical properties (0–15 cm) of the low P cane-growing soil from Nicotra, North Queensland, determined prior to steam pasteurisation at 70°C for 30 min

Samples were taken 21 September 1996. Determinations were performed at University of Queensland, except for Zn, Cu, and Mn, which were performed at Incitec. Methods are, unless otherwise stated, those found in Rayment and Higginson (1992)

Property	Unit	Value	Extractant	Method
pH		4.75	1:5 H ₂ O	4A1
EC	dS/m	0.25	1:5 H ₂ O	3A1
P	mg/kg	4	0.005 M H ₂ SO ₄	9G1
Ca	cmol(+)/g	0.16	1 M NH ₄ OAc	15E1
Mg	cmol(+)/g	0.08	1 M NH ₄ OAc	15E1
K	cmol(+)/g	0.06	1 M NH ₄ OAc	15E1
Zn	mg/kg	0.61	0.1 M HCl	Reghenzani (1993)
Cu	mg/kg	0.21	0.005 M DTPA	12A1
Mn	mg/kg	4	0.005 M DTPA	12A1
Effective P concentration	µg P/L	35.1		9J1
P buffer capacity	mg/kg per log ₁₀ µg/L	520.4	0.01 M CaCl ₂	9J1

After sowing, fortnightly applications of 40 kg N/ha equivalent as NH₄NO₃ in solution were made for the duration of the trial. Copper (Cu, as CuSO₄·5H₂O) and boron (B, as H₃BO₃) nutrient solutions were added to each pot during the course of the experiment to overcome suspected nutritional deficiencies by providing 5 and 0.5 kg/ha equivalent, respectively, at 21–28 days after planting (DAP).

Nematode assessments

Nematode extractions were undertaken to confirm that the pasteurisation event was successful, and that nematodes would not confound potential growth responses to VAM. Three 200-mL volumetric samples of soil, both fresh and steamed, were dispensed on Whitehead trays for nematode extraction (O'Brien and Stirling 1991). Nematodes retained on a 38-µm mesh sieve after 4 days were classified and counted by genus. Nematodes were not found in the steamed soil. A negligible number (<3 per 200 mL) of pathogenic nematodes (i.e. those with root-piercing stylets), and only low numbers of non-pathogenic nematodes (<40 per 200 mL), were found in the fresh soil. The low counts indicated that the risk of confounding growth responses due to pathogenic nematodes was minimal.

Planting material and preparation

Three host plant species were chosen to measure the response to colonisation by mycorrhizal strains from cane soils. Cultivars and species were chosen for their tolerance to the acidic cane-growing soil, ability to be propagated within a pot for 6–8 weeks, and inclusion in a sugarcane rotation.

The plant cultivar/species choices included (i) soybean cv. Manta, a low-pH manganese-tolerant cultivar bred in Grafton, northern NSW, and grown commercially in southern Queensland over the last 5–8 years (J. Rose, pers. comm.); (ii) maize cv. Pioneer 6875, an inbred field maize variety grown commercially in northern Queensland during the last 10–20 years (I. Martin, pers. comm.), and (iii) sugarcane cv. Q124, a moderately resistant cultivar to *Pachymetra* root rot and previously shown to be slightly dependent on VAM fungi under even moderate soil P conditions (Kelly *et al.* 1997).

Twelve soybean seeds inoculated with a commercial *Rhizobium* starter culture (Group H) (surface-sterilised by rinsing in 70% ethanol for 1 min followed by 2 rinses in SDW) were sown per pot, and thinned to 3 plants per pot at 7 DAP. Seven maize seeds, surface-sterilised by

the method described above, were sown per pot, and thinned to 2 plants per pot at 7 DAP. Sugarcane stems of Q124, found to be free from ratoon-stunt disease (D. Teakle, pers. comm.), were taken from a plant-cane site at Gilberton, south-east Queensland, and aseptically chopped into single-eye setts 5 cm in length. Setts (surface-sterilised in 1% sodium hypochlorite for 10 min, and rinsed twice in SDW) were then placed into an aseptic misting chamber for 3 days; 3 g of powdered gypsum (CaSO₄·2H₂O) was applied at this time to enhance germination. Single setts were planted per pot. Setts were stratified at planting to minimise any potential differences in P content according to node position, i.e. setts sown in replicate 1 pots were taken from the top of the stalk, while those sown into replicate 4 pots were taken from the base of the stalk.

Maize, soybean, and sugarcane pots were planted on 17, 18, and 21 October 1996, respectively. By 7 DAP, average germination rates of soybean, maize, and sugarcane were 92%, 95%, and 99%, respectively.

Experimental design

The experiment consisted of 3 host plant species each planted into 24 pots that consisted of 4 replicates with 6 fertiliser P rates split into those that did (+VAM) or those that did not (–VAM) receive VAM spores. The pots were arranged in crop species blocks within the glasshouse, each host block in a randomised complete block design with weekly bench randomisations.

Growth conditions

To prevent possible soil mite infestations and to protect against nematode and aphid damage but preserve VAM, Temik (a.i. aldicarb, 10% w/w) was incorporated into the top of all pots at a rate of 2 kg a.i./ha on 30 October. Plants were watered to 90% field capacity twice each week ($\theta_g = 32\%$) with daily applications to approximately that moisture point. Mean ambient temperature ranged between 33°C and 25°C for the duration of the trial (17 October 1996–23 January 1997).

Harvest methodology

Soybean, maize, and sugarcane plants were harvested at 52, 55, and 94 DAP, respectively. For each plant, nutritional symptoms were noted, while plant heights (base of the stem to the base of the youngest mature leaf) and final leaf areas were determined. Tops were then harvested and dried at 65°C over 7 days to determine dry weight (DW). Roots

were rinsed free of soil, and a 1.5-g subsample of fine roots was taken randomly from the root bolus to determine the level of root colonisation; the remainder was dried at 65°C over 7 days to determine root DW.

