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Carbohydrate allocation patterns in citrus genotypes as affected by phosphorus nutrition, mycorrhizal colonization and mycorrhizal dependency

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SUMMARY

Among closely related citrus genotypes growing in high phosphorus (P) orchard soils, there is a tendency for less mycorrhizal-dependent (M-dependent) species to have lower rates of root colonization than more M-dependent species. We hypothesized that the less M-dependent the citrus species the more limited is their carbohydrate (CHO) allocation to the M fungus. In a glasshouse study at low and high P supply, lower total incidence of *Glomus intraradices* FL 208, intensity of vesicles formation, and accumulation of 16: $1_{\omega 5}$ *cis* fungal fatty acid as measures of root colonization were related to lower mycorrhizal dependency (MD; M plant d. wt/non-mycorrhizal (NM) plant d. wt expressed at low P supply) in five citrus genotypes. At high P supply, when host carbon (C) production was not affected by P nutrition, less M-dependent genotypes had consistently lower starch concentrations in their root and shoot tissues than did more M-dependent genotypes, irrespective of M inoculation. At low P supply, M plants were more heavily colonized by *G. intraradices* and had lower starch levels than the M plants supplied with additional P. At high P, M plants of the more dependent genotypes allocated more C to starch pools relative to the NM plant than the least dependent genotype. Concentration of sucrose in tissues did not vary consistently with the dependency of citrus genotypes or with M inoculation except in high P NM plants, when less M-dependent genotypes had lower levels of sucrose in their roots than the more dependent species. At high P supply, sucrose concentrations were lower in colonized roots than in NM roots. Across citrus genotypes, sucrose in M fibrous roots decreased relative to that in NM roots with increase in root colonization suggesting that more sucrose was allocated for growth and maintenance of *G. intraradices* in roots of M-dependent species. The concentration of reducing sugars in root tissues varied in relation to MD of citrus genotypes in the same way as that of starch and sucrose, but was less responsive to M colonization. Responses of total non-structural CHO in tissues indicated that more heavily colonized plants at low P expended more C to acquire P than did M plants of similar biomass and P status grown at high P supply. Total CHO pools increased with MD of citrus genotypes, providing evidence than C allocation patterns in the host affect M colonization.

Key words: Fungal fatty acids, *Glomus intraradices*, non-structural carbohydrates.

INTRODUCTION

In orchard soil high in P, there is a tendency in citrus genotypes of lower mycorrhizal dependency (MD) to limit rate of colonization compared to genotypes of higher MD (Graham, Eissenstat & Drouillard, 1991). The rate rather than the ultimate extent of M colonization is limited, presumably, to prolong the plant's expenditure of carbon (C) for forming mycorrhizas. Thus, based on economic theory (Bloom, Chapin & Mooney, 1985), C could be expended for other competing needs likely to return a more immediate return on the investment, such as leaf area for C assimilation.

Reduced M colonization in citrus genotypes of lower MD might mean that fungal growth in the root represents a significant C cost and that there has been some evolutionary selection for this trait (Graham & Eissenstat, 1994). Growth of citrus

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species that have substantial M colonization at high P supply is depressed by the M fungus *Glomus intraradices* Schenck and Smith FL208 (Peng *et al.*, 1993). The cost of M colonization of the highly M-dependent citrus species, Volkamer lemon, at high P supply, includes a 50 % greater allocation of C to the roots than in non-mycorrhizal (NM) seedlings, an 11 % expenditure for building lipid rich fungal structures, and a 39 % greater respiratory cost for maintenance of fungal tissue in the root and for growth and maintenance of extramatrical hyphae (Peng *et al.*, 1993). Volkamer lemon had the highest incidence of colonization by *Glomus intraradices* FL208 among five citrus genotypes studied in field and greenhouse studies (Graham *et al.*, 1991; Graham & Eissenstat, 1994). In these studies, genotypes of lesser mycorrhizal dependency (MD, M plant d. wt/NM plant d. wt) showed substantially less colonization in roots than did more M-dependent genotypes (Graham & Eissenstat, 1994). At high P supply, mycorrhizal colonization by *G. intraradices* FL208 depressed growth of all citrus genotypes (by 1–9 %) but growth depression was not correlated with MD or fungal fatty acid content, a potential index of construction cost of the M fungus in citrus roots (Peng *et al.*, 1993; Graham & Eissenstat, 1994; Graham, Hodge & Morton, 1995). Thus, growth depression by *G. intraradices* FL208 and allocation for fungal construction were not clearly linked to MD of citrus genotypes as predicted by Graham *et al.* (1991).

It has been proposed that expenditure on the fungus by citrus genotypes is regulated by the amount of C available in pools of non-structural CHO in roots and other tissues (Graham *et al.*, 1991; Graham & Eissenstat, 1994). The role of CHO available in the root apoplast and exuding from roots in regulation of colonization dynamics has been recognized for several years (Graham *et al.*, 1981; Schwab, Menge & Tinker, 1991), but the relative importance of different types and sizes of CHO pools in roots has not been addressed in much depth until recently (Amijee, Stribley & Tinker, 1993). In many studies, methods for CHO measurement involve crude extractions and assays only of total anthrone-positive sugars (e.g. Thomson, Robson & Abbott, 1986; Pearson & Schweiger, 1993). Only a few studies examine starch and soluble sugar pools in relation to one another (Dixon, Garrett & Cox, 1988; Pacovsky, 1989), and none considers the effects of colonization completely independent of P supply when both M and NM plants receive sufficient P. This experimental approach is essential in order to exclude the very significant effects of P nutrition on C partitioning and CHO metabolism in the plant (Eissenstat *et al.*, 1993; Peng *et al.*, 1993).

