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Field evidence for the carbon cost of citrus mycorrhizas

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SUMMARY

Mycorrhizas can produce negative crop responses when phosphorus availability is sufficient in agricultural soils because the fungi are of no benefit in nutrient acquisition yet continue to colonize roots and invoke parasitic costs. Benomyl fungicide was used to test this prediction in the field by limiting mycorrhizal colonization of 2-yr-old Valencia orange trees (*Citrus sinensis* (L.) Osbeck) on four rootstocks of varying mycorrhizal dependency in P-deficient soil fertilized with and without phosphate. No known fungal pathogens of citrus roots controlled by benomyl were present on the trees or in the field soil. Young trees with or without P fertilization and benomyl treatment remained sufficient in P ($\geqslant 0{\cdot}10\,\%$ leaf P) throughout the 27-month study. Root zone drenches of benomyl reduced mycorrhizal colonization and leaf P status of Valencia orange trees on the three slower-growing rootstocks, trifoliate orange (*Poncirus trifoliata* (L.) Raf.), Swingle citrumelo (*Citrus paradisi* Macf. × *P. trifoliata*) and sour orange (*Citrus aurantium* L.), for the duration of three growing seasons. Benomyl affected root colonization and P status of trees on the faster-growing rootstock, Volkamer lemon (*Citrus volkameriana* Tan. and Pasq.), less than for trees on the slower-growing rootstocks and the effects were sustained for only two seasons. The shorter duration of benomyl effect for trees on Volkamer lemon rootstock compared with the slower-growing rootstocks was explained by the loss of inhibition of mycorrhizal activity when roots grew out of the drench zone and mycorrhizas were no longer in direct contact with the fungicide. Benomyl treatment increased growth rate of Valencia orange on the slow-growing rootstocks from 5 to 17 % after three seasons, and from 2 to 9 % on Volkamer lemon rootstock after two seasons compared with the non-benomyl treated trees. The benomyl effect was attributed to reduction of costs of root colonization over time, and consequently, a greater availability of carbon assimilate for shoot growth of trees. Since mycorrhizal fungi are ubiquitous in fertilized agricultural soils and obligate biotrophs on the roots of most crop species, these results indicate a need to further investigate whether negative growth responses of P-sufficient plants in the field occur because mycorrhizal fungi are no longer behaving as mutualists.

Key words: Mycorrhizal colonization, phosphorus supply, mutualism, parasitism, Valencia orange trees, citrus rootstocks.

INTRODUCTION

Mycorrhizas enhance P uptake when soil P supply is limiting, which results in large increases in growth of mycorrhiza-dependent plants (Jakobsen, Abbott & Robson, 1992). Most research in citrus on mycorrhizal effects on carbon status has been conducted with seedlings in the glasshouse or growth room. In P-deficient soil, *Glomus intraradices* isolate FL208 can increase P inflow by threefold over nonmycorrhizal (NM) roots, causing large increases in relative growth rate (RGR) of citrus seedlings (Eissenstat *et al.*, 1993). Besides promoting P acquisition, mycorrhizas affect below-ground C expenditure of citrus (Douds, Johnson & Koch, 1988; Eissenstat *et al.*, 1993; Peng *et al.*, 1993). Without a compensatory increase in shoot C assimilation of citrus seedlings, *G. intraradices* FL208 reduced RGR compared with nonmycorrhizal plants of equivalent P status, consistent with an increase in below-ground C expenditure (Eissenstat *et al.*, 1993).

Although high P supply reduces colonization of citrus seedlings by *G. intraradices*, considerable fungal colonization of citrus roots still occurs in

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potted and field soils (Graham, Eissenstat & Drouillard, 1991; Peng *et al.*, 1993). This colonization neither benefits plant-P sufficiency nor increases photosynthesis (Syvertsen & Graham, 1990). Glasshouse and growth room studies demonstrate that, owing to a substantial effect of *G. intraradices* on root C allocation, mycorrhizal colonization at high P supply can reduce seedling RGR by up to 12 %, (19 % higher root d. wt and 10 % higher daily rate of root growth); (Peng *et al.*, 1993).