Root samples were stained by soaking in a 1 M (10%) potassium hydroxide (KOH) solution at 90°C to remove cell nuclei (1.5, 2, and 4 h for soybean, maize, and sugarcane roots, respectively). Sugarcane roots were additionally soaked in a 1 M alkaline peroxide (0.5% NH₄OH and H₂O₂ v/v in water) solution for 20 min to remove any phenolic compounds. All root samples were then acid-soaked in 0.1 M HCl for 5 min, before being stained with a trypan-blue lactoglycerol solution for 10 min (Phillips and Hayman 1970). The percentage of the root colonised by VAM fungi was determined by the gridline intersect method across 100 root intersections observed under a dissecting microscope (×40) (Giovannetti and Mosse 1980).

A measure of plant responsiveness to VAM fungi was given by the relative mycorrhizal dependency (RMD) using the formula of Plenchette *et al.* (1983):

$$\text{RMD} = 100 \times [\text{DW}(+\text{VAM}) - \text{DW}(-\text{VAM})] / \text{DW}(+\text{VAM})$$

Nutrient concentrations were determined in the OD index leaf of each host species. The youngest mature blade (YMB; soybean, maize) and the middle 200 mm of the topmost visible dewlap (TVD) leaf (sugarcane) with the midrib removed were digested in a nitric/perchloric acid solution (Johnson and Ulrich 1959). The concentration of P in the digests was then determined by inductively coupled plasma-atomic emission spectroscopy. Phosphorus content in plant tops was determined by the acid digest of a sample of ground whole plant tops, and colorimetric analysis (at 420 nm) by the vanado-molybdate method (O'Neill and Webb 1970). Phosphorus recoveries from standard maize and peanut (*Arachis hypogaea*) tissue samples were 103% and 91% for the two species, respectively.

Statistical analysis

For each plant species, analysis of variance (ANOVA) was used to determine the effects of treatments. Means were compared using the protected least significant difference (l.s.d.) procedure where the *F*-test was found to be significant ($P < 0.05$). An arc-sin transformation prior to ANOVA was conducted on the percent root infection data to achieve homogeneity of variance around the means. The MPN and 95% confidence (or fiducial) limits were determined from statistical tables for the dilutions (Fisher and Yates 1963). Regression analysis was used to derive polynomial models to describe the P relationships.

Results

Most probable number study

The viable propagule density of the spore suspension was determined to be 188 propagules per tube (± 2.3 propagules within the 95% fiducial limits), or 0.9 propagules/g OD-equivalent soil. This density was only one-quarter of the number of spores thought to be added from the visual assessment of the spore suspension (0.9 v. 4 spores/g).

General symptoms

All plant species exhibited severe and marginal P-deficiency symptoms for the low-P fertiliser rates. For maize, -VAM plants that received nil P fertiliser produced a red-purple stem, the internodes failed to expand, and older leaves became necrotic; the most severely affected plants died at 40

DAP. Similarly, -VAM soybean plants that received nil P fertiliser exhibited abscission of the older leaf, interveinal necrotic streaks on younger leaves, and grossly reduced leaf areas. Such symptoms were indicative of a moderate or severe P-deficiency (Grundon 1987).

Sugarcane plants that received 0 or 2.7 mg P/kg showed poor tillering, shorter internodes, greater necrosis of older leaves, and redder stems than those that received 74 or 222 kg P/ha, irrespective of mycorrhizal status. Symptoms were similar to those of slightly P-deficient sugarcane plants (Calcino 1994).

Dry weight of plant tops

Maize plants responded positively to the application of P fertiliser (Fig. 1a). Responses to VAM depended on the rate of P applied. For example, responses were 5-fold (9.1 v. 1.8 g; $P < 0.05$) without added P whereas at 2.7 mg P/kg, responses were modest (16.4 v. 14.0 g; $P > 0.05$). Mean DW of +VAM plants that received 25 mg P/kg was equivalent to that of -VAM plants that received 74 mg/kg, indicating a P saving of 49 mg/kg (or 53 kg/ha). Similarly, mean DW of +VAM plants that received 74 mg P/kg was equivalent to that of -VAM plants that received 222 mg/kg, or a P saving of 148 mg/kg (or 160 kg/ha). There was no response to VAM when plants received 222 mg P/kg.

Soybean plants responded positively to the addition of P fertiliser (Fig. 2a). Response to VAM was greatest without added P (4.7 v. 11.2 g; $P < 0.05$), but there was no response when 222 mg P/kg was added (36.6 v. 34.4 g; $P > 0.05$). Mean DW of +VAM plants that received 25 mg P/kg was equivalent to that of -VAM plants that received 222 mg/kg, a P saving of 197 mg/kg (213 kg/ha).

Sugarcane plants responded positively to P fertiliser, but the response to VAM was poor compared with maize and soybean plants (Fig. 3a). At 2.7 mg P/kg, +VAM plants produced >30% more DW in tops than -VAM plants (29.0 v. 21.2 g; $P < 0.05$). However, no other positive responses to VAM were observed. In fact, at 222 mg P/kg, +VAM plants produced significantly less DW than -VAM plants (63.0 v. 70.2 g).

Root weight ratio

For maize and soybean plants, there was no significant response in root weight ratio to VAM or P fertiliser, and responses were not clear-cut (Figs 1b, 2b). For sugarcane, +VAM plants that received 8.2 or 74 mg P/kg had a significantly higher root weight ratio than -VAM plants (Fig. 3b).

Leaf area

Leaf area responses by all plant species to VAM matched the DW responses. For maize, leaf area responses to VAM tended to be positive, apart from when 222 mg P/kg was applied (Fig. 1c). Similarly, +VAM soybean plants produced