The objective of this study is to establish further the link between MD at low P supply with tendency for the plant genotype to limit colonization and, therefore, C expenditure on the fungus at high P supply. Previous studies have made detailed analysis of growth and C allocation using ^{14}C pulses (Eissenstat *et al.*, 1993), respiration and tissue construction costs, and fatty acid analysis (Peng *et al.*, 1993). No emphasis has been placed on investigation of responses in non-structural CHO pools as affected by M colonization and citrus genotype. In this study, effects of P nutrition and mycorrhiza are examined to provide additional insight into how mycorrhizal fungi and the host interact in the utilization of C in five citrus genotypes varying in root colonization and M dependency (Graham *et al.*, 1991; Graham & Eissenstat, 1994).

MATERIALS AND METHODS

Plant inoculation and growth conditions

Seeds of five genotypes, Volkamer lemon (VL, *Citrus volkameriana* Tan. and Pasq.), sour orange (SO, *C. aurantium* L.), trifoliate orange (TO, *Poncirus trifoliata* (L.) Raf.), Swingle citrumelo (SC, *C. paradisi* Macf. × *P. trifoliata*), and Carrizo citrange (CC, *C. sinensis* (L.) Osb. × *P. trifoliata*) were germinated in a commercial peat–perlite–bark medium with starter nutrients and grown in 150-cm^3 plastic containers for 3 months (2–4 true-leaf stage). The soilless medium was thoroughly washed from roots, and each seedling was transplanted into 1 l of autoclaved (121 °C, 1·1 kg cm^{-2}) Candler fine sand soil (pH 6·8) with 3·8 mg kg^{-1} of available P as determined by double-acid extraction (Mehlich, 1953). For M treatments, 0·2 g of Sudan grass (*Sorghum sudanense* (Staph.) Piper) roots containing 1000 propagules (determined by most-probable-number analysis) of *Glomus intraradices* FL208 were placed below each seedling at transplanting. Non-mycorrhizal (NM) soil received an extract of inoculum, passed through a 38-μm sieve, to establish the same microflora associated with Sudan grass roots. The M and NM plants were grown from April to August 1991 in a shaded glasshouse with a maximum photon flux density of 1000 μmol m^{-2} s^{-2}. Average day/night temperatures were 33/25 °C and r.h. was 60–100 %. Seedlings were watered to excess every other day with tap water and fertilized weekly with Hoagland's solution (Hoagland & Arnon, 1939) minus P or with 5 mM P added. There were two levels of M treatments (M, NM), two levels of P (0, 5 mM) five citrus genotypes (VL, SO, TO, SC, CC), and six replicate seedlings per treatment. Seedlings were located in the glasshouse in a completely randomized design and relocated on the bench every 3 wk until harvest.

Mycorrhizal dependency and colonization assessment

At 125 d after transplanting, roots had filled the pot but were not yet pot-bound. Roots were gently

Table 1. *Mycorrhizal incidence* (*MI*), *vesicle intensity* (*VI*), *concentration of the fungal fatty acid* $16:1_{w5}$ *cis and mycorrhizal dependency* (*MD*, *ratio of mycorrhizal plant dry weight and non-mycorrhizal plant d. wt*) *for five citrus genotypes grown at high P* (+*P*) *and low P* (−*P*) *supply in soil with or without* Glomus intraradices

Citrus genotype	MI (%)*		VI (0–5)**		$16:1_{w5}$ *cis* (relative unit)†		MD	
	−P	+P	−P	+P	−P	+P	−P	+P
Volkamer lemon	83 a‡	85 a	3·0 a	2·6 a	14·8 a	3·0 a	8·1 a	0·99 a
Sour orange	77 a	76 ab	3·0 a	2·3 a	14·3 a	3·3 a	7·4 a	0·98 a
Swingle citrumelo	73 ab	70 b	2·5 a	2·0 ab	7·3 b	2·4 ab	3·7 b	0·99 a
Carrizo citrange	61 bc	71 b	1·5 b	1·5 b	4·0 b	2·2 ab	3·0 b	0·96 a
Trifoliate orange	53 c	41 c	1·1 b	0·5 c	5·5 b	1·0 b	1·2 c	0·91 a

* MI is the percentage of 20 root segments (1-cm) with vesicles, arbuscules and hyphae present.
** VI is the mean intensity of vesicle formation estimated on a scale of 0–5 in 20 root segments.
† Concentration expressed as relative amount based on the integrated area of the total fatty acids in the profile.
‡ Means ($n = 6$) in each column followed by different letters are significantly different ($P \leqslant 0{\cdot}05$) according to Duncan's multiple range test.

washed free of soil with tap water. Roots (fibrous roots < 2 mm diameter, and tap roots > 2 mm), stems, and leaves were dried at 70 °C for 24 h. Mycorrhizal dependency (MD) was expressed as the ratio of M plant d. wt to the NM plant d. wt.