Other *Glomus* spp., which colonize roots aggressively and stimulate growth of citrus seedlings at low P supply, also colonize aggressively at high P supply but provide no additional P benefit and reduce RGR (Graham, Drouillard & Hodge, 1996). At low P supply, aggressiveness may be defined in terms of rate of root colonization since this character is a useful predictor of growth response to increased P uptake (Abbott & Robson, 1981). Less aggressive *Glomus* spp., that colonize roots more slowly, do not increase P acquisition at low P supply as effectively as do aggressive colonizers (Sanders *et al.*, 1977; Abbott & Robson, 1981). However, non-aggressive fungi do not colonize as rapidly, and are not as growth depressive, as aggressive fungi at high P supply (Graham *et al.*, 1996). These studies confirm that colonization rate is a potentially useful predictor of fungal cost, as well as fungal effectiveness, in P uptake.

Mycorrhizal fungi that aggressively colonize roots occur commonly in fungal populations in the field (Abbott & Robson, 1991; Brundrett, 1991), but the relevance of this character to mycorrhizal functioning in the field remains to be determined (Abbott & Gazey, 1994). Despite very high P fertility (160 $mg\ g^{-1}$ soil) in citrus orchards, mycorrhizal fungi colonize extensively citrus roots of mature trees (60–80 % incidence) (Graham *et al.*, 1991). The fungicide benomyl may be used to reduce mycorrhizal colonization under potted plant and field conditions (Fitter, 1986; Fitter & Nichols, 1988). However, control of plant pathogenic fungi by benomyl complicates the interpretation of fungicide effects on host plant RGR because of the interaction between communities of root-pathogenic fungi and mycorrhizal fungi (Newsham, Fitter & Watkinson, 1994). This interaction compromises the use of fungicides in the study of the relationship between cost and benefit of mycorrhizal symbiosis under field conditions (Johnson, Graham & Smith, 1997).

In the present study, we have determined that drench treatment of root systems with benomyl reduces mycorrhizal colonization and depresses P acquisition of young citrus trees. We predicted that limiting colonization with benomyl would reduce P uptake. In low P soil, this condition would result in P limitation and reduce the growth of young trees on mycorrhizal dependent rootstocks, assuming all other nutrients and water supply were sufficient. At high P supply, inhibition of mycorrhizas would not result in P limitation, but would reduce belowground C expenditure resulting in a positive growth response of young citrus trees.

MATERIALS AND METHODS

Study site, plant material and plot design

The study was conducted in an unfertilized field site at the Citrus Research and Education Center Tract 5 at Baseball City, FL, USA. The soil was a deep, uniform, Candler fine sand (Typic quartzipsamments: 96·5 % sand; 2 % silt; 1·5 % clay), that was very low in cation exchange and water-holding capacity. Soil pH was 6·8, organic matter content < 1 %, and Mehlich 1-extractable concentrations ($mg\ g^{-1}$) of P 3·5, Ca 156, Mg 8, K 4, Fe 3·6, Cu 2·6, Mn 0·92, and Zn 0·84 (Mehlich, 1953).

Four citrus rootstocks, Volkamer lemon, (VL, *Citrus volkameriana* Tan. and Pasq.), sour orange (SO, *C. aurantium* L.), trifoliate orange (TO, *Poncirus trifoliata* (L.) Raf.), and Swingle citrumelo (SC, *C. paradisi* Macf. × *P. trifoliata*), were grown from seed in a soil-less medium free of mycorrhizal fungi for 6 months in the glasshouse. Seedlings were transplanted to a heavily fertilized commercial field nursery in Avon Park, FL, USA, budded with a Valencia orange (*C. sinensis* (L.) Osbeck) scion, and trees grown for 1 yr. Seedling production and budding of TO commenced *c.* 1 yr earlier to compensate for the much slower growth rate of this deciduous citrus relative (*Poncirus*) that goes dormant in the winter. Trees were graded within and among rootstocks according to size and a representative root sample collected from 10 trees for evaluation of mycorrhizal colonization before transplanting. Trees were planted on 30 June 1993 at a spacing of 2·5 m in row and 6·5 m between rows. The design was a split plot, with two fertility treatments as the main plots (complete fertilizer with and without P), four rootstocks as the subplots and two fungicide treatments as the split plots (with and without benomyl drenches) with three trees per split plot and six replicate plots per treatment ($2 \times 2 \times 4 \times 6$ factorial).

