

Growth Depression in Mycorrhizal Citrus at High-Phosphorus Supply¹

Analysis of Carbon Costs

Shaobing Peng², David M. Eissenstat*, James H. Graham, Kimberlyn Williams, and Nancy C. Hodge

Citrus Research and Education Center, University of Florida, Institute of Food and Agricultural Sciences, Lake Alfred, Florida 33850 (S.P., D.M.E., J.H.G.); Department of Botany (K.W.), and Department of Plant Pathology (N.C.H.), University of Florida, Gainesville, Florida 32611

Mycorrhizal-induced growth depression of plants in high-P soil has been reported in many species. The carbon costs of factors contributing to this growth depression were analyzed in Volkamer lemon (*Citrus volkameriana* Tan. & Pasq.) colonized by the mycorrhizal (M) fungus *Glomus intraradices* Schenck and Smith. M and nonmycorrhizal (NM) plants were each grown at two P-supply rates. Carbon budgets of M and NM plants were determined by measuring whole-plant carbon assimilation and respiration rates using gas-exchange techniques. Biomass, M colonization, tissue-P concentration, and total fatty acid concentration in the fibrous roots were determined. Construction costs of the fibrous roots were estimated from heat of combustion, N, and ash content. Root-growth respiration was derived from daily root growth and root-construction cost. M and NM plants grown in high-P soil were similar in P concentration, daily shoot carbon assimilation, and daily shoot dark respiration. At 52 d after transplanting (DAT), however, combined daily root plus soil respiration was 37% higher for M than for NM plants, resulting in a 20% higher daily specific carbon gain ($\text{mmol CO}_2 [\text{mmol carbon}]^{-1} \text{ d}^{-1}$) in NM than M plants. Estimates of specific carbon gain from specific growth rates indicated about a 10% difference between M and NM plants. Absolute values of specific carbon gain estimated by whole-plant gas exchange and by growth analysis were in general agreement. At 52 DAT, M and NM plants at high P had nearly identical whole-plant growth rates, but M plants had 19% higher root dry weight with 10% higher daily rates of root growth. These allocation differences at high P accounted for about 51% of the differences in root/soil respiration between M and NM plants. Significantly higher fatty acid concentrations in M than NM fibrous roots were correlated with differences in construction costs of the fibrous roots. Of the 37% difference in daily total root/soil respiration observed between high-P M and NM plants at 52 DAT, estimated daily growth respiration accounted for only about 16%, two-thirds of which was associated with construction of lipid-rich roots, and the remaining one-third with greater M root growth rates. Thus, of the 37% more root/soil respiration associated with M colonization of high-P plants, 10% was directly attributable to building lipid-rich roots,

51% to greater M root biomass allocation, and the remaining 39% could have been used for maintenance of the fungal tissue in the root and growth and maintenance of the extramatrical hyphae.

Growth depression of plants colonized by VAM fungi at a high level of soil-P availability has been reported in several studies (Cooper, 1975; Crush, 1976; Buwalda and Goh, 1982; Hall et al., 1984; Son and Smith, 1988). It has been suggested that the requirement of host-plant carbohydrates by mycorrhizae may cause the depression in plant growth (Cox et al., 1975). Hepper (1977) estimated that the biomass of the M fungus within roots can represent up to 17% of the dry weight of roots. Histological analysis of M roots revealed abundant lipid in the arbuscules, vesicles, and hyphae (Cox et al., 1975). Furthermore, Nagy and Nordby (1980) found that citrus roots with VAM contained significantly more phospholipid and triglycerides than did noninfected roots. The large biomass of the fungus plus the high concentrations of lipid within the fungal structures suggest appreciable host-fungus competition for carbon.

Consistent with these observations, studies commonly indicate higher below-ground respiration for M than for NM plants (Pang and Paul, 1980; Snellgrove et al., 1982; Harris et al., 1985). Amounts of carbon required by the M fungus have been estimated in several studies using whole-plant ¹⁴C-labeling techniques; values range from 4 to 17% of fixed carbon (Pang and Paul, 1980; Kucey and Paul, 1982; Snellgrove et al., 1982; Koch and Johnson, 1984; Harris et al., 1985). These studies were conducted at low-P supply for M

¹ Florida Agricultural Experiment Station Journal Series No. R-02500. This research was supported in part by National Science Foundation grant BSR-9111824.

² Present address: Agronomy, Plant Physiology, Agroecology Division, International Rice Research Institute, P.O. Box 933, 1099 Manila, Philippines.

* Corresponding author; fax 1-813-956-4631.

Abbreviations: A, net carbon assimilation rate; CER, carbon exchange rate; C_r, daily root-construction cost; C_w, root-construction cost per unit dry weight of new root; DAT, days after transplanting; M, mycorrhizal; NM, nonmycorrhizal; PVC, polyvinylchloride; R_{G(t)}, daily root-growth respiration; R_{G(w)}, growth respiration per unit dry weight of new root; R_{M(t)}, daily "maintenance" respiration of root/soil system; R_{M(w)}, daily "maintenance" respiration per unit root dry weight; R_{T(t)}, daily total respiration of the root/soil system; R_{T(w)}, daily total-root/soil respiration per unit root dry weight; SGR, specific growth rate; TDW, total dry weight; VAM, vesicular-arbuscular mycorrhiza; ΔW_C, rate of increase in carbon content; ΔW_w, rate of increase in dry weight.

plants. Additional P was supplied only for NM plants to produce plants of approximately similar size and P nutrition. Another approach for assessing carbon requirements of mycorrhizae is to grow both M and NM plants at high-P supply, provided that appreciable M colonization still occurs. Because P supply is saturated for both M and NM plants, this approach has the advantage that equivalence between M and NM plants in P nutrition throughout the growth period is relatively easy to achieve, greatly simplifying evaluation of the carbon cost on the M fungus (Eissenstat et al., 1993).

Using gas-exchange techniques, we examined causes for the differences in daily carbon gain between M and NM plants at high-P supply. Plants at low-P supply were included for background information on the costs of mycorrhizae under conditions where the symbiosis increased P acquisition. As will be shown, the lower daily carbon gain of M than NM plants at high-P supply was due to greater below-ground respiration. Root fatty acid content and root construction costs were then estimated to assess how mycorrhizae may have chemically altered the fibrous roots. Using information from construction costs and total daily below-ground respiration, we then partitioned root/soil respiration into growth and "maintenance" (i.e. residual not accounted for by growth) to determine if construction of lipid-rich M roots was the primary reason for the differences in net carbon gain. In so doing, we provide the first estimates of the components of root respiration in M plants.

