

# Carbon Cost of the Fungal Symbiont Relative to Net Leaf P Accumulation in a Split-Root VA Mycorrhizal Symbiosis<sup>1</sup>

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## ABSTRACT

Translocation of <sup>14</sup>C-photosynthates to mycorrhizal (+ +), half mycorrhizal (0 +), and nonmycorrhizal (00) split-root systems was compared to P accumulation in leaves of the host plant. Carrizo citrange seedlings (*Poncirus trifoliata* [L.] Raf. × *Citrus sinensis* [L.] Osbeck) were inoculated with the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices* Schenck and Smith. Plants were exposed to <sup>14</sup>CO<sub>2</sub> for 10 minutes and ambient air for 2 hours. Three to 4% of recently labeled photosynthate was allocated to metabolism of the mycorrhiza in each inoculated root half independent of shoot P concentration, growth response, and whether one or both root halves were colonized. Nonmycorrhizal roots respired more of the label translocated to them than did mycorrhizal roots. Label recovered in the potting medium due to exudation or transport into extraradical hyphae was 5 to 6 times greater for (+ +) versus (00) plants. In low nutrient media, roots of (0 +) and (+ +) plants transported more P to leaves per root weight than roots of (00) plants. However, when C translocated to roots utilized for respiration, exudation, etc., as well as growth is considered, (00) plant roots were at least as efficient at P uptake (benefit) per C utilized (cost) as (0 +) and (+ +) plants. Root systems of (+ +) plants did not supply more P to leaves than (0 +) plants in higher nutrient media, yet they still allocated twice the <sup>14</sup>C-photosynthate to the mycorrhiza as did (0 +) root systems. This indicates there is an optimal level of mycorrhizal colonization above which the plant receives no enhanced P uptake yet continues to partition photosynthates to metabolism of the mycorrhiza.

ranged from 4 to 17% of fixed C (5, 12, 13, 17, 22). Mycorrhizal roots of several plant species have been shown to have greater rates of respiration (5, 17, 22) and fewer starch grains (11, 16) than nonmycorrhizal roots.

Differences in physiology between mycorrhizal and nonmycorrhizal roots due solely to the presence or absence of the fungus have been difficult to measure. Shoots of VAM plants are usually more vigorous and contain higher levels of phosphorus than those of nonmycorrhizal plants. Any difference in carbohydrate content, respiration, or other physiological parameters between mycorrhizal and nonmycorrhizal roots on different plants could therefore be ascribed to differing shoot physiology. A remedy to this problem is the use of plants grown with root systems divided between two pots (12). One can describe differences in root physiology without compounding effects of different shoots by inoculating one half-root system with a mycorrhizal fungus. In such a system, mycorrhizal root halves of sour orange and Carrizo citrange seedlings accumulated 3 to 5% more of total <sup>14</sup>C-photosynthate than the paired nonmycorrhizal half-root system (12).

The following experiments were conducted to detail C partitioning in below ground fractions of split-root Carrizo citrange and to quantify C cost relative to net P acquisition in leaves. The first portion of this objective was accomplished through the development of a method of wet acid digestion and oxidation of soil organic matter. Below ground respiration and partitioning within roots also were measured. Thus, all components of below ground partitioning of carbon have been quantified in the present study.

## MATERIALS AND METHODS

**Plant Material.** Split-root Carrizo citrange seedlings (*Poncirus trifoliata* [L.] Raf. × *Citrus sinensis* [L.] Osbeck) were grown as described earlier (12). Stem cuttings of 2-week-old seedlings were treated with 4 mg ml<sup>-1</sup> indole butyric acid. Adventitious roots were fully developed in 2 to 3 weeks, after which plants were potted in paired 500 ml square plastic pots. Inorganic potting media (calcined clay and washed coarse sand in Exp I and washed medium-grain sand in Exp II) were used to facilitate recovery of labeled organic matter. Plants 25 cm or less in height have shown no evidence of P taken up by one half-root system being localized in leaves on that side of the shoot (12).

Three treatment combinations were applied. Five g of soil containing spores, hyphae, and infected roots from a pot culture of the VAM fungus *Glomus intraradices* (Schenck and Smith) on Bahiagrass (*Paspalum notatum* Flugge) were added to one (0 +) or both (+ +) sides of split-root seedlings. Others were left uninoculated (00). Inoculum filtrate was added to the uninoculated pots to equilibrate nonmycorrhizal soil microflora.