Fertilization and fungicide application methods

The fertilization programme was adjusted as the trees increased in size over three growing seasons. In 1993, 1994 and 1995, nitrogen was applied at 150, 150 and 300 g N yr^{-1} per tree as urea (39-0-0), potassium was applied at 68, 68 and 136 g K yr^{-1} per tree as potassium sulphate (0-0-48), and P was applied at 45, 80 and 90 g P yr^{-1} per tree as triple superphosphate (0-46-0). Micronutrients were supplied as a minor element additive Esmigran® (The Scotts Co., Marysville, OH, USA) at 80 g yr^{-1} per

tree. In 1993, 57 g Ca and 7 g Mg per tree were applied as calcium sulphate and magnesium sulphate, respectively. Trees were irrigated during dry periods on a weekly schedule to minimize interaction of water supply as a factor limiting tree growth.

Benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) was applied as Benlate® 50WP (Dupont, Wilmington, DE, USA) to each tree site as a soil drench at 23 g active ingredient per 38 l which was retained in a 1-m diameter ring of soil around each tree. This rate of benomyl had no phytotoxic or plant growth regulator activity when drenched on non-mycorrhizal sour orange seedlings in Candler fine sand in a preliminary glasshouse trial (Eissenstat & Graham, unpublished). As demonstrated previously (Hale & Sanders, 1982), benomyl inhibited further development of root colonization by *Glomus intraradices* FL208, a species common in this field site (Graham & Fardelmann 1986), and presumably reduced mycorrhizal-mediated P uptake in low P Candler fine sand from the study site.

The drench volume of benomyl and area of soil treatment was designed to contact as much of the developing fibrous root system as possible during the three seasons of tree growth. Benomyl applications were made every 45 d during the growing season between May and September except in year one after transplanting (1993) when they were applied from August to October.

Soil-borne pathogen assessment

Thielaviopsis basicola is the only known pathogen of citrus roots potentially controlled by benomyl, but it is normally important only in artificial nursery soils and not persistent as a pathogen in field soils (Graham, 1994). Nevertheless, to assure that responses in the experiment were not a result of pathogen control in soil from the nursery or field site, a soil core (2·5 × 20 cm) was taken from each tree location and composited into one sample per plot in a resealable plastic bag for mixing. Ten 1-cm^3 samples of soil were mixed with 90 cm^3 of sterile 0·25 % agar. After an additional 1 : 10 dilution with water agar, 1-ml aliquots of each dilution were spread on the surface of five Petri plates of *T. basicola*–carrot–etridiazol–nystatin agar (Specht & Griffin, 1985). Plates were inspected 7–10 d later and no colonies of *T. basicola* were found.

Growth, nutrient and root colonization measurements

Stem diameter was measured with calipers twice per year in the spring and autumn before and after vegetative growth periods. Repeated measurements were taken at a painted mark 6 cm above the bud union. Fibrous roots ($\leqslant$ 2·0-mm diameter) were sampled in November–December after the final root growth flush of the root system was produced. Roots were excavated to a depth of 10 cm from a 20–40-cm diameter area around each tree and pooled into one sample per three-tree subplot. Roots were cleared with KOH followed by NaOCl as previously described for field roots of citrus (Graham *et al.*, 1991), and stained with trypan blue and mounted on slides for microscopic evaluation at × 50–250 magnification. Incidence of mycorrhizal colonization per sample was estimated as the percentage of 20 1-cm root segments with vesicles, arbuscules and hyphae present.