MATERIALS AND METHODS

Plant Culture and Inoculation

Seed of Volkamer lemon (*Citrus volkameriana* Tan. & Pasq.) were germinated in autoclaved Candler fine sandy soil with 3.8 mg kg^{-1} of available P as determined by double-acid extraction (Mehlich, 1953). One-month-old seedlings grown in the greenhouse were transplanted into 125-cm^3 conetainers filled with autoclaved Candler soil. For the M treatment, a pad of air-dried sudangrass (*Sorghum bicolor* [L.] Moench var *sudanense*) roots (about 0.1 g) containing 5000 chlamydo-spores of *Glomus intraradices* Schenck and Smith was placed in the middle of the soil profile in the conetainer during transplanting. The NM control received an extract of inoculum passed through a $38\text{-}\mu\text{m}$ sieve to remove the M fungal propagules but otherwise retained similar microflora. Decomposition of the root pad should have represented little additional CO_2 after 52 DAT because the simple sugars and starches of the small pad should have decomposed within the first few weeks, and the structural carbon materials (e.g. celluloses, hemicelluloses, and lignins) apparently decomposed little during the experiment because the root pad was observed to be largely intact for seedlings harvested at the end of the experiment (92 DAT).

Seedlings were grown in a growth chamber with PPFD of $450 \mu\text{mol m}^{-2} \text{ s}^{-1}$, photoperiod of 14 h, and day/night temperatures of $30/24^\circ\text{C}$. Wind velocity in the chamber averaged 0.3 m s^{-1} (model HH-30, Omega Engineering, Inc., Stamford, CT) at different positions among the plant shoots. Watering was provided in excess every day, and fertilization was conducted weekly with standard Hoagland (Hoagland

and Arnon, 1939) solution (1 mM KH_2PO_4 , 5 mM KNO_3 , 5 mM $\text{Ca}[\text{NO}_3]_2$, 2 mM MgSO_4 , and micronutrients; designated 1P) or with standard Hoagland solution modified with 5 mM KH_2PO_4 (designated 5P) added for both M and NM plants.

Physiological Measurements

Whole-plant gas exchange was measured four times during the experiment on the same six plants for each treatment during the period from 2 to 5 h after the beginning of the light period. A diurnal relationship of whole-plant gas exchange was determined 52 DAT on 10 plants for just the two treatments at high P. Net carbon assimilation (A) and dark respiration rates of shoots were determined with a portable photosynthesis system (LI-6200, Li-Cor Inc., Lincoln, NE). This gas-exchange system was operated as a closed system to measure A over a 20-s period. A 4-L cuvette was used to cover the entire shoot. When seedlings were too big to fit inside the cuvette, net assimilation of the upper and lower portions of the shoot were measured separately. Within the measurement cuvette, wind velocity was 3 m s^{-1} , PPFD was $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$, leaf temperature was $30 \pm 2^\circ\text{C}$, RH was $65 \pm 5\%$, and CO_2 concentration was $350 \pm 10 \mu\text{L L}^{-1}$. Assimilation rates of these growth chamber-grown seedlings saturated at 400 PPFD (data not shown). The 20-s measurement duration resulted in an air temperature increase of only 1°C inside the cuvette. The boundary layer conductance of plants in the growth chamber, estimated from wind velocity and leaf length (Nobel, 1991), was used to correct cuvette assimilation rates to growth-chamber conditions. Corrections reduced A 6 to 12%, depending on the relative magnitude of stomatal conductance. No boundary-layer adjustments were made for shoot dark respiration measured in the cuvette. Leaf length and width were measured to estimate leaf area ($r^2 = 0.99$) to express photosynthesis on a leaf-area basis. After gas-exchange measurements of the shoot, the conetainer was inserted into a PVC chamber 25 cm deep and with an inside diameter of 4.5 cm, which sealed around the base of the stem (Eissenstat et al., 1993). Air, scrubbed free of moisture and CO_2 by passage through silica gel and soda lime, entered the bottom of the PVC chamber at a flow rate of $5 \text{ cm}^3 \text{ s}^{-1}$. The majority of the air circulated through the space between the conetainer and PVC chamber, mixed with the air above the soil surface of the conetainer, and exited via a port at the top of the PVC chamber. This pattern of air flow was used to minimize the disturbance of the soil atmosphere. To determine the combined root and soil respiration rate, CO_2 concentration of outcoming air, which ranged from 50 to $100 \mu\text{L CO}_2 \text{ L}^{-1}$, was measured with an Anarad IRGA after 1-d equilibration in the growth room.

Plants were harvested 33, 65, and 92 DAT for 5P, and 33, 58, and 92 DAT for 1P. Gas-exchange plants were harvested at 92 DAT. Six plants were harvested for each treatment on each date, except at 65 DAT, when 10 M and 10 NM 5P plants were harvested. Fibrous roots, tap roots, stems, and leaves were dried at 70°C for 3 d. Length of fibrous roots was determined by the line-intersect technique (Newman, 1966; Tennant, 1975). Roots were cleared in KOH and stained with trypan blue for assessing VAM colonization. Twenty individual 1-cm root segments from each sample were

mounted on slides for examination under a microscope. The incidence of colonization was determined as the percentage of the 20 segments (scored individually) that contained vesicles or arbuscules as described previously (Graham et al., 1991).

Tissue P concentration was determined with an inductively coupled plasma atomic emission spectrometer after the tissue had been ashed (500°C, 4 h) and resuspended in 1 M HCl. Fatty acids of fibrous roots were determined with a Hewlett-Packard 5890 gas-liquid chromatograph after methanol extraction of the roots and fatty acid saponification and derivatization to methyl esters (Sasser, 1990). Concentrations of individual fatty acids and their total were expressed relative to the highest value using the integrated areas of the fatty acid profiles.

The construction cost of fibrous roots, or the amount of simple carbohydrate (e.g. Glc) required to build all of the organic molecules in a tissue sample (the inverse of "production value"; Penning de Vries et al., 1974) was estimated from heat of combustion, N content, and ash content:

$$C_w = [(0.06968 \times \Delta H_c - 0.065) (1 - \text{ash}) + kN/14 \times 180/24] (1/0.89) (6000/180) \quad (1)$$

where C_w is construction cost of tissue in mmol carbon (g dry weight)⁻¹, ΔH_c is the ash-free heat of combustion in kJ g⁻¹, ash is the ash fraction of the sample (g ash [g dry weight]⁻¹), k is the reduction state of the N substrate (+5, nitrate in this study), and (6000/180) converts units of g Glc (g dry weight)⁻¹ to mmol carbon (g dry weight)⁻¹ (Williams et al., 1987). Heat of combustion was measured using a Phillipson-type microbomb calorimeter. Organic N content was measured as Kjeldahl N by a semi-automated microkjeldahl procedure and an Alpkem RFA/2 continuous flow analyzer. Ash content was determined by ashing samples in a muffle furnace (500°C, 4 h).