Colonization was assessed as incidence of vesicles, arbuscules and hyphae in 20 1-cm fibrous root segments per plant (Graham *et al.*, 1991), and intensity of vesicle formation was visually estimated on a scale of 0 to 5 (0 = no vesicles present and 5 = entire length of root segments with vesicles present). These measures tended to overestimate colonization intensity because of the multiple cell layers in the root cortex of citrus species (Graham *et al.*, 1991). Colonization in roots was quantitatively estimated by measurement of the fungal fatty acid, $16:1_{w5}$ *cis*, a fatty acid that is not found in host tissue (Graham *et al.*, 1995). The content of fungal $16:1_{w5}$ *cis* was determined on a 15 mg subsample of dried fibrous roots with a Hewlett-Packard 5890 gas-liquid chromatograph after methanol extraction of roots, fatty acid saponification, and derivatization to methyl esters. Concentrations of $16:1_{w5}$ *cis* were expressed relative to the highest value using the integrated areas of the total fatty acid profile (Peng *et al.*, 1993).

Non-structural carbohydrate and P analysis

The remainder of fibrous roots, tap roots, stems, and leaves were separately ground to pass through a 40-mesh screen (2·0 mm opening size). A 30 mg subsample of each plant tissue was suspended in 15 ml of water, boiled for 2 min, and centrifuged at 800 ***g*** for 2 min, as previously described (Eissenstat & Duncan, 1992). The supernatant was analysed for total reducing sugars using arsenomolybdate (Nelson, 1944), and for sucrose using anthrone sulphuric acid after alkali treatment (Van Handel, 1968). Soluble and insoluble starch in the supernatant and pellet, respectively, were measured using amyloglucosidase after correcting for free glucose (Haissig & Dickson, 1979). Glucose was used as the standard for analysis of starch and sucrose. Fructose was used as the standard in the Nelson test because free fructose exceeded glucose concentrations in tissue samples (Eissenstat & Duncan, 1992).

Leaf P concentration was determined on a 100 mg subsample of leaf tissue using inductively coupled plasma atomic emission spectrometry after the tissue had been ashed (500 °C, 4 h) and resuspended in 1 mM HCl.

Data analysis

Plant biomass, CHO parameters, and P concentrations were subjected to three-factor (M, P levels, citrus genotype) analysis of variance (completely randomized design), and individual paired *t*-tests to evaluate the significance of M vs. NM treatment effects. Linear correlation analyses were used to determine relationships between expressions of CHO status and M colonization as measured by fungal fatty acid content of roots for *G. intraradices*-inoculated treatments. The effect of M treatment on CHO allocation to fibrous roots relative to NM roots was examined as the ratio of tissue CHO concentrations in M and NM plants.

RESULTS

Phosphorus and biomass responses

At low P supply, inoculation with *G. intraradices* FL208 increased leaf P status of citrus genotypes from deficiency ($< 0{\cdot}10$ % P) to moderate sufficiency ($> 0{\cdot}19$ % P). At high P supply, M treatment significantly increased leaf P status of the more M-dependent VL and SO but not of the other citrus genotypes. All values of leaf P concentration at high P supply were within the moderate to high sufficiency range, similar to those in the M treatment at low P supply.

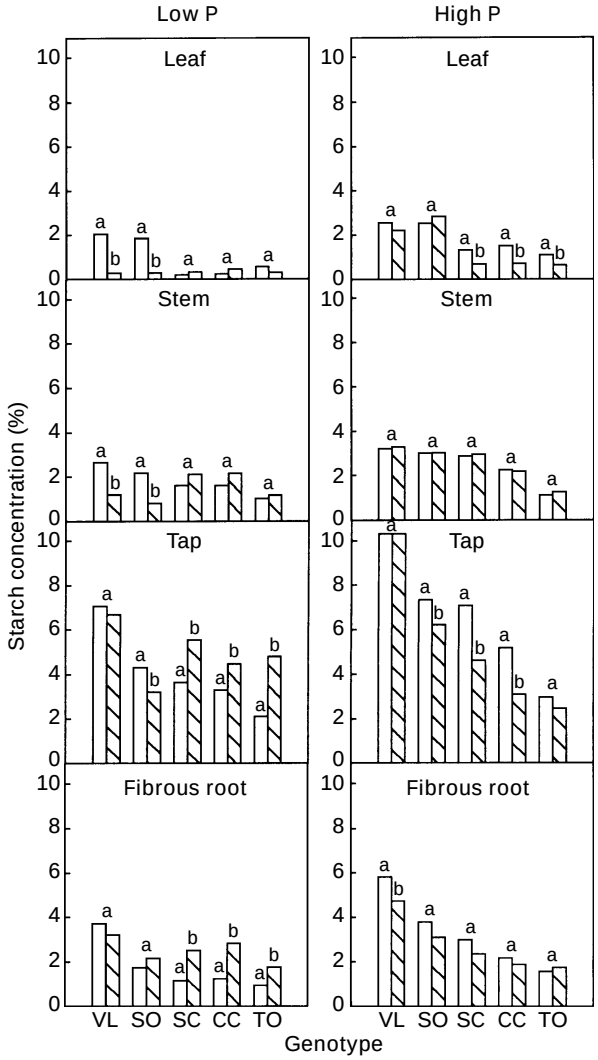

Figure 1. Starch concentration (% of tissue d. wt) in tissues of five citrus genotypes inoculated with *Glomus intraradices* FL208 (M, □) or non-inoculated (NM, ▨) and grown at low and high P supply. Differences between M and NM are indicated by unlike letters according to paired *t*-tests ($P \leqslant 0{\cdot}05$). (VL, Volkamer lemon; SO, sour orange; SC, Swingle citrumelo; CC, Carrizo citrange; TO, trifoliate orange.) Ranking of mycorrhizal dependency (MD): VL > SO ⩾ SC > CC > TO (see Table 1).