In Exp I, seedlings were potted on May 3 and grown in a greenhouse in Gainesville, FL, under natural photoperiods through

Vesicular-arbuscular mycorrhizal (VAM)<sup>4</sup> fungi have been shown to increase growth and P uptake in a wide variety of forest and fruit trees (14, 19, 20) as well as agronomic crops (9, 10, 16). These fungi are assumed to be obligate symbionts because they have not been cultured axenically. As obligate symbionts they require organic compounds from their hosts, and indeed, the transfer of <sup>14</sup>C-labeled compounds from host plant to VAM fungus has been noted (6, 13). Estimates of the amount of C required by the VAM fungus and fungus-induced metabolism such as increased metabolic activity of host tissue (2, 11), have

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<sup>4</sup> Abbreviations: VAM, vesicular-arbuscular mycorrhizal; Exp, experiment; Pi, inorganic phosphate.

September 10, 1984. Plants reached the desired height (approximately 20 cm) for labeling studies on July 5. For the first 10 weeks, plants were fertilized three times weekly. Fifty ml of half-strength Hoagland's macronutrient solution minus P (7) were applied to each pot twice weekly. Macronutrients plus P and micronutrients (50 ml complete half-strength Hoagland's solution) were applied once each week. Half-strength complete solution was applied weekly thereafter.

In Exp II, 50 ml of half-strength complete solution were applied to each pot per week. In addition, 50 ml of 1.5 mM  $\text{KH}_2\text{PO}_4$  were added twice each week to each pot of the (00) treatment to enhance P nutrition of the nonmycorrhizal plants. This experiment was conducted from October 2 through December 13, 1984. Plants reached an appropriate size for labeling studies on November 6.

The medium-grain sand used in Exp II had greater water holding capacity than the washed coarse sand and calcined clay used in Exp I. Therefore, the potting medium of Exp II was considered to retain more of the water-soluble nutrients applied to the roots than was the medium of Exp I.

**Labeling Studies.** Plants selected for uniformity were utilized for labeling studies. Before each exposure to  $^{14}\text{CO}_2$ , holes were cut in the sides of pots to allow air flow. The paired pots were placed in a divided plexiglass chamber that sealed the root systems in individual 1.027 L compartments. Air was circulated within each by an electric fan. Artificial illumination (Lucalox LU400, high pressure sodium lamps<sup>5</sup>) began between 8:00 and 9:00 h EST with a photosynthetic photon flux density at mid plant level of 600 to 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Air, bubbled through 250 ml 0.2 N NaOH to reduce the  $\text{CO}_2$  concentration to approximately 30  $\mu\text{l CO}_2 \text{L}^{-1}$ , was pumped through the root chambers at 0.8 to 1.0  $\text{L min}^{-1}$ . After 1 h, the shoot was enclosed in a third compartment (2.310 L) with its own air flow system and fan.  $\text{CO}_2$ -free air was pumped through the shoot compartment for 5 min prior to  $^{14}\text{CO}_2$  exposure.

The shoot chamber and air flow system were filled with 327  $\mu\text{l CO}_2 \text{L}^{-1}$  containing 34  $\mu\text{Ci}$  (Exp I) or 15  $\mu\text{Ci}$  (Exp II)  $^{14}\text{CO}_2$  and a balance of dry air. Uptake of  $\text{CO}_2$  during the 10 min exposure period was monitored with an infrared gas analyzer. A 2-h chase period followed in which ambient air was flushed through the shoot chamber and low- $\text{CO}_2$  air was passed through the root chambers. Efflux from each root chamber that contained respired  $^{14}\text{CO}_2$  was bubbled through separate flasks with 200 ml 0.2 N NaOH to trap  $\text{CO}_2$ . Effectiveness of this procedure was monitored by infrared gas analysis. Soil temperatures ranged from 32.2 to 36.0°C for the beginning and end, respectively, of the chase period. Shoot temperatures ranged from 33.5 to 37.7°C for the beginning and end, respectively, of the chase period. These temperatures are similar to those the plants experienced in the greenhouse (to 38 and 35°C for root and shoot, respectively) and typical of citrus nursery conditions.

**Sampling and Analysis.** Plants were dissected into leaves, stems, and root halves after each experiment. A sample of each root system was scored for percentage root length infected by the VAM fungus (18). Plant parts were sliced, weighed, frozen in liquid  $\text{N}_2$ , boiled in 80% (v/v) ethanol, and ground in a Polytron (Brinkmann Instruments). Insoluble matter was filtered and rinsed thoroughly with 80% ethanol. Replicate samples of pooled ethanol extracts were added to scintillant, and radioactivity was determined by liquid scintillation spectrometry. Label incorporated into structural and storage compounds was measured by the same procedures with insoluble residues.

Carbon dioxide in the NaOH traps was precipitated as  $\text{BaCO}_3$  with saturated  $\text{BaCl}_2$  and allowed to settle overnight. The  $\text{BaCO}_3$  was collected in tubes, washed with deionized water, centrifuged,

and dried at 75 to 100°C for 5 d.