Comparing Gas Exchange with Plant Growth

A main objective of this study was to partition below-ground respiration of M and NM plants into respiration associated with growth and that associated with maintenance, which was done at the time of the diurnal gas-exchange measurements, 52 DAT. One important assumption in this process was that our gas-exchange measurements, as indicated by specific carbon gain (net daily carbon gain [carbon content of the plant]⁻¹, mmol mmol⁻¹ d⁻¹) reasonably reflected our growth measurements as indicated by SGR (g⁻¹ g⁻¹ d⁻¹; same as relative growth rate). We assumed that soil respiration was from recent photosynthate and was not highly buffered by large soil pools of carbon (organic matter of soil was less than 1%). Previous experiments using ¹⁴C under these growth conditions indicated that soil carbon was not a large sink for current photosynthate, i.e. soil carbon pools after 72 h represented less than 2% of total carbon exported below ground (Eissenstat et al., 1993).

Daily carbon gain (mmol plant⁻¹ d⁻¹) at 52 DAT was determined by integrating the diurnal estimates of shoot CER and root plus soil respiration. Specific carbon gain was then determined from the whole-plant dry weight at 52 DAT

(determined from curve fitting the destructive harvests) and carbon content of the tissue (MS of similar tissue). To determine SGR at 52 DAT for each treatment, we fitted a polynomial equation of the natural log of whole-plant dry weight versus DAT and then determined its first derivative.

Plant growth rates (g d⁻¹) from 48 to 92 DAT were also compared with daily rates of carbon gain. Whole-plant and root growth rates were determined by fitting a polynomial equation of dry weight versus time and then calculating the first derivative. To determine daily rates of carbon gain from the single-point estimates of shoot A and root plus soil respiration measured in the morning at 48, 57, 71, and 85 DAT, we assumed that the diurnal patterns at 52 DAT were consistent across the growing period and P treatment. At 52 DAT, for 1 h beginning 4 h after the beginning of the light period, shoot A represented 7.55% of total daily carbon assimilation and root plus soil respiration represented 4.38% of $R_{(t)}$; shoot respiration during the dark period represented 11.42% of total daily carbon assimilation. M and NM plants differed by less than 1% in these percentages (i.e. $[M/NM - 1] \times 100$).

Partitioning Total Below-Ground Respiration

Using estimates of C_r , root tissue carbon content, root growth rates, and total below-ground respiration, we determined the daily increase in root carbon content and daily growth and "maintenance" respiration at 52 DAT.

Root carbon content (mmol C [g dry weight]⁻¹) was calculated from heat of combustion and N and ash contents following the method of Gnaiger and Bitterlich (1984). In this method, lipid, carbohydrate, and protein fractions of the tissue were estimated stoichiometrically from the N content and heat of combustion. Carbon content of the tissue was obtained by multiplying these fractions by the average carbon content for each class of compound. (Values of carbon content calculated in this manner were similar to carbon contents measured by MS on similar tissue.)

To estimate root biomass at 52 DAT, a polynomial equation was fitted to the data of total root biomass as a function of DAT for each treatment using the three destructive harvests. Root growth rates, ΔW_w (mg d⁻¹), at 52 DAT were estimated from the first derivatives of these equations. The change in root carbon content from 52 to 53 DAT, ΔW_c (μmol d⁻¹), was estimated from the product of root carbon content and ΔW_w , assuming that tap roots had the same carbon content as the fibrous roots.

$R_{G(t)}$ (μmol CO₂ d⁻¹) represents the respired carbon associated with biosynthesis of new tissue. Growth respiration was calculated as follows:

$$R_{G(t)} = C_t - \Delta W_c \quad (2)$$

The carbon required for daily construction of new tissue, C_t (μmol CO₂ d⁻¹), was calculated by multiplying the root growth rate (ΔW_w , mg dry weight d⁻¹) by the tissue construction cost (C_w , mmol CO₂ [g dry weight]⁻¹). Growth respiration was also expressed per unit weight of new root or the growth respiration coefficient, $R_{G(w)}$ (mmol CO₂ [g dry weight]⁻¹):

$$R_{G(w)} = R_{G(t)}/\Delta W_w \quad (3)$$

Table I. Mycorrhizal incidence (MI), whole-plant P content (Total P), leaf P concentration (Leaf P), leaf N concentration (Leaf N), fibrous root length (RL), leaf area (LA), and dry weight (DW) of M and NM plants grown in high- (5) and low- (1) P soil

DAT	P	VAM	MI	Total P	Leaf P	Leaf N	LA	RL	Fib Rt DW	Tap Rt DW	Stem DW	Leaf DW	Total DW
			%	mg plant ⁻¹	mg g ⁻¹	mg g ⁻¹	cm ²	m	g	g	g	g	g
33	1	M	37	0.24 a ^a	1.21 a		18 a	1.00 a	0.034 a	0.025 a	0.033 a	0.088 a	0.18 a
		NM	0	0.13 b	0.93 a		14 a	0.66 b	0.024 a	0.020 b	0.022 b	0.064 b	0.13 b
33	5	M	35	0.55 p	1.82 p		27 p	1.33 p	0.048 p	0.033 p	0.046 p	0.146 p	0.27 p
		NM	0	0.43 p	2.01 p		22 p	1.29 p	0.037 p	0.026 q	0.036 p	0.112 q	0.21 q
58	1	M	54	1.94 a	2.55 a	31.9 b	80 a	2.95 a	0.092 a	0.107 a	0.161 a	0.457 a	0.82 a
		NM	0	0.45 b	1.12 b	43.0 a	40 b	1.86 b	0.050 b	0.054 b	0.074 b	0.226 b	0.40 b
65	5	M	63	4.51 p	2.15 p	20.7 p	120 p	5.28 p	0.247 p	0.268 p	0.394 p	0.883 p	1.79 p
		NM	0	4.14 p	2.02 p	21.4 p	114 p	4.80 p	0.209 p	0.232 p	0.400 p	0.857 p	1.70 p
92	1	M	99 ^b	4.18 a	1.24 a	19.8 b	199 a	8.24 a	0.494 a	0.516 a	0.810 a	1.417 a	3.24 a
		NM	0	1.22 b	0.85 b	35.4 a	101 b	4.67 b	0.177 b	0.239 b	0.344 b	0.668 b	1.43 b
92	5	M	60	9.30 p	1.80 p	17.3 p	249 p	8.93 p	0.472 p	0.752 p	1.115 p	1.759 q	4.10 q
		NM	0	9.59 p	1.87 p	17.1 p	262 p	8.66 p	0.480 p	0.684 p	1.271 p	2.003 p	4.44 p

^a At each P treatment, means followed by the same letter (within a column) are not significantly different at the 0.05 probability level according to *t* test. ^b Significantly different from 5P 92 DAT at the 0.05 probability level.