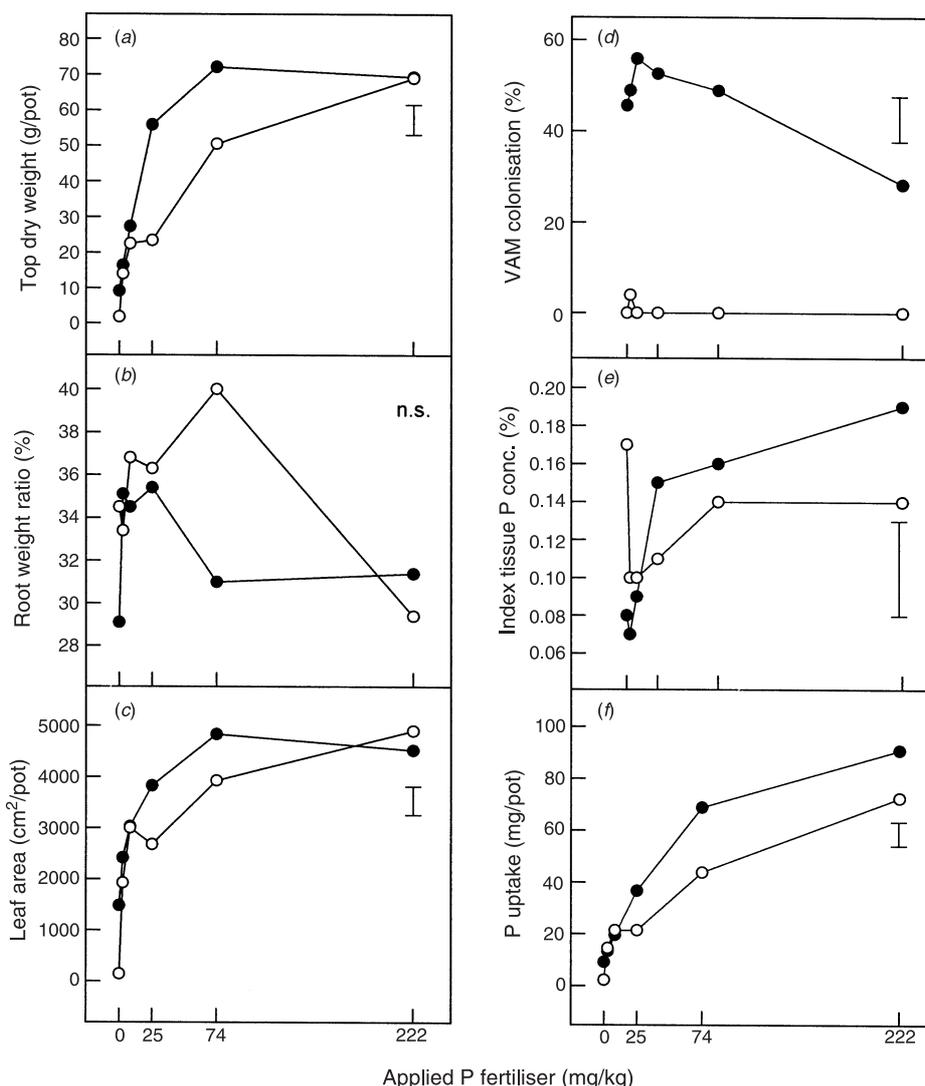


Fig. 1. Effect of applied P fertiliser (mg/kg OD soil) on (a) top dry weight, (b) root weight ratio, (c) leaf area, (d) VAM colonisation, (e) P concentration in the YMB, and (f) P uptake for maize plants at 55 DAP when inoculated with (●) or without (○) VAM spores taken from cane-growing soil. Vertical bars represent the l.s.d. ($P = 0.05$), and refer to the comparison of means within each figure. Neither main nor interaction effects were significant for (b). The l.s.d. in (c) refers to the VAM effect ($P = 0.05$).

more leaf area ($P < 0.05$) than VAM plants when P was added at 0, 25, or 74 mg P/kg (Fig. 2c).

Sugarcane +VAM plants that received 2.7 or 25 mg P/kg produced more leaf area than -VAM plants (Fig. 3c). However, at 222 mg P/kg, +VAM plants produced nearly 10% less leaf area than -VAM plants (2767 v. 3054 cm²; $P < 0.05$).

Mycorrhizal colonisation

For all 3 host species, roots of +VAM plants were colonised for all P rates, and the levels of infection dropped significantly with high P application. Colonisation of maize

roots tended to increase with P until a peak of 56% infection was reached with a rate of 8.2 mg P/kg (Fig. 1d). Higher P rates led to reductions in root infection, such that colonisation fell below 30% with the addition of 222 mg P/kg.

Root colonisation of soybean plants increased with the rate of P fertiliser from 32% without P to 71% when 25 mg P/kg was added (Fig. 2d). Root colonisation was reduced below 50% only when 222 kg P/kg was added.

Sugarcane +VAM plants also were colonised by VAM for all rates of P, though to a lesser extent than maize and soybean plants (Fig. 3d). Roots of plants that received 0 or