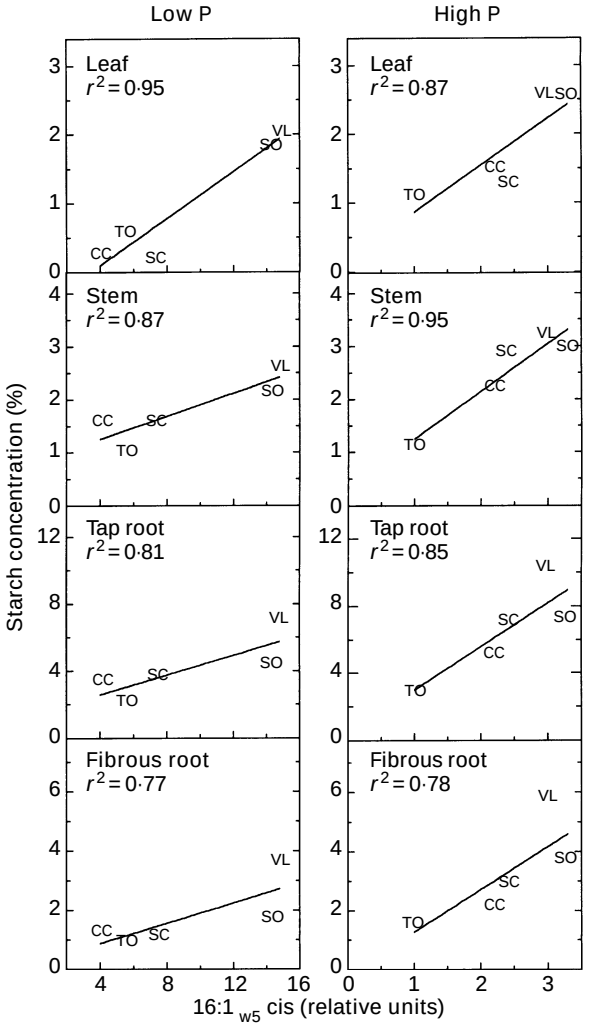

Figure 2. Correlation between starch concentration in tissues of five citrus genotypes inoculated with *Glomus intraradices* FL208 and grown at low and high P supply, and concentration (relative units) of the fungal fatty acid $16{:}1_{\omega5}$ *cis* in roots at the respective P supply. Correlation coefficients (r^2) are significant at the $P \leqslant 0{\cdot}05$ level. See Figure 1 for identification of citrus genotypes. Ranking of mycorrhizal dependency (MD): VL > SO ⩾ SC > CC > TO (see Table 1).

Plant biomass responses to M inoculation were significantly ($P \leqslant 0{\cdot}001$) affected by P supply and citrus genotype. At low P supply, VL and SO showed significantly ($P \leqslant 0{\cdot}05$) greater biomass responses in all plant tissues to inoculation with *G. intraradices* than SC, CC, and TO (see MD values in Table 1). At high P supply, total biomass responses of citrus genotypes to M inoculation were slightly negative (MD values $< 1{\cdot}0$ in Table 1) and not significantly different from NM responses.

Root colonization and fungal fatty acid responses

Visual estimates of mycorrhizal colonization incidence (MI) and vesicle intensity (VI) were not significantly reduced by high P supply, but relative content of fungal fatty acid, $16{:}1_{\omega5}$ *cis*, was significantly ($P \leqslant 0{\cdot}001$) lower in high P plants (Table 1). All measures of colonization were significantly lower in the least M-dependent citrus genotype, TO, than in the most M-dependent, VL. Ranking of genotypes was less well defined by fungal $16{:}1_{\omega5}$ *cis* content at high P because of the narrower range in fatty acid content than in visual assessments (which tended to overestimate colonization). However, all estimates of fungal colonization were well correlated (r^2 values from 0·72 to 0·90) with each other and with MD among citrus genotypes at the respective P levels as previously reported (Graham & Eissenstat, 1994; Graham *et al.*, 1995).

Non-structural carbohydrate responses

Starch. At low P supply, responses of starch concentration to *G. intraradices* FL208 varied with