Daily "maintenance" respiration, $R_{M(t)}$, represented all root/soil respiration not included in the estimate of growth respiration:

$$R_{M(t)} = R_{T(t)} - R_{G(t)} \quad (4)$$

where $R_{T(t)}$ or daily total respiration was calculated from gas exchange. Specific rates of total ($R_{T(w)}$) and maintenance ($R_{M(w)}$) respiration ($\text{mmol CO}_2 [\text{g dry weight}]^{-1} \text{d}^{-1}$) also were determined:

$$R_{T(w)} = R_{T(t)}/W \quad (5)$$

$$R_{M(w)} = R_{M(t)}/W \quad (6)$$

where W is the total root dry weight (g).

RESULTS

Both 1P and 5P plants were infected to a similar extent 60 d after inoculation with *G. intraradices*, although 1P had

significantly higher incidence of colonization than 5P at 92 DAT (Table I). The noninoculated control plants remained free of infection. Mycorrhizae increased whole-plant P content, leaf P concentration, root length, leaf area, and TDW at 1P but not at 5P. On the contrary, TDW of M plants 92 DAT was 8% lower than that of NM plants at 5P.

Diurnal gas-exchange measurements conducted 52 DAT at 5P indicated little difference between M and NM shoots in carbon assimilation and dark respiration (Fig. 1). However, combined root plus soil respiration was consistently higher for M than for NM plants (Fig. 2). Daily carbon budget 52 DAT was determined by integrating the CER data in Figures 1 and 2. M plants had only 2% higher shoot carbon gain but 37% higher root and soil respiration over NM plants, which resulted in an 8% higher daily carbon gain for NM plants (Table II). M plants were slightly larger than NM plants 52 DAT; consequently, their carbon gain per unit plant carbon or specific carbon gain ($\text{mmol carbon mmol}^{-1} \text{d}^{-1}$) was about 20% less than NM plants. The SGR of M plants was about

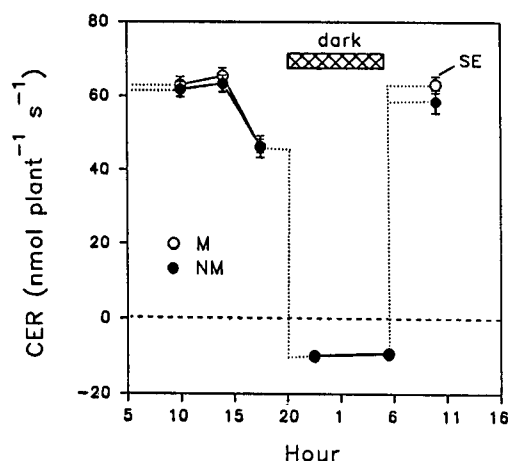


Figure 1. Diurnal CER of M and NM shoots at 5P determined 52 DAT ($n = 10$ per treatment). Dotted lines represent anticipated response based on previous studies in citrus. For three points, M and NM symbols overlapped.

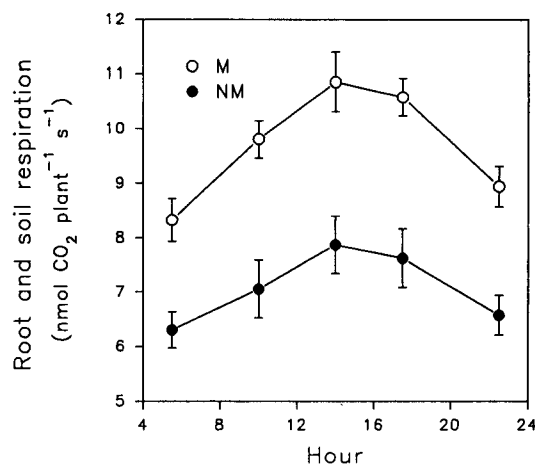


Figure 2. Diurnal root and soil respiration rates of M and NM plants at 5P determined 52 DAT ($n = 10$).

10% greater than that of NM plants. No estimates of variation were made of specific carbon gain because CER and plant carbon content at 52 DAT were estimated from separate plants and SGR was determined by solving the differential equations of the biomass data. Nonetheless, the fairly close agreement between SGR and specific carbon gain (e.g. 0.0516 versus 0.0561, Table II) provides support that the integrated estimates of the gas-exchange data reasonably accounted for plant growth.

During the entire growth period, mycorrhizae enhanced net carbon assimilation rate per unit leaf area at 1P but not at 5P (Fig. 3). From 50 to 70 DAT, rates declined in a similar manner in all four treatments.

On a whole-plant basis, carbon gain was increased substantially by the M fungus at low-P supply (Fig. 4). At 5P supply, NM plants initially were smaller than M plants, presumably because 5P addition was delayed 1 week following transplanting. After about 50 DAT, NM plants had higher daily carbon gain than M plants at 5P supply because mycorrhizae increased root plus soil respiration but not shoot carbon assimilation (Table II). There was 15% higher daily carbon gain of NM than M plants at 5P during late stages of growth (Fig. 4), which was partially attributable to slightly less leaf area in M plants (Table I). The enhancement in carbon gain at 1P and the reduction in carbon gain at 5P by the M fungus were reflected by the differences in growth rates between M and NM plants at 1P and 5P, respectively (Fig. 4, insets).

Two fatty acids, 16:0 and 16:1_{n-7}cis, represented the principal fatty acids in the fibrous roots of Volkamer lemon. Fibrous roots of M plants had more total fatty acids (Fig. 5) and proportionately more of the fatty acid 16:0 (Table III), than those of NM plants. The concentration of 16:1 increased more following infection than that of 16:0 or total fatty acids (Table III).

Estimated construction costs of fibrous roots were positively correlated with their total fatty acid concentration (Fig. 5); consequently, fibrous roots of M plants had significantly higher construction costs than those of NM plants at both P levels. Within a M or NM treatment, P concentration was negatively related to construction cost of the fibrous roots. M colonization affected fatty acid concentrations much more than construction costs. At 5P, M colonization of the fibrous roots increased lipid content 227% and construction costs

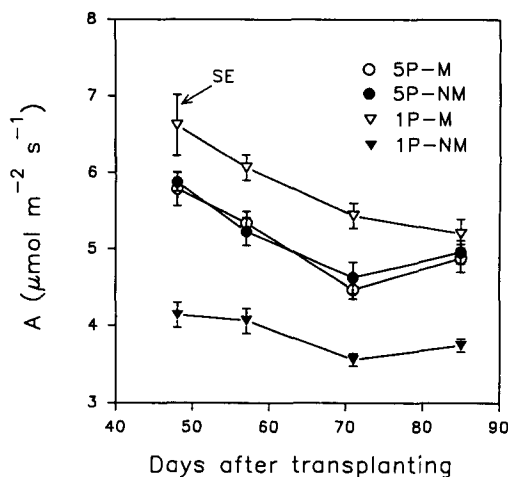


Figure 3. A of M and NM plants at 1P and 5P determined 9 to 11 AM during the growing period ($n = 6$).