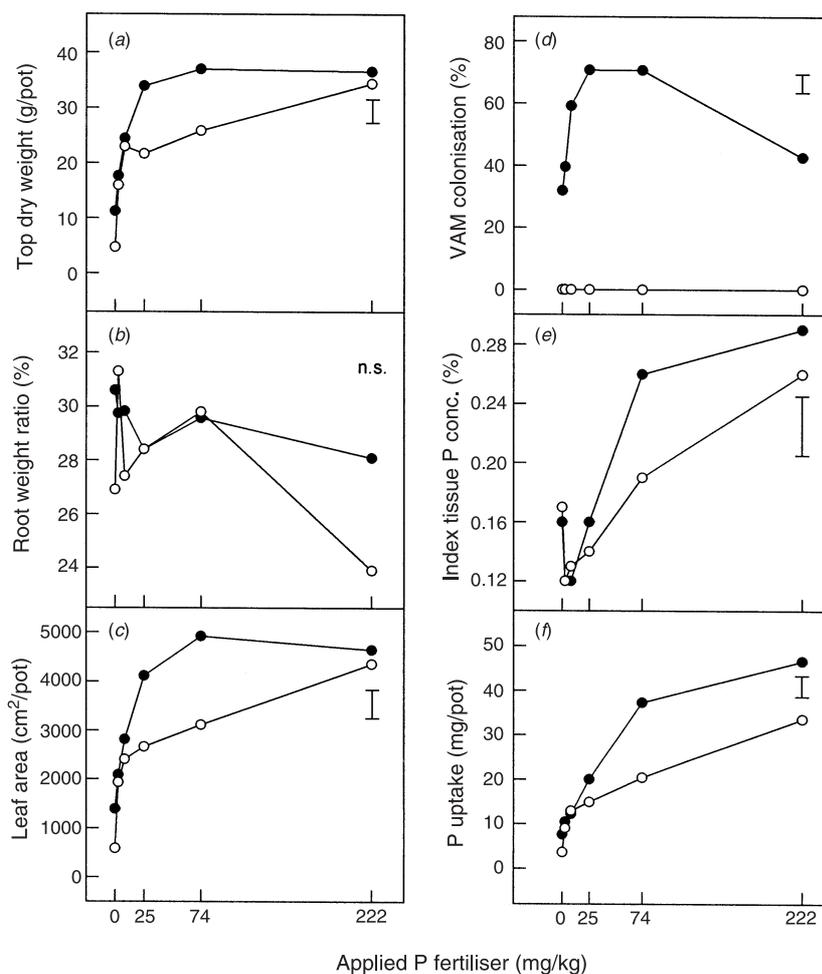


Fig. 2. Effect of applied P fertiliser (mg/kg OD soil) on (a) top dry weight, (b) root weight ratio, (c) leaf area, (d) VAM colonisation, (e) P concentration in the YMB, and (f) P uptake for soybean plants at 52 DAP when inoculated with (●) or without (○) VAM spores taken from cane-growing soil. Vertical bars represent the l.s.d. ($P = 0.05$), and refer to the comparison of means within each figure. Neither the main nor interaction effects were significant for (b).

222 mg P/kg were only slightly infected (15% v. 4%, respectively), but plants that received 2.7 or 8.2 mg/kg had strong root colonisation (>50%).

Tissue P concentration

Phosphorus concentration in the index tissue of all host plants decreased as the rate of P increased from 0 to 2.7 mg P/kg, but from this point on, tissue P rose with increasingly higher rates of P. For maize, as the P rate increased from 2.7 to 222 mg P/kg, P concentration in the YMB rose from 0.07% to 0.19% and from 0.10% to 0.14% for +VAM and -VAM plants, respectively (Fig. 1e).

For soybean, as the P rate increased from 8.2 mg P/kg, P concentration in the YMB rose from 0.12% to 0.29% and from 0.13% to 0.26% for +VAM and -VAM plants,

respectively (Fig. 2e). Phosphorus concentration was significantly higher in the index tissue of +VAM plants than -VAM plants over the 3 highest rates of P.

Phosphorus concentration in TVD leaves of sugarcane did not respond, for any P rate, to VAM colonisation (Fig. 3e). However, index tissue P rose from 0.06% to 0.22% as the rate of P increased from 0 to 222 mg P/kg.

Phosphorus uptake by plants

Phosphorus uptake by maize and soybean plants increased significantly in response to VAM over the highest 3 rates of P (Figs 1f, 2f). Soybean +VAM plants with nil P fertiliser took up twice as much P as -VAM plants (7.6 v. 3.6 mg; $P < 0.05$). Sugarcane plants did not respond to VAM fungi for any P rate except for 222 mg P/kg; in this case, +VAM

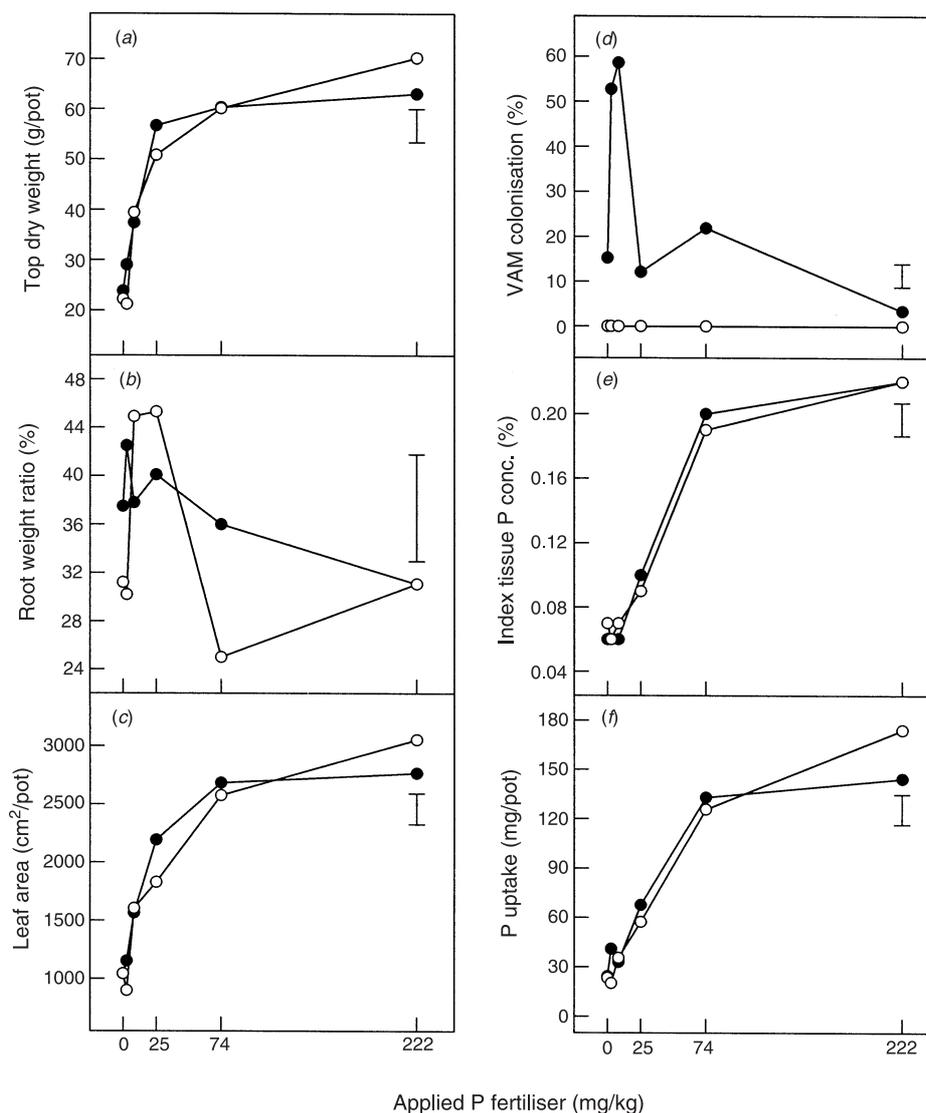


Fig. 3. Effect of applied P fertiliser (mg/kg OD soil) on (a) top dry weight, (b) root weight ratio, (c) leaf area, (d) VAM colonisation, (e) P concentration in the TVD, and (f) P uptake for sugarcane plants at 94 DAP when inoculated with (●) or without (○) VAM spores taken from cane-growing soil. Vertical bars represent the l.s.d. ($P = 0.05$), and refer to the comparison of means within each figure. The l.s.d. in (e) refers to the P effect ($P = 0.05$); for (f), the interaction was significant at $P = 0.08$.

plants took up 20% less P than -VAM plants (144 v. 174 mg; $P = 0.08$) (Fig. 3f).