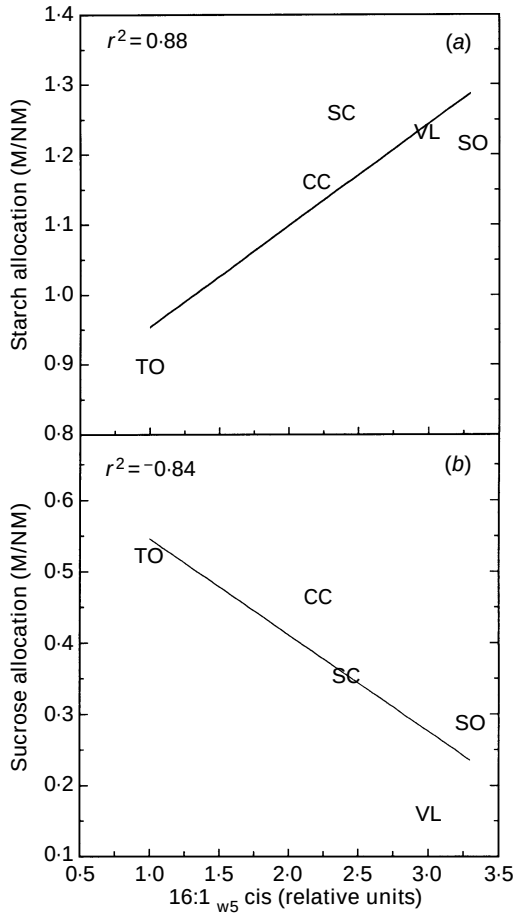

Figure 3. Correlation between the relative concentrations of (*a*) starch and (*b*) sucrose allocated to fibrous roots in mycorrhizal (M) versus non-mycorrhizal (NM) roots (expressed as the ratio [M]/[NM]) of five citrus genotypes grown at high P supply, and the concentration (relative units) of the fungal fatty acid $16:1_{\omega 5}$ *cis* in roots (as a measure of colonization by *Glomus intraradices* FL208). Correlations (R2) are significant at the $P \leqslant 0{\cdot}05$ level. See Figure 1 for identification of citrus genotypes. Ranking of mycorrhizal dependency (MD): VL > SO ⩾ SC > CC > TO (see Table 1).

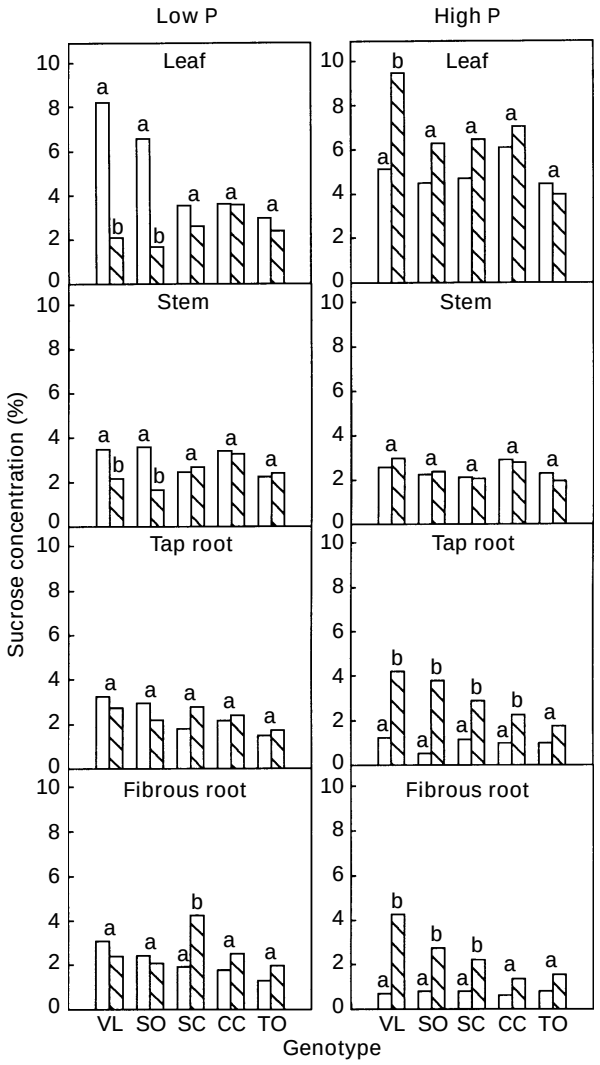

Figure 4. Sucrose concentration (% of tissue d. wt) in tissues of five citrus genotypes inoculated with *Glomus intraradices* FL208 (M, □) or non-inoculated (NM, ▨) and grown at low and high P supply. Differences between M and NM are indicated by unlike letters according to paired *t*-test ($P \leqslant 0{\cdot}05$). See Figure 1 for identification of citrus genotypes. Ranking of mycorrhizal dependency (MD): VL > SO ⩾ SC > CC > TO (see Table 1).

citrus genotype and tissue type (Fig. 1). Starch concentration was consistently higher in leaf, stem, tap, and fibrous root tissues of M plants of VL and SO, except in fibrous roots of SO. Generally, the opposite condition occurred in the less M-dependent SC, CC, and TO; i.e. tissue levels of starch were lower in M plants.

When both M and NM plants received sufficient P, responses of starch concentration to M colonization were more subtle than at low P (Fig. 1). In M fibrous roots, VL had significantly more starch than NM roots, with decreasing difference between M and NM plants as MD of the citrus genotype decreased. Tap roots of M plants tended to have higher starch concentrations, except in VL. Mycorrhizal colonization had little effect on starch concentration in stem tissues, but there were greater starch levels in leaves of M plants of less MD citrus genotypes.

Starch concentrations in tissues of citrus genotypes for *G. intraradices*-inoculated treatments were correlated with mycorrhizas as measured by fatty acid content of roots in order to observe relationships between tissue starch status and M colonization (Fig. 2). Positive correlations between starch and colonization were seen in all tissues of M plants in all citrus genotypes. Mycorrhizal plants had 10–50% higher starch levels in tissues at high P supply than M plants grown at low P supply in spite of similar P status and plant biomass (data not shown). Except in leaves, the difference in starch concentration between tissues at high and low P supply was greatest for M-dependent genotypes and diminished in less dependent genotypes (Fig. 2). At high P, M plants of more dependent citrus genotypes with more fungal tissue in the roots tended to allocate more C to the