Leaf nutrient analysis was performed on the spring-shoot flush collected from the tree in August–September after the leaves had fully matured. Five leaves per tree were collected then ground, ashed (500 °C, 4 h) and resuspended in 1·0 mM HCl. Leaf concentrations of P, K, Ca, Mg, Fe, Cu, Mn, and Zn were determined by inductively coupled plasma atomic emission spectrometry. Leaf N was determined by the micro-Kjeldahl method.

Data analysis

Increase in stem diameter was subjected to the General Linear Model (GLM) for analysis of variance with time as a repeated measure. Linear contrast of the rate of increase in stem diameter was made in the univariate mode. Responses of tissue nutrients and mycorrhizal colonization were subjected to ANOVA for each year. Comparisons within treatments (rootstocks, fertilization, benomyl) were made with Student–Newman–Keuls multiple range test.

RESULTS

Effects of phosphorus fertilization and benomyl on mycorrhizal colonization and phosphorus nutrition

In the first season after trees were transplanted into the orchard (1993), the incidence of mycorrhizal colonization increased from low levels in the heavily-fertilized nursery site (SO, 18·4 %; VL, 10·0 %; SC, 18·4 %; TO, 23·4 %) to levels approaching those in mature citrus orchards (Graham *et al.*, 1991) (Table 1). Fertilization with phosphate did not suppress the development of mycorrhizal colonization compared with the −P fertility condition for the duration of three seasons. In all rootstocks, development of mycorrhizal colonization was reduced by drenches of benomyl fungicide with and without P fertilization in each season (1993, $P \leqslant 0{\cdot}0001$; 1994, $P \leqslant 0{\cdot}0003$; 1995, $P \leqslant 0{\cdot}0002$). Within rootstock treatments, mycorrhizal colonization was significantly ($P \leqslant 0{\cdot}05$) reduced by benomyl for SO +P fertilization in 1993, for TO −P fertilization in 1994, and for SC +P fertilization in 1995 (Table 1).

Although there were significant ($P \leqslant 0{\cdot}0001$) effects of P fertilization and benomyl on leaf con-

Table 1. *Intraradical mycorrhizal colonization (% incidence in 20 1·0-cm root segments with vesicles, arbuscules and hyphae present) of fibrous roots from young Valencia orange trees on four rootstocks grown with and without added phosphorus, and drenched with and without, benomyl to inhibit mycorrhizal development for three growing seasons (1993–1995)*

	+P fertilization		−P fertilization	
Season/rootstock	+Benomyl	−Benomyl	+Benomyl	−Benomyl
1993				
Volkamer lemon	60·9 a[z]	82·5 a	54·6 a	78·9 a
Swingle citrumelo	58·1 a	66·0 ab	41·9 a	61·4 ab
Trifoliate orange	42·5 ab	65·4 ab	36·9 a	41·5 b
Sour orange	21·6 b*	54·6 b	34·2 a	45·8 b
1994				
Volkamer lemon	76·4 a[z]	78·0 a	57·5 a	80·8 a
Swingle citrumelo	69·5 a	78·3 a	58·5 a	71·9 a
Trifoliate orange	62·1 a	73·6 a	34·6 a*	62·0 a
Sour orange	59·4 a	64·7 a	42·5 a	60·0 a
1995				
Volkamer lemon	87·0 a[z]	90·0 a	86·9 a	90·0 a
Swingle citrumelo	69·5 a*	90·0 a	76·0 a	82·5 a
Trifoliate orange	71·4 a	83·7 ab	75·9 a	83·9 a
Sour orange	65·9 a	76·4 b	67·9 a	76·4 a

[z] For each season, means in columns with the same letter are not significantly different ($P \leqslant 0{\cdot}05$) by Student–Newman–Keuls multiple range test. An asterisk (*) denotes significant difference ($P \leqslant 0{\cdot}05$) between benomyl treatments within rootstock and fertilization treatment.