Relationship between DW and P fertiliser

Dry weight responses by host plants to P fertiliser were best described using the Mitscherlich equation:

$$y = a - b.c^x$$

where y refers to the DW of tops (g/pot) and x refers to the rate of P (mg/kg). The value of 'a' refers to the maximum DW of tops, and $(a - b)$ is the DW of the plant tops without P. Surprisingly, +VAM and -VAM maize treatments had similar

model parameters: +VAM plants reached the same plateau as -VAM plants (Table 2). For soybean, the DW of tops without P for +VAM plants was twice that of -VAM plants (11.6 v. 5 g), and the maximum DW was greater with VAM inoculation (36.8 v. 27.2 g). For sugarcane, only slightly more DW of tops was produced, without added P, by +VAM plants than -VAM plants (22.9 v. 21.0 g), and maximum DW was less with VAM inoculation (62.3 v. 66.6 g).

Relationship between DW and tissue P concentration

Maize and soybean -VAM plants exhibited a C-shaped relationship between DW of tops and P concentration in the

Table 2. Parameters for Mitscherlich response surfaces that describe responses in dry weight of tops to the application of P fertiliser

Parameters refer to the model $y = a - b.c^x$, where y is DW (g/pot) and x is P fertiliser applied (mg/kg)

Crop species	Treatment	Model parameters			
		a	b	c	r^2
Maize	+VAM	71.5	63.7	0.95	0.96
	-VAM	71.4	63.1	0.99	0.89
Soybean	+VAM	36.8	25.2	0.92	0.97
	-VAM	27.2	22.2	0.81	0.73
Sugarcane	+VAM	62.3	39.4	0.94	0.94
	-VAM	66.6	45.6	0.96	0.90

YMB (Fig. 4a,b). For maize, critical P concentrations in the YMB that corresponded to 90% of maximum top DW were 0.15–0.16% and 0.14–0.15% for +VAM and -VAM plants, respectively. All maize plants had P concentrations in the YMB <0.25%, the critical concentration cited by Reuter *et al.* (1997). For soybean, the critical P concentration in the YMB was 0.15–0.16% and 0.21–0.23% for +VAM and -VAM plants, respectively. These concentrations were also below 0.29–0.34%, the range of critical values cited by Reuter *et al.* (1997).

For sugarcane, the critical P concentration in the TVD was 0.09–0.11% and 0.16–0.18% for +VAM and -VAM plants, respectively (Fig. 4c). Again, these values are below the critical concentration of 0.19% for sugarcane cited by Calcino (1994), which was only reached, or exceeded, in this study by those plants that received ≥ 74 mg P/kg.

Responsiveness to VAM fungi

The degree to which plant species responded to VAM fungi, as measured by the RMD (%), varied among species and for different P rates. For example, maize and soybean plants were most responsive to VAM fungi when nil P was applied (78% and 57%, respectively) (Table 3). Although RMD for both plant species was low when 2.7, 8.2, or 222 mg P/kg was applied (0–15%), RMD was greater with P application of 25 or 74 mg P/kg (30–54%). Sugarcane plants, overall, were less responsive to VAM fungi than the other species (Table 3). Plants that received 2.7 mg P/kg were most responsive (27%) to VAM. However, negative responses to VAM were found for plants that received 8.2 or 222 mg P/kg (-6% and -12%, respectively).

Relationship between plant growth and VAM fungal colonisation

Root colonisation of +VAM plants that received ≥ 25 mg P/kg was positively correlated with DW of tops. For maize, the variation was largely unexplained by the linear relationship ($r^2 = 0.03$; $P > 0.05$; $n = 12$) (Fig. 5a). However, the positive response in DW of tops of soybean ($r^2 = 0.84$;

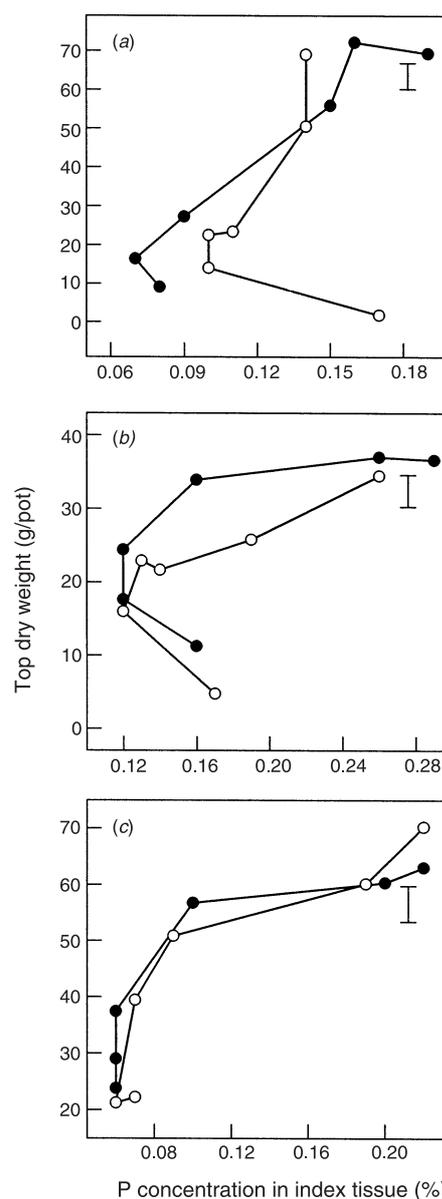


Fig. 4. Relationship between top dry weights of (a) maize, (b) soybean, and (c) sugarcane at harvest and P concentrations in the index tissue for 6 rates of applied P (0, 2.7, 8.2, 25, 74, 222 mg/kg OD soil) and with (●) or without (○) VAM spores (4 spores/g). Vertical bars represent the l.s.d. ($P = 0.05$), and refer to the comparison of means within each graph

$P < 0.001$; $n = 16$) and sugarcane ($r^2 = 0.57$; $P < 0.01$; $n = 12$) to root colonisation was significant (Fig. 5a,b).