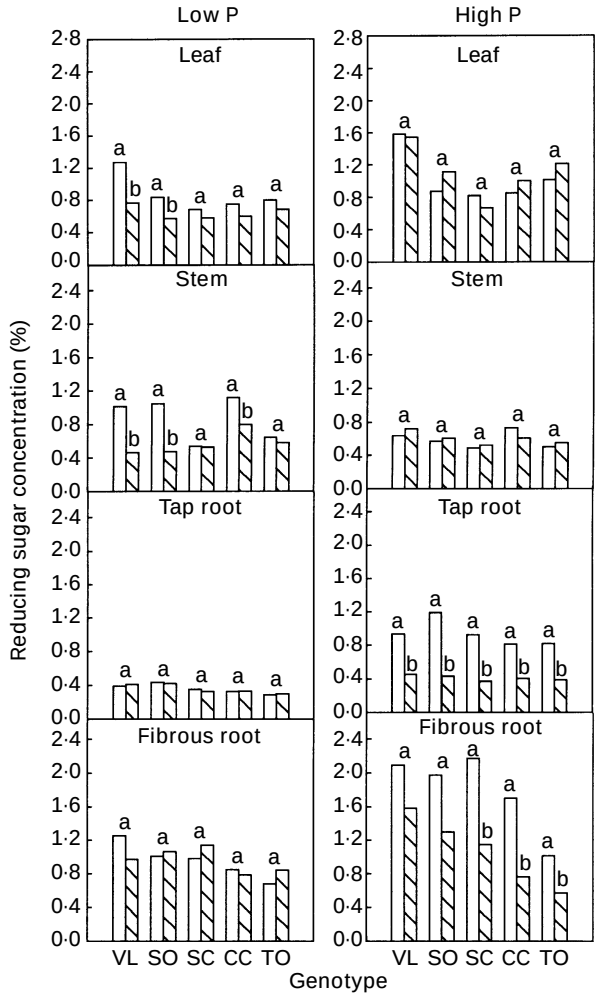

Figure 5. Reducing sugar concentration (% of tissue d. wt) in tissues of five citrus genotypes inoculated with *Glomus intraradices* FL208 (M, □) or non-inoculated (NM, ▨) and grown at low and high P supply. Differences between M and NM indicated by unlike letters according to paired *t*-test ($P \leqslant 0{\cdot}05$). See Fig. 1 for identification of citrus genotypes. Ranking of mycorrhizal dependency (MD): VL > SO ⩾ SC > CC > TO (see Table 1).

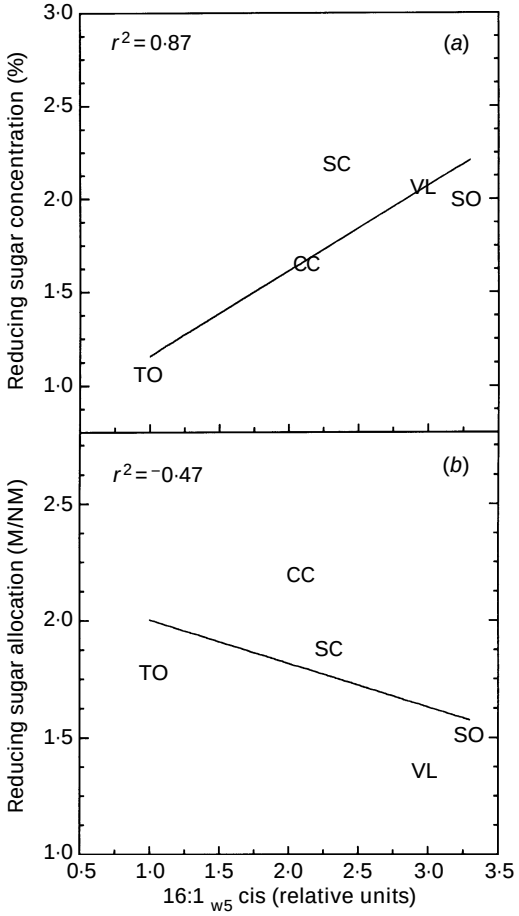

Figure 6. (*a*) Correlation between reducing sugar concentration (% of tissue d. wt) and concentration (relative unit) of the fungal fatty acid $16{:}1_{w5}$ *cis* in roots of mycorrhizal (M) plants of five citrus genotypes grown at high P supply. (*b*) Correlation between the relative reducing sugar allocation to fibrous roots of M versus NM plants (expressed as the ratio [M]/[NM]. Correlations (r^2) are significant at the $P \leqslant 0{\cdot}05$ levels and non-significant for (*a*) and (*b*), respectively. See Fig. 1 for identification of citrus genotypes. Ranking of mycorrhizal dependency (MD): VL > SO ⩾ SC > CC > TO (see Table 1).

starch pool in fibrous roots relative to NM plants than the least dependent genotype, TO (Fig. 3*a*).

Sucrose. Sucrose concentrations in tissues of M plants were generally similar to those in NM plants at low P supply, with exception of elevated levels in stems and leaves of VL and SO (Fig. 4). At high P supply, however, sucrose levels were lower in *G. intraradices*-colonized fibrous roots of VL, SO and SC, and in tap roots of all citrus genotypes except TO. Mycorrhizal effects on stem tissue concentrations of sucrose were slight at high P. In leaf tissue, sucrose concentrations shifted from significantly lower levels in M plants of VL to slightly higher levels in M plants of TO.

When the ratio of sucrose concentration in roots of M and NM plants (relative allocation) was correlated with fungal fatty acid content across genotypes as a measure of colonization, there was a strongly negative relationship (Fig. 3*b*). The decrease in sucrose in M roots relative to NM roots with increase in colonization suggests that more sucrose was allocated to the growth of *G. intraradices* in roots of the more M-dependent genotypes.