Table 2. *Phosphorus concentration (%) in leaves from young Valencia orange trees on four rootstocks grown with and without added P, and drenched with and without benomyl to inhibit mycorrhizal development sampled for three growing seasons (1993–1995)*

	+P fertilization		−P fertilization	
Season/rootstock	+Benomyl	−Benomyl	+Benomyl	−Benomyl
1993				
Volkamer lemon	0·15 b*[z]	0·18 ab	0·14 a*	0·16 b
Swingle citrumelo	0·17 a*	0·19 a	0·15 a*	0·17 a
Trifoliate orange	0·14 b*	0·18 a	0·14 a*	0·17 ab
Sour orange	0·11 c*	0·16 b	0·10 b*	0·16 ab
1994				
Volkamer lemon	0·15 a*[z]	0·19 b	0·13 b*	0·15 b
Swingle citrumelo	0·15 a*	0·25 a	0·13 b*	0·18 a
Trifoliate orange	0·14 a*	0·24 a	0·13 b*	0·18 a
Sour orange	0·14 a*	0·25 a	0·15 a*	0·19 a
1995				
Volkamer lemon	0·18 a*[z]	0·17 a	0·15 a	0·16 a
Swingle citrumelo	0·16 a*	0·18 a	0·15 a	0·16 a
Trifoliate orange	0·16 a	0·16 a	0·13 a	0·14 a
Sour orange	0·15 a*	0·16 a	0·13 a*	0·15 a

[z] For each season, means in columns with the same letter are not significantly different ($P \leqslant 0{\cdot}05$) by Student–Newman–Keuls multiple range test. An asterisk (*) denotes significant difference ($P \leqslant 0{\cdot}05$) between benomyl treatments within rootstock and fertilization treatment.

centration of young Valencia orange trees, all treatments remained sufficient in P ($\geqslant 0{\cdot}10$ % leaf P concentration) throughout the 27-month study. Benomyl treatment significantly ($P \leqslant 0{\cdot}0001$) reduced leaf P concentration of trees on all rootstocks from 1993 to 1995 (Table 2). There was also a significant rootstock interaction with benomyl each year (1993, $P \leqslant 0{\cdot}0006$; 1994, $P \leqslant 0{\cdot}0001$; 1995, $P \leqslant 0{\cdot}04$) because leaf P of trees on the slower-growing rootstocks, SO, SC and TO, was more greatly reduced by benomyl than for trees on faster-growing VL. Trees on SO treated with benomyl approached