7.9% (Fig. 5). At 1P, fibrous roots of M plants had a 307% higher lipid content and an 8.9% higher construction cost than NM plants.

Using the data from gas exchange, root growth, and root-construction costs, we estimated how much of the greater below-ground carbon expenditure in M than NM plants was due to differences in fibrous-root construction costs (Table IV). Total respiration, R_T , can be subdivided into that used for growth of new tissue, R_G , and that used for maintenance of existing tissue, R_M (Thornley and Johnson, 1990). In calculating respiration associated with growth of root tissue, we assumed all N in the roots came from nitrate (the only form of N in Hoagland solution) and was reduced in the roots, construction cost of the fibrous roots equaled that of the tap roots, and energy required to reduce nitrate for incorporation in shoot tissue was not reflected in respiration (based on studies that show abundant nitrate reductase activity in the shoots of citrus; Kato, 1986).

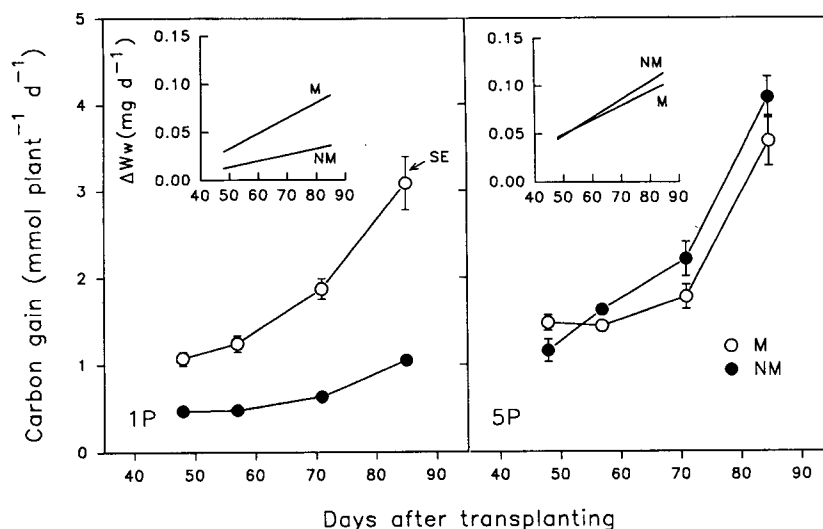
Comparing just 5P plants, higher construction costs, C_w , of the lipid-rich fibrous roots of M than NM plants (Fig. 5) only partly accounted for differences in daily growth respiration 52 DAT; M plants also had 10% higher root growth rates at this time (Table IV). Second, growth respiration of the root

Table II. Daily carbon budgets at 52 DAT of M and NM plants at 5P calculated by gas exchanges and by SGR (Figs. 2 and 3)

Parameter	M	NM	M/NM
<i>(mmol CO₂ plant⁻¹ d⁻¹) ± SE</i>			
Net assimilation (light period only)	3.00 ± 0.06	2.95 ± 0.08	1.02
Dark respiration of shoot	0.34 ± 0.02	0.34 ± 0.02	1.00
Carbon gain of shoot	2.66 ± 0.05	2.61 ± 0.07	1.02
Root and soil respiration	0.82 ± 0.03	0.60 ± 0.03	1.37
Carbon gain of whole plant	1.84 ± 0.04	2.00 ± 0.06	0.92
<i>(d⁻¹)</i>			
Specific carbon gain (gas exchange) ^a	0.0516	0.0644	0.80
SGR	0.0561	0.0624	0.90

^a Assumes ash-free biomass at 52 DAT had a carbon content of 48%.

Figure 4. Daily carbon gain and growth rate, ΔW_w (insets), of M and NM plants at 1P and 5P. Daily carbon gain was calculated based upon diurnal patterns of gas-exchange measurements of shoot assimilation rate and root plus soil respiration rate (Figs. 1 and 2). Estimates of daily growth rate were based on the first derivative of the polynomial equation describing whole-plant biomass as a function of time based on three harvests.



system 52 DAT represented only about 17 to 20% of the daily total respiration that evolved from the root/soil system, regardless of treatment. Proportional differences between M and NM plants in daily growth respiration were roughly equivalent to proportional differences in total respiration (Table IV). As a result, growth respiration per gram of new root (i.e. growth coefficient) of M plants was 18% higher than that of NM plants at 5P (Table IV). Daily "maintenance" respiration per gram dry weight of the total root system, which would include respiration associated with ion uptake, maintenance of root and intramatrical fungal tissue, and growth and maintenance of extramatrical hyphae and soil microbes, was 16% higher in M than NM plants.

At 1P soil supply, comparison of M and NM plants includes both direct M effects and indirect nutritional effects. M roots at 1P had a 17% higher growth respiration coefficient, $R_{G(w)}$, and a 33% higher specific rate of maintenance respiration, $R_{M(w)}$. The direct effect of P can be estimated by comparing

1P and 5P NM plants (Table IV). P deficiency increased $R_{G(w)}$ 16% and increased $R_{M(w)}$ 20%. Consequently, if NM plants at 1P were not more P-deficient than 1P M plants, then differences in $R_{G(w)}$ and $R_{M(w)}$ associated with M colonization would be even greater.

At 52 DAT, the difference in total daily root/soil respiration between M and NM plants at 5P was $220 \mu\text{mol CO}_2 \text{ d}^{-1}$ (Table IV). The relative effects of M and NM differences in root dry weight, root growth rate, growth respiration coefficient, and specific rate of maintenance respiration, which are illustrated in Figure 6, were determined by first estimating the percentage of total root/soil respiration attributable to $R_{G(t)}$ and $R_{M(t)}$ and then estimating the relative contributions of ΔW_w and $R_{G(w)}$ to $R_{G(t)}$ and W and $R_{M(w)}$ to $R_{M(t)}$. M effects on root biomass allocation, W and ΔW_w , accounted for 51% of the differences in root/soil respiration. Growing lipid-rich M roots (i.e. $R_{G(w)}$) accounted for only 10% of the differences in respiration measured at this time.