Reducing sugars. At low P supply, reducing sugars in M and NM plants did not vary consistently across citrus genotypes, although M plants of more dependent genotypes had significantly higher levels of reducing sugars in stems and leaves than the NM plants (Fig. 5). At high P supply, M plants had higher levels of reducing sugars in fibrous and tap roots. These responses of reducing sugars were similar to those of starch, although concentrations of reducing sugar in tissues were 2–5 times lower than starch levels (Figs 1, 5). When reducing sugar concentrations in fibrous roots of M plants at high P were correlated with M colonization at high P, the relationship with MD of citrus genotypes was similar to that for starch (Figs 2, 6*a*). However, unlike

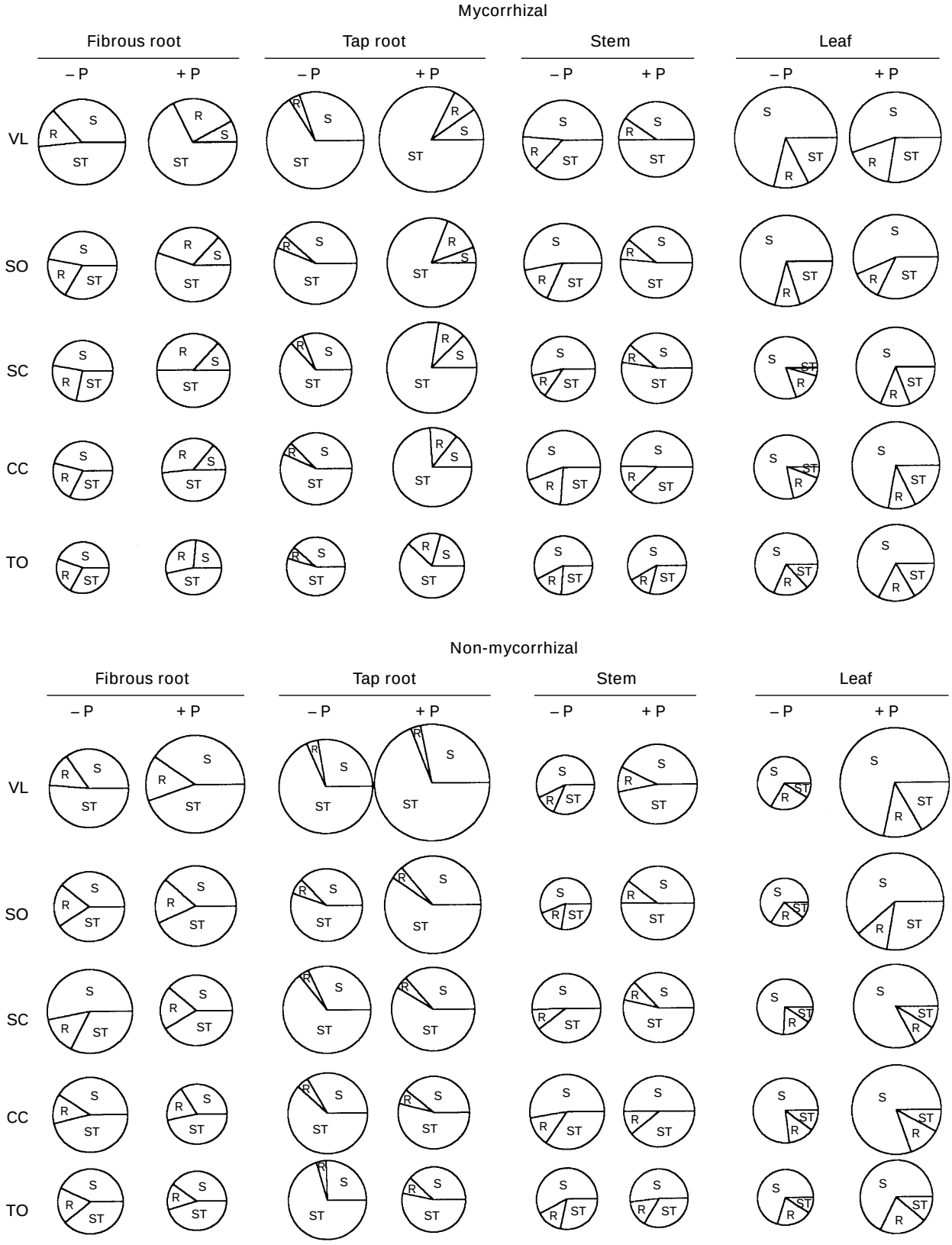

Figure 7. Total non-structural carbohydrates (area of circle) and the proportion of the pool that is starch (ST), sucrose (S), and reducing sugars (R) in tissues of five citrus genotypes grown at high P supply (+P) and in low P (−P) soil inoculated with *Glomus intraradices* or noninoculated (nonmycorrhizal). See Fig. 1 for identification of citrus genotypes. Ranking of mycorrhizal dependency (MD): VL > SO ⩾ SC > CC > TO (see Table 1).

starch, the allocation of reducing sugars to fibrous roots of M plants relative to NM plants at high P was negatively and weakly related to M colonization of citrus genotypes (Fig. 6*b*).