Plant heights

Maize plants increased exponentially in height with time, with greater responses to VAM evident at the lower rates of P (Fig. 6a). Height of soybean plants increased sigmoidally with time according to P fertiliser rate (Fig. 6b). Plants that received 8.2 mg P/kg had the greatest response to VAM fungi

Table 3. Responsiveness (%) of three plant species (maize, soybean, sugarcane) to VAM fungi for a range of P applications (0, 2.7, 8.2, 25, 74, and 222 mg P/kg)

Plants were harvested at 55, 52, and 94 DAP, respectively. Within a column, values followed by the same letter are not significantly different ($P = 0.05$). Responsiveness was measured by relative mycorrhizal dependency (RMD; Plenchette *et al.* 1983) as indicated earlier

P fertiliser (mg/kg)	Responsiveness (%) of plant species		
	Maize	Soybean	Sugarcane
0	78 c	57 c	5 ab
2.7	10 a	10 a	27 c
8.2	15 a	6 a	-6 ab
25	54 bc	36 b	9 bc
74	30 ab	30 b	0 ab
222	0 a	6 a	-12 a

in height, evident from as soon as 27 DAP until harvest. There was a linear increase in height of sugarcane with time (Fig. 6c), and no significant response to VAM fungi for the entire trial period.

Discussion

Numbers of viable propagules

The viable propagule density, as measured by the MPN study, was only one-quarter of the number of spores thought to be added from the visual assessment of the spore suspension (0.9 v. 4 spores/g). This discrepancy can best be explained by the likelihood that non-germinating spores, either due to dormancy or non-viability (Tommerup 1992), were present in the sample, and unable to contribute to root infection. Similarly, An *et al.* (1990) found that, for some mycorrhizal species detected in cropping fields, the proportion of viable spores, determined by a vital stain, was often about half that of total spores.

The rate of 4 spores/g was intended as a compromise between the limited availability of spore material, the volume of soil necessary for the plants to reach a stage where responses to VAM were evident, and a propagule density that would elicit a growth response. Although other investigations of crop response to VAM have introduced an inoculum density of >20 spores/g (e.g. Thompson 1990, 1994b), previous studies on sugarcane have shown that a rate of 4 spores/g was sufficient to mimic colonisation rates observed in the field (Kelly *et al.* 1996).

Effect of phosphorus on root colonisation and responsiveness to VAM fungi

Four factors appear to confound the response by plants to VAM fungi: density of propagules, the effectiveness of the mycorrhizal strain, the soil nutrient status, and the responsiveness (or dependency) of the host species to VAM fungi (Johnson and Pflieger 1992; Thompson 1994a). For

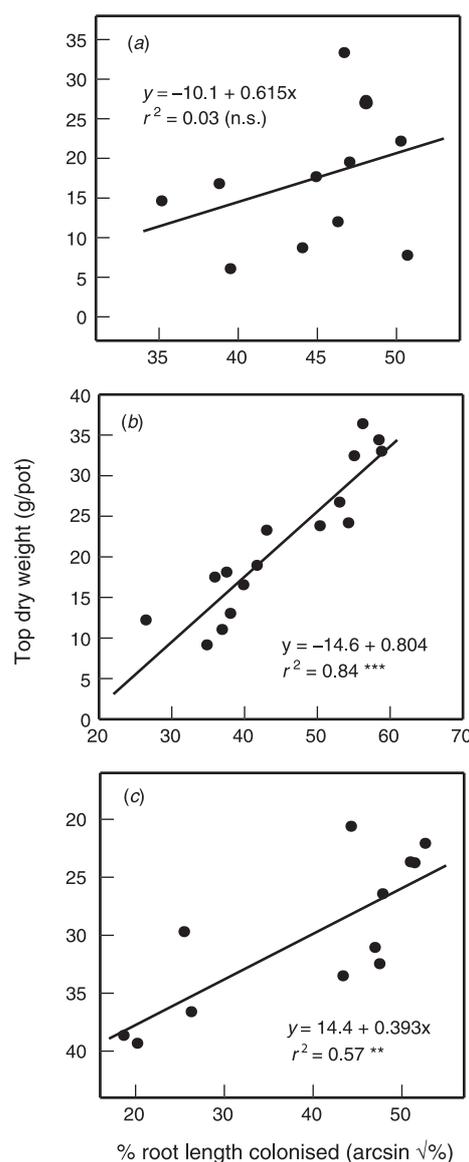


Fig. 5. Relationship between top dry weights of +VAM plants of (a) maize, (b) soybean, and (c) sugarcane at harvest and colonisation of roots by VAM. Data presented include the 4 replicates for the 0, 2.7, 8.2 [for (a) and (c)], and 25 [for (b)] mg P/kg applications.

example, it would be unclear whether poor growth responses by plants to VAM fungi were due to the ineffectiveness of the strain or to a poor responsiveness by the host species.

In this study we maintained a constant propagule density, inoculated with a single mycorrhizal strain, and tested 2 alternative host species known to be responsive to VAM colonisation (maize, soybean), in addition to the species in question (sugarcane), for a number of P levels. In this way, response by sugarcane, and the other host species, to VAM was able to be logically ascribed to one, or more, of these 4 influencing factors.