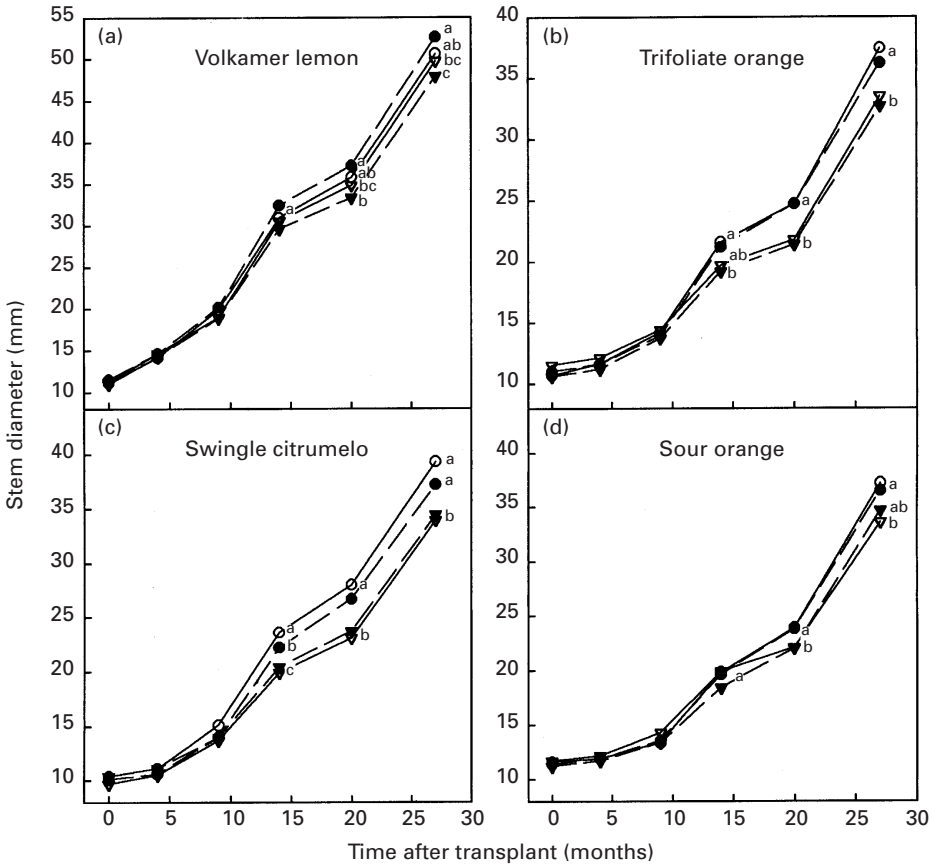

Figure 1. Response of stem diameter to benomyl drench treatments (B) of 2-yr-old Valencia orange trees on four rootstocks over 27 months after transplant in low phosphorus (P) soil with and without (+/−) P fertilization. Treatments are +P+B (○), +P−B (▽), −P+B (●), −P−B (▼). Points followed by unlike letters are significantly different according to linear contrast analysis ($P \leqslant 0{\cdot}05$).

the deficiency threshold in 1993 but the trees recovered to moderately sufficient leaf P status in 1994 and 1995 (Table 2). Leaf P concentration was reduced by benomyl for all individual treatment comparisons in 1993 and 1994. In 1995, reduction of leaf P by benomyl was only significant ($P \leqslant 0{\cdot}05$) for a few comparisons within rootstock treatments.

Neither P fertilization nor benomyl treatments affected leaf N or concentrations of other macronutrients and micronutrients (data not shown). All tissue levels were within the sufficiency range for optimum growth of young citrus trees under Florida conditions. (Tucker *et al.*, 1995).

Effects of phosphorus fertilization and benomyl on tree growth

The effect of P fertilization on the increase in stem diameter of trees on the four rootstocks from 1993 to 1995 (Fig. 1) was not significant. The lack of response to P fertilization was confirmed by leaf analysis that showed P status never dropped below the threshold for sufficiency for maximum tree growth (Table 2). Benomyl significantly ($P \leqslant 0{\cdot}0001$) increased stem diameter growth beginning at 14 months for trees on all rootstocks (Fig. 1*a–d*). The benomyl effect was similar for treatments with and without P fertilization, therefore the interaction was non-significant ($P \leqslant 0{\cdot}42$). After three growing seasons, the enhancement of tree growth by benomyl was significant ($P \leqslant 0{\cdot}05$) for all rootstock and fertilization treatments, except VL +P fertilization. For this treatment, the stem diameter for +benomyl treated trees was only 2·0 % greater than that for the −benomyl treatment after 27 months; the difference between the +benomyl and −benomyl treatments for −P fertilized trees on VL was 9·0 % (Fig. 1*a*). The benomyl effect was greatest for SC rootstock, 17·4 % for the +P treatment, and 11·5 % for the −P treatment (Fig. 1*c*). For TO and SO rootstocks, the increase in stem caliper by benomyl for +P treatment was 10·5 and 9·6 %, and for −P treatment was 9·8 and 4·9 %, respectively (Fig. 1*b*, *d*). Benomyl enhancement of tree growth was increasing through the third season (27 months) for trees on the slower-growing rootstocks, SO, SC and TO, but only through the second season (20 months) for trees on the faster-growing VL.

DISCUSSION

Our data are consistent with the hypothesis that benomyl reduced both the ability of the mycorrhizal fungus to acquire P and the cost of the fungal

colonization of citrus roots under field conditions for different rootstock genotypes. Although P deficiency did not occur with consequent reduction of young tree growth, positive growth response of trees to limitation of mycorrhizal colonization by benomyl was achieved under sufficient plant P status. The growth response observed in the field for young Valencia orange trees was predicted to range from 9 to 14% based on previous estimates of growth depressions attributed to C costs of *Glomus* spp. on citrus seedlings (Peng *et al.*, 1993; Graham *et al.*, 1996). This prediction from glasshouse and growth room studies is remarkably consistent with the results of the present field study which indicated 5–17% growth enhancement of young Valencia orange trees after three growing seasons of benomyl treatment.