DISCUSSION

In this study, we applied additional P (5P) to both M and NM plants, which essentially eliminated nutritional differences between the two treatments and, thus, were able to assess the influences of VAM per se (rather than nutrition)

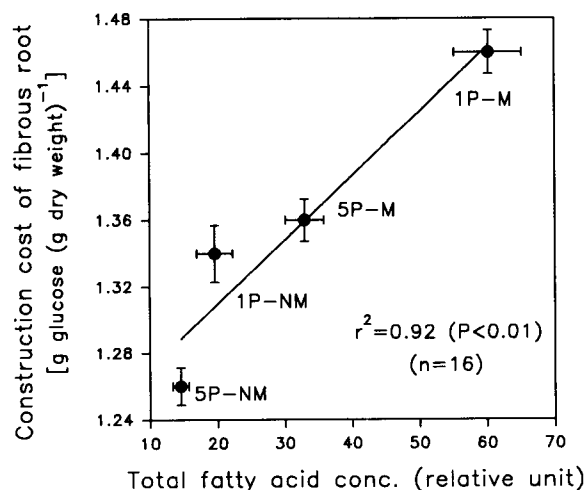


Figure 5. Relationship of construction cost of fibrous roots of M and NM plants at 1P and 5P to their relative total fatty acid content.

Table III. Concentration of major fatty acids (relative unit) of fibrous roots of M and NM plants grown in high- (5) and low- (1) P soil at final harvest ($n = 4$)

P	VAM	16:0	Percent ^a	16:1 _{w7cis}	Percent
1	M	15.3 a ^b	25	32.2 a	54
	NM	4.6 b	24	6.7 b	34
5	M	7.9 p	24	12.3 p	37
	NM	3.4 q	23	1.7 q	12

^a Percentage of total fatty acid concentration of fibrous roots. ^b At each P treatment, means followed by different letter (within a column) are significantly different at the 0.05 probability level according to an analysis of variance.

Table IV. Analysis of below-ground respiratory components at 52 DAT in M and NM plants grown in high- (5P) and low- (1P) P soil

	1P			5P		
	M	NM	M/NM	M	NM	M/NM
Root dry wt, W (mg)	134	78	1.72	273	230	1.19
Root growth (dry wt) ΔW_w (mg d ⁻¹)	9.62	3.90	2.46	14.8	13.4	1.10
Daily cost ($\mu\text{mol CO}_2 \text{ d}^{-1}$)						
Construction cost, C_t	468	174	2.69	671	563	1.19
Root growth (carbon), ΔW_c^a	356	135	2.63	518	446	1.16
Growth respiration, $R_{G(t)}^b$	114	40	2.85	153	117	1.31
Total respiration, $R_{T(t)}^c$	555	234	2.37	820	600	1.37
"Maintenance" respiration, $R_{M(t)}^d$	441	194	2.27	667	483	1.38
Cost per unit root dry wt [mmol CO ₂ (g new root) ⁻¹]						
Construction cost, C_w	48.7	44.7	1.09	45.3	42.0	1.08
Growth respiration coefficient, $R_{G(w)}$	11.8	10.1	1.17	10.3	8.7	1.18
Specific respiration rates [mmol CO ₂ (g whole-root system) ⁻¹ d ⁻¹]						
Total respiration, $R_{T(w)}$	4.14	3.00	1.38	3.00	2.61	1.15
"Maintenance" respiration, $R_{M(w)}$	3.33	2.51	1.33	2.44	2.10	1.16

^a Calculated based on ash content, N content, and heat of combustion.

^b $C_t - \Delta W_c$.

^c From Table II.

^d $R_{T(t)} - R_{G(t)}$.

on host-plant carbon economy and relate these to host-plant growth depression. M and NM plants at 1P were included to show general effects of mycorrhizae under low-P conditions.

A major shortcoming of examining the carbon cost of the mycorrhizae at high-P supply is the direct effect of P on M colonization. High tissue-P concentrations generally inhibit M colonization (Menge et al., 1978b), and this is a major reason that most previous investigators grew M plants at low P when estimating the carbon cost on M fungus. This greatly complicates assessment of overall carbon cost, however, because mycorrhizae under low-P conditions provide a P benefit (Eissenstat et al., 1993). This problem was avoided in this study by using Volkamer lemon, which, like many species highly dependent on M colonization when grown in low-P soil, maintains relatively high rates of M colonization even at high-P supply (Graham et al., 1991).

The overall cost of M colonization in 5P plants in terms of diminished SGR or diminished specific carbon gain ranged from 10 to 20% (Table II). We emphasize that these estimates are for 52 DAT; the relative costs of M colonization may change appreciably depending on the growth stage of the plant or on the stage of M colonization (Eissenstat et al., 1993). Although our study was different in approach and theory from previous experiments, our values were in general agreement with differences in below-ground costs estimated by ¹⁴C-labeling techniques using M plants grown in low-P soil (Pang and Paul, 1980; Kucey and Paul, 1982; Snellgrove et al., 1982; Koch and Johnson, 1984; Harris et al., 1985). Moreover, overall carbon cost of M colonization in 5P plants likely would be higher if colonization were as high as in the 1P condition, where fatty acid concentration was more than twice that of the 5P M fibrous roots (Fig. 5).

At 5P supply, SGR of M plants was 10% lower than that of NM plants 52 DAT (Table II). This growth depression was in general agreement with the specific carbon gain estimated by gas exchange. This implies that the bulk of the substrates for root/soil respiration were likely from fairly recent photosynthate, since it is unlikely that the growth and gas-ex-

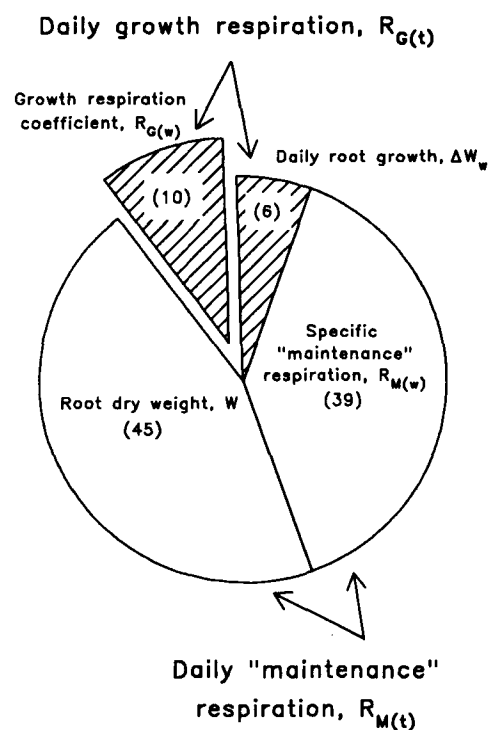


Figure 6. Differences in total root/soil respiration between 5P M and NM plants at 52 DAT ($220 \mu\text{mol CO}_2 \text{ d}^{-1}$) attributable to ΔW_w , $R_{G(w)}$, W , and $R_{M(w)}$. The percentage of total additional M respiration that was attributable to the construction of lipid-rich M roots is indicated by $R_{G(w)}$. Data are from Table IV.

change data would have been in such close agreement over the duration of the experiment if this were not true (Table II, Fig. 4). M plants had lower daily carbon gain because they expended approximately 37% more carbon to their roots. Several factors were responsible for the greater below-ground carbon expenditure in M than in NM plants.