Label partitioned to the potting media by root exudation or transport of organic compounds to extraradical VAM hyphae was quantified by a method we developed utilizing oxidation of organic C to  $^{14}\text{CO}_2$ , solubilization of  $^{14}\text{CO}_2$  in NaOH, and precipitation as  $\text{Ba}^{14}\text{CO}_3$ . Immediately after harvest, two (Exp I) or three (Exp II) 5-cm<sup>3</sup> samples of potting medium from each pot were each digested in 20 ml of concentrated  $\text{H}_2\text{SO}_4$  in a 125 ml sidearm flask to oxidize organic matter, notably labeled exudates and hyphae, to elemental C. After a 4-h digestion period, each of these flasks was connected to a 250-ml sidearm flask containing 200 ml 0.2 N NaOH, a sintered glass aeration tube, and a magnetic stir bar. The entire system was evacuated, and 4 to 5 ml of 20%  $\text{H}_2\text{O}_2$  were added to the digestion flask from a burette to oxidize C to  $\text{CO}_2$ . Total  $\text{CO}_2$  was trapped in the stirred NaOH, precipitated and processed as described for respiratory  $\text{CO}_2$ . A standard addition experiment showed complete recovery of C from cellulose.

Barium carbonate from potting media digestions and respiration was ground to a powder and suspended in scintillation cocktail using fine silica (4% [w/v] Cab-O-Sil, Eastman Kodak) (1). Replicate samples of 0.05 g  $\text{BaCO}_3$  were analyzed by liquid scintillation spectrometry to determine  $^{14}\text{CO}_2$  respired from the root chambers or released upon oxidation of organic matter in the potting media.

Dry weights of plant parts were determined from dry weight: fresh weight ratios of plants grown under the conditions of these experiments. Phosphorus content of leaves was determined by combustion in a muffle furnace and quantified by the ascorbic acid-molybdenum blue method (15). Starch was quantified by amyloglucosidase digestion of ethanol-insoluble residues of leaves. Data were analyzed by analysis of variance ( $p = 0.05$ ). Significant treatment effects were characterized further by Tukey's method of multiple comparisons.

## RESULTS

**Growth of Plants.** Half-root systems inoculated with *G. intraradices* averaged  $74 \pm 6$  (mean  $\pm$  SEM) and  $77 \pm 11\%$  root length infected for (0+) plants in Exps I and II, respectively, and  $82 \pm 2$  and  $71 \pm 10\%$  for (+ +) plants in Exps I and II, respectively.

Inoculation of both root halves with *G. intraradices* increased the growth of Carrizo citrange over that of (00) plants (Table I). The addition of extra  $\text{KH}_2\text{PO}_4$  to (00) plants in Exp II enabled them to attain heights, leaf areas, dry weights, and partition dry matter in proportions that were not statistically different from those of (0+) plants.

Leaves of plants in Exp II had higher P concentrations and contents than those of Exp I due to the addition of  $\text{KH}_2\text{PO}_4$  to (00) plants and, possibly, increased nutrient retention in the potting media of Exp II (see "Materials and Methods" and Table II). Leaves of (00) plants in Exp I had a mean P concentration 45% lower than leaves of mycorrhizal plants, while those of Exp II had P concentrations equivalent to (0+) plants.

**Recovery and Distribution of  $^{14}\text{C}$ -Photosynthates.** There were no significant differences in photosynthetic rates between treatments in Exp I (Table II). In Exp II, however, (00) plants had higher rates of net photosynthesis than mycorrhizal plants.

Mycorrhizal fungi affected the distribution of  $^{14}\text{C}$ -photosynthates during the 2-h translocation period in both experiments. A significantly greater percentage of label was translocated out from leaves of (0+) and (+ +) plants than from those of (00) plants (Table II). This enhanced C export occurred independently of leaf P and starch concentrations or the season in which the experiments were conducted.

The majority of  $^{14}\text{C}$ -photosynthates translocated to the roots in (0+) plants went to the mycorrhizal half (Fig. 1, a and b).

<sup>5</sup> Use of product names does not imply an endorsement by the Florida Institute of Food and Agricultural Sciences.