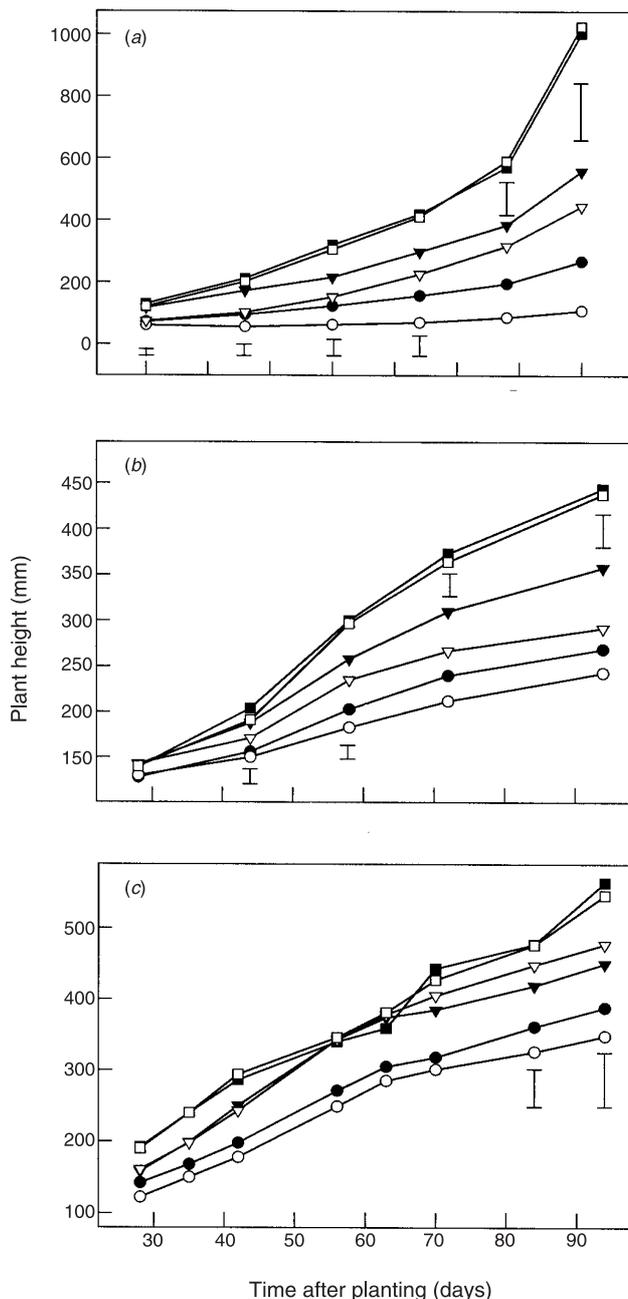


Fig. 6. Plant heights of (a) maize, (b) soybean, and (c) sugarcane after planting at 3 rates of applied P (0, 8.2, 222 mg P/kg; P₁, P₃ and P₅ respectively) and with (+) or without (-) VAM spores (4 spores/g); viz P₁+ (●); P₁- (○); P₃+ (■); P₃- (□); P₅+ (◆); P₅- (◇). Vertical bars represent the l.s.d. ($P = 0.05$), and refer to the comparison of means at a time after planting within each graph; missing l.s.d. values indicate that the P × VAM interaction at that time was not significant ($P = 0.05$).

Responses to VAM were largely determined by the effects of P fertiliser. Without P fertiliser, the responsiveness (or RMD) of maize and soybean to VAM peaked (RMD, 78% v. 57%), even though root colonisation was less than that at higher rates of P (Figs 1d, 2d). It appears that for these hosts,

but not sugarcane, root colonisation by the VAM strain led to an improved uptake of P, and this directly promoted plant growth despite a dilution effect, for maize, on the concentration of P within the index tissue. Evidently, the lack of growth by -VAM plants ensured that any responses, though slight in actual terms, would still appear relatively large.

When 2.7 mg P/kg was added, plant responsiveness was significantly modified. Sugarcane peaked in response to VAM (27%), whereas RMD for maize and soybean actually declined, despite an improvement in root colonisation, from that when nil P was added (Table 3). In a review of root colonisation responses to P fertiliser, Johnson and Pflieger (1992) indicated that, when soils are so nutrient-poor that root infection is itself limited by poor root growth, small additions of P can stimulate colonisation. That this, in itself, did not contribute to a maintenance of high plant responsiveness could be due to one, or both, of two factors.

Firstly, the timeliness of harvest can influence the apparent growth responses to VAM. For example, sequential harvesting of cowpea (*Vigna unguiculata*) indicated that plant responsiveness to VAM could vary considerably from 3% (at 28 DAP) to >80% (at 42 DAP) (Ikombo *et al.* 1991). Initial stages in the colonisation of roots, and the ensuing carbon (C) drain on the plant photosynthetic mechanisms, can even temporarily lead to a cost in terms of yield for +VAM plants compared with -VAM plants (Smith and Read 1997). We attempted to overcome this temporary effect by growing each host species until growth differences between mycorrhizal treatments, as indicated by plant height (Fig. 6) and leaf area (data not shown), appeared obvious.

Secondly, low rates of P may have stimulated root growth of -VAM plants, to a greater extent than +VAM plants, such that the amount of P taken up by these roots was equivalent to that of the inoculated plants. The effect of a C drain on +VAM plants, and the competition between roots, shoots, and VAM for C, would also account for such a response. A lack of significance in RWR data, due to poor consistency in response, prevented this hypothesis from being verified. Other studies, such as for sweet corn (Olsen *et al.* 1996) and subterranean clover (*Trifolium subterraneum*) (Smith and Read 1997), confirm that slight additions of P to -VAM plants can enhance root length to a greater extent than for +VAM plants.

The responsiveness by the 3 host plants varied considerably over the intermediate range of P (viz. 8.2, 25, and 74 mg P/kg). Maize and soybean plants were highly responsive to root colonisation; addition of spores led to greater shoot DW and leaf area, and higher tissue concentration and uptake of P (although not all responses were significant). In contrast, sugarcane plants were not responsive to VAM.

When all 3 host species received 222 mg P/kg, root colonisation and RMD of +VAM plants declined from when

74 mg P/kg was added. These findings are consistent with those of Menge *et al.* (1978), who, using a split-pot arrangement, demonstrated that the high tissue P concentration within the host plant, rather than soil P, led to the reduction in root colonisation. For sugarcane, it is unclear whether the significant reduction in DW, at this P rate, due to inoculation (63.0 v. 70.2 g) is reversible.

The variation in response between highly responsive species (maize, soybean) and poorly responsive hosts (sugarcane) over the intermediate rates of P can be related to 3 mechanisms. Firstly, root architecture can predetermine plant responsiveness to VAM. Poorly responsive plants often have relatively slender and lengthy root hairs (1–2 mm), and/or a high frequency of root hairs (Baylis 1975). However, improved P nutrition can, for some plant species, alter root architecture. As the rate of applied P increased from nil to 92.7 mg P/kg, roots of sweet corn (cv. Snosweet) grew longer (0.6 v. 2.6 km/plant) and thicker, as indicated by a drop in the specific root length (200 v. 140 m/g) (Olsen *et al.* 1996). Asher and Loneragan (1967) showed that for a number of temperate annual pasture species, root DW increased for some species by 2–4-fold but decreased for blue lupin (*Lupinus digitatus*) as the P concentration in a flowing solution culture increased from 0.04 to 1 μ M. Variation in root architecture, in response to added P, may have led to the variation in plant responsiveness to VAM.