One factor accounting for about 10% of the difference in root/soil respiration was that the lipid-rich M roots had greater construction costs than NM fibrous roots (Fig. 5), which led to higher coefficients of growth respiration, $R_{C(w)}$ (Fig. 6). The M fungus used in this study, *G. intraradices*, extensively forms lipid-rich vesicles, which in citrus occurs even at high-P concentrations. Consequently, at a given P supply, total fatty acid concentration of M fibrous roots was more than twice that of NM roots (Fig. 5). Fatty acid, 16:1, was mainly responsible for the higher total fatty acid of M fibrous roots (Table III), which agrees with Pacovsky and Fuller's (1988) suggestion that 16:1 was probably the principal storage fatty acid in fungal vesicles.

Another important factor contributing to the greater below-ground carbon expenditure in M than NM plants at 5P was the greater root biomass allocation in M plants. Root growth rate, ΔW_w , at 52 DAT was 10% higher in M than in NM plants (Table IV), whereas whole-plant growth rates were nearly identical (Fig. 4, insets). Root biomass, W , which had accumulated by 52 DAT, was 19% greater in M than in NM plants. Together, W and ΔW_w accounted for about 51% of the difference in root/soil respiration between M and NM plants (Fig. 6). Several other studies in citrus have also found greater root biomass allocation in M than in NM plants under conditions of equivalent P status (Menge et al., 1978a; Graham and Syvertsen, 1985; Eissenstat et al., 1993). Other species, such as the legumes *Trifolium subterraneum* (Thomson et al., 1986) and *Glycine max* (Fredeen and Terry, 1988), however, exhibit reduced root biomass allocation following colonization by M fungi. Whether or not a particular species of M fungus affects root biomass allocation of a particular plant species (independent of nutrition) will profoundly influence the overall carbon cost of the symbiosis.

Construction costs represent the sum of carbon used in growth respiration and carbon retained in the new root material. By using daily construction costs and subtracting out the carbon retained in new root growth (ΔW_c), we estimated that daily growth respiration accounted for about 16% of the difference in total root/soil respiration between M and NM plants at 5P (Fig. 6). Approximately one-third of the difference in growth respiration was attributed to differences in root growth rate (ΔW_w); the other two-thirds was attributed to building more expensive root tissue. The remaining 84% of the daily total root/soil respiration was considered "maintenance" respiration. The higher "maintenance" respiration, $R_{M(t)}$, in M than in NM plants at 52 DAT, was due to both a larger root system, W , and to apparently greater specific rates of "maintenance" respiration, $R_{M(w)}$ (Fig. 6). Because $R_{M(t)}$ represents residual respiration not associated with growth, it includes respiration associated with ion uptake, microbial respiration, growth respiration of extramatrical hyphae, as well as maintenance respiration of the roots and M fungus.

Maintenance and growth respiration have been determined in most studies by linear regression of root respiration rate as

a function of root SGR (e.g. Szaniawski and Kielkiewicz, 1982); growth respiration is the slope of this regression and maintenance respiration is the y intercept (respiration when there is no growth). Consequently, using regression techniques, growth respiration includes root respiration associated with ion uptake and possibly some aspects of shoot growth (e.g. nitrate reduction) (Johnson, 1983; Poorter et al., 1991). Root growth respiration estimated by these techniques ranges from 18 to 37 mmol g⁻¹ (refs. in Szaniawski and Kielkiewicz, 1982), which is much higher than our estimates of 8.7 to 11.8 mmol g⁻¹. Studies more similar to ours, which used a theoretical construction-cost approach, found that roots of desert succulents (Nobel et al., 1992) and roots of 24 noncrop species (Poorter et al., 1991; assumed respiratory quotient of 1.2) had growth respiration coefficients that ranged from 6.6 to 9.6 mmol CO₂ g⁻¹. All these plants were NM and well supplied with nutrients; thus, they were quite comparable to the 8.7 mmol g⁻¹ growth respiration of NM citrus at 5P (Table IV). M colonization or P stress, however, can increase the growth coefficient of respiration, $R_{C(w)}$, 16 to 36% (Table IV).

Respiration associated with ion uptake was included in the "maintenance component" in this study. Estimated costs of the ion uptake component have ranged from 0.63 to 1.5 mol CO₂ (mol N)⁻¹ (Veen, 1981; Johnson, 1983; Poorter et al., 1991). Taking the highest values of 1.5 mol CO₂ (mol N)⁻¹ and assuming that the rate of N uptake represents about 90% of total anion uptake (Veen, 1981), we estimated ion-uptake respiration from the change in whole-plant N content at 52 DAT. Using these assumptions, we estimated daily respiration associated with ion uptake at 52 DAT to be 111, 56, 109, and 111 μ mol CO₂ d⁻¹ for M-1P, NM-1P, M-5P, and NM-5P plants, respectively. Consequently, respiration associated with ion uptake accounted for about 14 to 24% of total root/soil respiration, $R_{T(t)}$ (Table IV). M plants at 1P and 5P and NM plants at 5P had similar rates of respiration associated with ion uptake because of nearly identical rates of N uptake. Thus, separating daily total respiration into three components, growth, ion uptake, and maintenance, did not affect relative differences in specific rates of maintenance respiration between M and NM plants at 5P.

Some studies have indicated that higher carbon expenditures below ground following M colonization can be offset by higher net assimilation rates (Pang and Paul, 1980; Harris et al., 1985). We observed higher assimilation rates of M plants at low P, which might have been attributed to greater sink strength of the 1P M roots. A more likely explanation, however, was that N was less limiting to A in the slower-growing 1P M plants, which at the second harvest had about 52% higher N concentration than the M and NM 5P plants (Table I).

In conclusion, at high-P supply, mycorrhizae depressed plant growth of Volkamer lemon. Growth depression in the M seedlings was attributed to greater carbon expenditure to M colonized roots than NM roots (per root system). M colonization greatly increased the fatty acid content of the fibrous roots, and a higher fatty acid content was directly linked to a higher root construction cost. Therefore, we assessed whether growth of lipid-rich M roots could account for the 37% higher respiration of the whole-root system of M than

NM plants. Only 10% of the greater root/soil respiration could be attributed to growing M roots rich in lipids. Higher "maintenance" respiration, associated with larger root systems, higher specific rates of "maintenance" respiration, and, to a lesser extent, higher root growth rates, accounted for the majority of the difference in below-ground carbon use by M than by NM plants.

ACKNOWLEDGMENTS

We wish to thank J.P. Syvertsen, M.L. Smith, Jr., D.L. Drouillard, and N. Timmer for helpful discussions and technical assistance, and J. Amthor and R. Koide for critical reviews.