Total non-structural CHO. Total pool sizes (area of circles) and proportional response of the three component sugar pools (size of sectors) to M colonization were examined in the different tissues of citrus genotypes (Fig. 7). At low P supply, M colonization represented a greater fungal biomass than at high P (Table 1), and plants had smaller pools of non-structural CHO in fibrous, tap, and leaf tissues than M plants at high P supply (Fig. 7). This M colonization effect was generally consistent for all citrus genotypes except VL. Greater colonization at low P than in M plants at high P was also related to the presence of proportionately more sucrose and of less starch in root and shoot tissues of all genotypes. In NM plants, P supply had little effect on the proportion of sucrose and starch, although P had some effect on sucrose in stem tissue.

Less dependent citrus genotypes had relatively smaller pools (size of circles) of non-structural CHO in fibrous and tap roots than more dependent citrus genotypes (Fig. 7). The trends in total pool sizes across genotypes were less clear in stems but were also apparent in leaves. The more M-dependent genotypes had proportionally higher starch pools in fibrous roots, tap and leaf tissues than less M-dependent genotypes. This trend was most clear in tap roots of P-sufficient plants irrespective of their mycorrhizal status.

DISCUSSION

Non-structural carbohydrate pools in citrus plants responded strongly to colonization by *G. intraradices* FL208 through altered tissue allocation of storage CHO in the form of starch and in availability of readily mobile and utilizable sugars in the roots in the form of sucrose and reducing sugars, respectively. At high P supply, starch levels in root and shoot tissues of M plants were usually higher than in NM plants, especially in the most M-dependent citrus genotypes. More heavily colonized plants grown at low P supply and, as a result, of similar biomass and P status to high P plants, had consistently lower starch concentrations in tissues than the P-fed M plants that acquired soluble P at a lower C cost of uptake (Eissenstat *et al*., 1993).

Comparison of starch concentrations in fibrous roots between citrus genotypes provided additional support for the hypothesis that plant genotypes differ in their C allocation for fungal colonization. The less M-dependent genotypes with substantially lower root colonization had less starch in their root and shoot tissues than did more dependent genotypes. The consistency of this relationship among citrus genotypes at low and high P supply establishes a link between availability of non-structural CHOs and control of M colonization, as previously suggested (Graham *et al*., 1991; Graham & Eissenstat, 1994).

Sucrose was consistently lower in root tissues of P-fed M plants than NM plants indicating utilization of sucrose by the fungus and supporting root tissue for growth and maintenance (Peng *et al*., 1993). In more heavily colonized roots at low P supply, sucrose represented a greater proportion of the total non-structural CHO pool, suggesting greater mobilization of sugars from insoluble starch to soluble pools in response to mycorrhizas. Thus, the fungus apparently altered CHO metabolism in the roots and throughout the plant in response to increased C demand. The observation that sucrose pools did not consistently vary between citrus genotypes might reflect current C demand (metabolic activities of the fungus and plant) rather than plant genotypic control of C supply as represented by starch pools.

Reducing sugars are probably the most readily utilizable pool and, therefore, potentially responsive to M colonization (Shachar-Hill *et al*., 1995). However, concentrations of reducing sugars represented only 0·5–2 % of tissue dry weight; whereas starch varied from 2 to 10 % and sucrose from 1 to 9 %. Relatively greater allocation to reducing sugar pools in M root tissues compared with NM roots at high P supply followed the starch responses. The effect of citrus genotype on reducing sugar availability to mycorrhizas was less well defined. Perhaps because of rapid turnover, reducing sugars appeared to be least informative among the non-structural CHOs measured in this study. This observation might partly account for conflicting reports of relationships between anthrone-positive CHOs (reducing sugars) in root extracts and M colonization dynamics (Jasper, Robson & Abbott, 1979; Graham, Leonard & Menge, 1981; Johnson *et al*., 1982; Same, Robson & Abbott, 1983; Thomson *et al*., 1986).

Responses of total non-structural CHO pools appeared to demonstrate best the influence of mycorrhizas on C supply and demand. Heavily colonized M plants at low P supply, which expended more C to acquire P, had consistently lower total non-structural CHO pools in root tissues than M plants of similar biomass and P status grown at high P supply. Total CHO pools in citrus genotypes were more consistently related to MD than was each component of the pool separately (Fig. 7). Although colonization was only evaluated at a late stage, the relationship for citrus genotypes is preliminary evidence that CHO allocation affects colonization rate and that this process is apparently under plant genetic control (Graham *et al*., 1991; Graham & Eissenstat, 1994). Nevertheless, to determine more directly the mechanism(s) for genotypic control,

CHO pools and colonization need to be further assessed at several stages utilizing isogenic lines of plants that principally differ in their allocation of C below ground.

Exact forms of CHO and mechanisms for C transfer from root cells to the fungus are not well characterized (Smith *et al*., 1994). The assumption is that the phloem is responsible for delivery of reduced C to the roots in the form of sucrose (Patrick, 1989) and C is transferred from host to fungus principally as glucose (Shachar-Hill *et al*., 1995). The mechanism of transport across the plasma membranes of the plants at the interface with the fungal arbuscule or intracellular hyphae may occur by either passive or facilitated diffusion and is dependent on the solute concentration in the cytoplasm of the root cells. Fungal modification of C allocation that increases CHO concentration in the plant cytoplasm will presumably result in greater efflux of C to the fungus. This might be effected by fungal conversion of transported sugar into the osmotically inactive, and non-plant-available CHOs, glycogen and trehalose (Shachar-Hill *et al*., 1995). The precise plant controls of this efflux process await characterization of the principal plant and fungal cellular sites at which membrane transport occurs (Smith *et al*., 1994).

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