Secondly, the level of P present in the planting material could influence the response to VAM. For example, when grown from small cuttings (2 cm long), cassava (*Manihot esculenta*) was highly dependent on VAM fungi, but when large cuttings (18 cm long) were planted, plants were only marginally dependent (Habte and Byappanahalli 1994). This loss of dependency was attributed to greater P reserves in the larger cuttings. Although there are clearly more reserves of P in the sugarcane sett than in maize or soybean seeds, it is not apparent that this led directly to a loss of root colonisation for sugarcane.

Thirdly, as iterated above, the timing of harvest can significantly alter the observed benefits, or otherwise, received from a mycorrhizal association. Sequential harvests would clarify when responses to mycorrhizal fungi would be greatest.

The variation in response concurs with the view that plant responsiveness to mycorrhizal colonisation is genetically and environmentally determined. The actual response will depend on the balance between the capacity to absorb P independently, on the one hand, and its capacity to support the symbiont with excess photosynthate, on the other (Smith and Read 1997).

Root colonisation and plant growth response

The positive correlation, and the steep slope, between root colonisation and DW of soybean tops is indicative of a highly

responsive species (Fig. 5b), and is similar to that of other grain legumes, linseed (*Linum usitatissimum*), and maize (Thompson 1994a). However, the less than expected response for maize in the present study highlights the limitations when matching harvested DW with the root colonisation at that same time. Thompson (1994b) showed a highly significant positive correlation between root colonisation of linseed at 36 DAP and harvested DW at 80 DAP. Root colonisation can vary throughout the duration of crop growth as the association develops, and as the plant approaches physiological maturity. The timing of root infection, then, may be the cause for a poor relationship between DW of maize and root infection. A time sequence that follows the development of infection and the later response in DW would be a useful procedure to identify relationships between root colonisation and plant response.

Of the 3 host species, the relationship between DW of tops and root colonisation for sugarcane had a positive intercept, suggesting that, although VAM can promote plant growth, colonisation rates would need to be relatively high for these results to be observed. This analysis supports the earlier conclusion that sugarcane is less responsive to mycorrhizal colonisation than maize or soybean.

Phosphorus nutrition of host plants

The critical P concentrations for the 3 host species in this study, for both +VAM and –VAM plants, were less than the critical concentrations for P deficiency reported elsewhere. None of these critical concentrations report an adjustment due to a mycorrhizal association (Reuter *et al.* 1997). Given that responsiveness to VAM is genetically and environmentally determined (Smith and Read 1997), the discrepancy in this instance could be due to the use of different cultivars that have lower critical P concentrations than those given by Reuter *et al.* (1997). One example is indicated in Reuter *et al.* (1997): for sugarcane, the critical P concentration for cv. NCO367 is 0.19%, whereas that for cv. N12 is only 0.16%.

The fact that interception of irradiation, critical for the provision of photosynthates for both the host and symbiont, is likely to vary between studies may further accentuate the discrepancies in critical P concentration. Consideration of the applicability of these results to the field must take into account the effects of pot and/or glasshouse culture. These factors would lower the confidence that such critical values are directly applicable to the field.

It is worth noting that a number of studies on mycorrhizal associations have failed to reproduce positive responses from glasshouse experiments in the field. This is, in part, due to the suppression of mycorrhizal activity by soil microflora (Hetrick *et al.* 1988). The difference between the critical P concentration of +VAM and –VAM treatments may then be less obvious if these studies were field-based.

Conclusions

A number of implications for long-term cane-growing soils arise in response to these findings. Firstly, this study confirms that although VAM are present in these soils, the *Glomus clarum* association led to a promotion of growth of the highly responsive crops, maize and soybean, over a range of P levels with substantial savings achievable on P fertiliser. As for other highly responsive crops such as linseed (Thompson 1994b), these crops, then, should not be sown into situations where the availability of VAM is low, such as after clearing or a clean long-fallow, unless an adequate rate of P fertiliser can be applied. Alternatively, if spore populations are low, it may be preferential to sow a less responsive crop such as sugarcane. Maize and soybeans, in rotation, could provide additional benefits for the ongoing sugarcane crop by preventing the build-up of less beneficial species of VAM fungi (An *et al.* 1993) and, in the case of soybean, provide sufficient N to the soil such that the next cane crop does not require fertilisation with N (Garside *et al.* 1997).

Secondly, the tested VAM strain did not contribute to YD in sugarcane plants and, in one instance for plants that received 2.7 mg P/kg, actually promoted growth by >30% over those without VAM. Further testing is needed over other long-term cane-growing soils to establish whether this finding is specific or general in nature. The observations in this study generally support the notion that sugarcane is poorly responsive to VAM association rather than that the VAM strain is less effective, or parasitic. These findings concur with that of Pinchin (1986).

Thirdly, the results have implications for P fertilisation in the sugarcane industry. This study indicated that, for this Tully-Coom soil, application of P fertiliser was not necessary unless P levels were <47 mg/kg, or <30 mg/kg if VAM fungi are present. The average of these results is similar to the current industry guideline that recommends the application of P fertiliser when P levels are <40 mg/kg (Calcino 1994). A more cautious approach by the industry to P application would help to minimise, in the short-term, the downstream and near-shore effects that are related to the loss of fertiliser P through erosion and surface runoff (Bramley and Wood 1996). The fact that the +VAM sugarcane plants produced less DW than -VAM plants at 222 mg P/kg in this study suggests that where VAM fungi exist, sugarcane yields may be compromised in over-fertilised cane-growing soils.

Acknowledgments

The authors wish to thank the Sugar Research and Development Corporation for the provision of research funds towards this study, Mrs J. Mercer, Mr G. Walters (UQ), and Mrs J. Bull (BSES) for technical assistance, and Mr D. Reid (QDPI) for biometrical advice.

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Manuscript received 22 September 2000, accepted 1 March 2001