As previously demonstrated in studies evaluating the functioning of mycorrhizas for other plant species (Fitter, 1986; Fitter & Nichols, 1988), benomyl is a fungitoxicant capable of inhibiting mycorrhizal colonization development and reducing acquisition of P by citrus rootstock genotypes. Based on our preliminary assessments of citrus seedlings, benomyl had no known confounding effects on host growth or control of pathogens of citrus roots in the nursery or planting site. Likewise, Merryweather & Fitter (1996) found few if any side effects of benomyl application on bluebell (*Hyacinthoides non-scripta*) in the field. They clearly demonstrated effective control of mycorrhizal colonization and reduction of P concentration in tissues of this highly obligate bulb plant with a limited root system development. After two seasons of benomyl treatment, P concentration in seed was reduced to a level critical for fecundity in the low P availability field site.

Analogous to the bluebell system, benomyl inhibited development of mycorrhizal colonization, and consequently reduced P acquisition for three seasons' growth of Valencia orange trees on four rootstocks of varying mycorrhizal dependency. This reduction in P acquisition had no negative effect on growth of trees because tissue P status never dropped below the critical level of leaf P for citrus (0·10%) during this 27-month study. Adequate P status of citrus trees was maintained with or without P fertilization and despite benomyl inhibition of extraradical and intraradical activity of mycorrhizas (Hale & Sanders, 1982; Larsen *et al.*, 1996; Sukarno, Smith & Scott, 1993, 1996). There are several possible reasons to explain why trees did develop P deficiency: (1) trees were planted from the commercial nursery with stored P ($\geqslant$ 0·20% leaf P) that was re-allocated for growth; (2) root systems had pre-established mycorrhizal colonization when transplanted, and even though further development and activity was inhibited by benomyl, some mycorrhizal-mediated uptake of P occurred even at low P supply; (3) slow-growing perennial *Citrus* on the four rootstocks had a relatively low P demand compared with supply of P that the expanding root system and mycorrhiza hyphal network had access to from the soil volume; (4) trees were sufficient in nutrient content and otherwise well-watered and fertilized with other macronutrients and micronutrients; or (5) soil-water status and nutrient availability in the low P-fixing Candler fine sand was not as restrictive to diffusion and mass flow of P as might be expected in finer textured soils of higher buffering capacity. The second possibility is supported by studies that indicate benomyl inhibited P uptake, transport and possibly transfer through the plant–mycorrhizal fungus interface (Sukarno *et al.*, 1993, 1996), but benomyl did not reduce fungal alkaline phosphatase activity of *G. caledonium* colonizing cucumber (Larsen *et al.*, 1996). This raises the possibility that different mycorrhizal species in the soil population vary in their sensitivity to benomyl.

Benomyl apparently reduced the parasitic cost of the symbiosis under conditions where mutualism was precluded by plant nutrient status and soil P supply (Johnson *et al.*, 1997). This alteration of cost:benefit occurred for trees on citrus genotypes that vary widely in mycorrhizal dependency, colonization rates and C allocation responses to mycorrhizal development under P deficiency and sufficiency (Graham *et al.*, 1991; Graham, Duncan & Eissenstat, 1997). Even though mycorrhiza-mediated P uptake in low availability conditions was inhibited by benomyl, young citrus trees did not experience P deficiency on any of the rootstocks despite their wide range in dependency on mycorrhizas for P uptake in low P soils (Menge, Johnson & Platt, 1978; Graham & Syvertsen, 1985). Thus, P fertilization of young trees could be substantially reduced from current horticultural recommendations (Tucker *et al.*, 1995) without limiting growth of trees on mycorrhizal-dependent and less-dependent rootstocks alike.