Received June 26, 1992; accepted November 19, 1992.

Copyright Clearance Center: 0032-0889/93/101/1063/09.

LITERATURE CITED

- Buwalda JG, Goh KM (1982) Host-fungus competition for carbon as a cause of growth depressions in vesicular-arbuscular mycorrhizal ryegrass. *Soil Biol Biochem* 14: 103–106
- Cooper KM (1975) Growth responses to the formation of endotrophic mycorrhizas in *Solanum*, *Leptospermum* and New Zealand ferns. In FE Sanders, B Mosse, PB Tinker, eds, *Endomycorrhizas*. Academic Press, London, pp 391–407
- Cox G, Sanders FE, Tinker PB, Wild JA (1975) Ultrastructural evidence relating to host-endophyte transfer in vesicular-arbuscular mycorrhiza. In FE Sanders, B Mosse, PB Tinker, eds, *Endomycorrhizas*. Academic Press, London, pp 297–312
- Crush JR (1976) Endomycorrhizas and legume growth in some soils of the Mackenzie Basin, Canterbury, New Zealand. *NZ J Agric Res* 19: 473–476
- Eissenstat DM, Graham JH, Syvertsen JP, Drouillard DL (1993) Carbon economy of sour orange in relation to mycorrhizal colonization and phosphorus status. *Ann Bot* 71: 1–10
- Fredeen AL, Terry N (1988) Influence of vesicular-arbuscular mycorrhizal infection and soil phosphorus level on growth and carbon metabolism of soybean. *Can J Bot* 66: 2311–2316
- Gnaiger E, Bitterlich G (1984) Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. *Oecologia (Berlin)* 62: 289–298
- Graham JH, Eissenstat DM, Drouillard DL (1991) On the relationship between a plant's mycorrhizal dependency and rate of vesicular-arbuscular mycorrhizal colonization. *Funct Ecol* 5: 773–779
- Graham JH, Syvertsen JP (1985) Host determinants of mycorrhizal dependency of citrus rootstock seedlings. *New Phytol* 101: 667–676
- Hall IR, Johnstone PD, Dolby R (1984) Interactions between endomycorrhizas and soil nitrogen and phosphorus on the growth of ryegrass. *New Phytol* 97: 447–453
- Harris D, Pacovsky RS, Paul EA (1985) Carbon economy of soybean-*Rhizobium-Glomus* associations. *New Phytol* 101: 427–440
- Hepper CM (1977) A colorimetric method for estimating vesicular-arbuscular mycorrhizal infection in roots. *Soil Biol Biochem* 9: 15–18
- Hoagland DR, Arnon DI (1939) The Water-Culture Method for Growing Plants without Soil. University of California, Agricultural Experiment Station Circular 347, Berkeley, CA
- Johnson IR (1983) Nitrate uptake and respiration in roots and shoots: a model. *Physiol Plant* 58: 145–147
- Kato T (1986) Nitrogen metabolism and utilization in citrus. *Hortic Rev* 8: 181–216
- Koch KE, Johnson CR (1984) Photosynthate partitioning in split-root citrus seedlings with mycorrhizal and non-mycorrhizal root systems. *Plant Physiol* 75: 26–30
- Kucey RMN, Paul EA (1982) Carbon flow, photosynthesis, and N₂ fixation in mycorrhizal and nodulated faba bean (*Vicia faba* L.). *Soil Biol Biochem* 14: 407–412
- Mehlich A (1953) Determination of P, Ca, Mg, K, Na, NH₄ by the North Carolina Soil Testing Laboratory. North Carolina State University, Raleigh
- Menge JA, Labanauskas CK, Johnson ELV, Platt RG (1978a) Partial substitution of mycorrhizal fungi for phosphorus fertilization in the greenhouse culture of citrus. *J Soil Sci Soc Am* 42: 926–930
- Menge JA, Steirle D, Bagyaraj DJ, Johnson ELV, Leonard RT (1978b) Phosphorus concentration in plant responsible for inhibition of mycorrhizal infection. *New Phytol* 80: 575–578
- Nagy S, Nordby HE (1980) Composition of lipids in roots of six citrus cultivars infected with the vesicular-arbuscular mycorrhizal fungus, *Glomus mosseae*. *New Phytol* 85: 377–384
- Newman EI (1966) A method of estimating the total length of root in a sample. *J Appl Ecol* 3: 139–145
- Nobel PS (1991) *Physicochemical and Environmental Plant Physiology*. Academic Press, San Diego
- Nobel PS, Alm DM, Cavelier J (1992) Growth respiration, maintenance respiration and structural-carbon costs for roots of three desert succulents. *Funct Ecol* 6: 79–85
- Pacovsky RS, Fuller G (1988) Mineral and lipid composition of *Glycine-Glomus-Bradyrhizobium* symbioses. *Physiol Plant* 72: 733–746
- Pang PC, Paul EA (1980) Effects of vesicular-arbuscular mycorrhiza on ¹⁴C and ¹⁵N distribution in nodulated fababeans. *Can J Soil Sci* 60: 241–250
- Penning de Vries FWT, Brunsting AHM, van Laar HH (1974) Products, requirements and efficiency of biosynthesis: a quantitative approach. *J Theor Biol* 45: 339–377
- Poorter H, van der Werf A, Atkin OK, Lambers H (1991) Respiratory energy requirements of roots vary with the potential growth rate of a plant species. *Physiol Plant* 83: 469–475
- Sasser M (1990) Identification of bacteria through fatty acid analysis. In Z Klement, K Rudolf, D Sands, eds, *Methods in Phytobacteriology*. Akademiai Kiado, Budapest, pp 199–204
- Snellgrove RC, Splittstoesser WE, Stribley DP, Tinker PB (1982) The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. *New Phytol* 92: 75–87
- Son CL, Smith SE (1988) Mycorrhizal growth responses: interactions between photon irradiance and phosphorus nutrition. *New Phytol* 108: 305–314
- Szaniawski RK, Kielkiewicz M (1982) Maintenance and growth respiration in shoots and roots of sunflower plants grown at different root temperatures. *Physiol Plant* 54: 500–504
- Tennant D (1975) A test of a modified line intersect method of estimating root length. *J Ecol* 63: 995–1001
- Thomson BD, Robson AD, Abbott LK (1986) Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. *New Phytol* 103: 751–765
- Thornley JHM, Johnson IR (1990) *Plant and Crop Modelling: A Mathematical Approach to Plant and Crop Physiology*. Oxford University Press, New York
- Veen BW (1981) Relation between root respiration and root activity. *Plant Soil* 63: 73–76
- Williams K, Percival F, Merino J, Mooney HA (1987) Estimation of tissue construction cost from heat of combustion and organic nitrogen content. *Plant Cell Environ* 10: 725–734