Merryweather & Fitter (1996) stressed that in natural ecosystems, benomyl needs to be thoroughly drenched into the root zone for effective fungitoxic activity against root colonization of bluebell and reduction of P uptake by extramatrical hyphae. In this study of citrus rootstocks, we confirmed that direct contact activity of benomyl in the mycorrhizosphere is probably crucial to reducing P uptake and C cost of mycorrhizas. A large volume of benomyl applied as a drench to a homogeneous sand profile was effective for reduction of physiological activity of the symbionts as long as the root system was fully contacted in the drench zone. Although this situation existed for at least three seasons for the three slow-growing rootstocks, TO, SC and SO, the effective period of benomyl application was apparently less for faster-growing VL. During the 27-month study period, there were smaller reductions in P status and lower positive growth responses to benomyl for the VL +P fertilization treatment compared with other

rootstocks. The loss of benomyl activity against mycorrhizas for VL is attributed to the root system growing out of the influence of the drench zone sooner than for the other rootstocks. This relationship between P uptake and growth response of trees on the rootstocks and degree of control of mycorrhizas with benomyl substantiates the premise that progress of colonization over time is a useful indicator of activity and below-ground C cost of the symbiosis (Graham *et al.*, 1991, 1996; Peng *et al.*, 1993).

Fertilization and crop monoculture might alter relative abundance and species composition of mycorrhizal communities possibly resulting in less mutualistic populations in terms of nutrient uptake and host growth. (Schenck & Sequeira, 1987; Johnson *et al.*, 1991, 1992). Johnson (1993) demonstrated that a composite fungal population from 8-yr fertilization plots produced a smaller growth response of big bluestem grass (*Andropogon gerardi*) under P limitation and was parasitic at high soil P supply, compared with a fungal population from non-fertilized plots. Colonization by the fungal assemblage from fertilized soil had a higher cost : benefit than the fungi from unfertilized soil as defined by vesicle development relative to arbuscules and hyphae.

If the prevailing conditions in fertilized agricultural fields select for more parasitic fungal communities, strategies to reduce the early colonization rate of indigenous fungi might be warranted, particularly for low dependency crops with annual production cycles. For example, the yield of maize grown under high phosphate fertility (> 50 kg P ha^{-1}) was increased by 1000 kg ha^{-1} in the midwest of North America if the soils were conventionally tilled compared with no-till crop practice (Vivekanandan & Fixen, 1991; McGonigle & Miller, 1996). Tillage would reduce the integrity of hyphal networks and rate of colonization, thus potentially reducing their C costs to plants. Analogous to the present study using benomyl to mitigate fungal colonization of P sufficient citrus, tillage-induced reduction in mycorrhiza-mediated uptake of P was of no consequence to maize growth because additional P was being supplied under luxury uptake conditions in the fertilized, no-till treatment. Alternatively, the positive growth response of maize to tillage was attributed to altered physical and microbiological properties of the soil, such as increase in seed bed temperature (McGonigle & Miller, 1996). The range of citrus growth response in this study (5–17 %) is quite consistent with the magnitude of positive growth and yield responses of maize to tillage in high P fertility soils over multiple seasons in several studies (Vivekanandan & Fixen, 1991; McGonigle & Miller, 1993, 1996).

Findings for maize support the hypothesis that if symbiotic costs are controlled by reduction in early-season mycorrhizal activity, positive yield effects will develop owing to re-allocation to shoot production during subsequent exponential growth stages as long as P is supplied adequately. By analogy for wheat grown in eastern Australia, undetermined 'biological factors' controlled by tillage or soil fumigation have been implicated in the consistently positive yield response of wheat, rather than other factors such as higher bulk density of soil or pathogen pressure that might limit growth under no-till conditions (Chan & Mead, 1992). In a glasshouse study, wheat growth was either unaffected or reduced by eight of 10 mycorrhizal fungal genotypes, including isolates of *Glomus*, *Acaulospora*, *Gigaspora* and *Scutellospora*, from Australian agricultural and undisturbed soils (Graham & Abbott, unpublished).

These studies of tillage-induced growth responses indicate the potential for mycorrhizal fungi to be non-mutualists and even parasites on low dependency crops during the early stages of crop development. This phenomenon deserves much further investigation using methods to manipulate cost : benefit of mycorrhizas in the field as recently outlined by Johnson *et al.* (1997).

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