Table I. *Physical Characteristics of Carrizo Citrange*

Treatment	Dry Weight			Root:Shoot	Leaf Area	Height
	Leaves	Stem	Roots <sup>a</sup>			
	<i>g</i>			<i>ratio</i>	<i>cm<sup>2</sup></i>	<i>cm</i>
A. Exp I. All plants received 1.55 mg P each week <sup>b</sup>						
noninoculated	0.152 ±0.021	0.201 ±0.024	0.376 ±0.052	1.08 ±0.09	21.61 ±3.26	11.0 ±0.6
Single inoculation	0.451 ±0.028	0.512 ±0.042	0.632 ±0.066	0.65 ±0.04	71.82 ±6.09	19.9 ±1.1
Double inoculation	0.612 ±0.069	0.622 ±0.053	1.004 ±0.140	0.82 ±0.10	82.06 ±4.65	23.9 ±1.2
B. Exp II. Noninoculated plants received 9.0 mg P and others received 1.55 mg P each week <sup>c</sup>						
Noninoculated	0.323 ±0.041	0.226 ±0.053	0.495 ±0.110	0.88 ±0.08	49.29 ±7.33	15.2 ±2.0
Single inoculation	0.365 ±0.020	0.301 ±0.001	0.661 ±0.030	1.00 ±0.07	60.61 ±3.97	17.3 ±0.4
Double inoculation	0.433 ±0.038	0.401 ±0.025	0.800 ±0.075	0.96 ±0.05	79.11 ±7.55	21.1 ±0.08

<sup>a</sup> Sum of both root halves. <sup>b</sup> Numbers represent the means of seven observations ± SEM. <sup>c</sup> Numbers represent the mean of four observations ± SEM.

Table II. *Photosynthesis and Phosphorus, Starch, and <sup>14</sup>C-Photosynthates in Carrizo Citrange Leaves*

Treatment	Phosphorus in Leaves <sup>a</sup>		Starch <sup>a</sup>	Photosynthesis	dpm Remaining in Leaves
	%	total (mg)			
			% dry wt	$\mu\text{mol C cm}^{-2} \text{ h}^{-1}$	% of total recovered <sup>14</sup> C
A. Exp I. 1.55 mg P week <sup>-1</sup> to all plants <sup>b</sup>					
Noninoculated (00)	0.060 ± 0.020	0.118 ± 0.051	7.7 ± 1.1	1.53 ± 0.29	85.7 ± 1.8
Single inoculation (0+)	0.105 ± 0.005	0.478 ± 0.034	5.2 ± 0.7	1.83 ± 0.12	75.4 ± 1.2
Double inoculation (++)	0.110 ± 0.020	0.623 ± 0.056	9.0 ± 1.5	1.61 ± 0.10	72.3 ± 1.4
B. Exp II. 1.55 mg P week <sup>-1</sup> to (0+) and (++), 9.0 mg P week <sup>-1</sup> to (00) <sup>c</sup>					
Noninoculated (00)	0.305 ± 0.029	1.017 ± 0.211	2.1 ± 0.1	1.66 ± 0.13	80.6 ± 2.8
Single inoculation (0+)	0.293 ± 0.029	1.050 ± 0.059	2.7 ± 0.6	1.35 ± 0.07	73.5 ± 2.1
Double inoculation (++)	0.248 ± 0.014	1.057 ± 0.059	2.3 ± 0.8	1.24 ± 0.04	68.2 ± 4.1

<sup>a</sup> P and starch data are expressed as percentage of air-dried, ethanol-insoluble matter. <sup>b</sup> Mean of two representative samples for P, others seven, ± SEM, potting medium was a mixture of calcined clay and coarse sand. <sup>c</sup> Mean of four observations ± SEM, potting medium was medium grain sand.

Mycorrhizal roots also had greater dpm g<sup>-1</sup> dry weight in the soluble fraction than did nonmycorrhizal half-root systems (data not shown). Half-root systems of (0+) plants received similar percentages of total labeled photosynthates as halves of (00) and (++) plants with the same colonization status. Longer (22 h) pulse-chase experiments showed that photosynthate distributions were proportional to those at 3 h (data not shown).

Nonmycorrhizal half-root systems respired a greater percentage of the <sup>14</sup>C translocated to them than did mycorrhizal half-root systems (Fig. 1, c and d). More <sup>14</sup>C-photosynthate was translocated to VAM root halves, however, so total respiratory losses of recently fixed <sup>14</sup>CO<sub>2</sub> were nearly equal for both halves.

Soil containing extraradical hyphae from VAM root systems (++) had approximately twice the percentage of label translocated to the root half as did soil from nonmycorrhizal roots (00) (Fig. 1, e and f). This was not evident when mycorrhizal and nonmycorrhizal root systems were supported by the same shoot (0+) in Exp II. The method used to recover radioactivity from the soil does not distinguish between label resulting from root exudation and transport into extraradical mycelium. Values for the latter cannot be obtained by subtracting label in nonmycorrhizal soil from that in mycorrhizal soil because VAM associations reportedly influence root exudation (4).

When grown in the low nutrient-holding medium of Exp I, mycorrhizae increased the P content of Carrizo citrange leaves (Fig. 2a). Leaf P contents g<sup>-1</sup> total root system for (0+) and (++) plants were equivalent and higher than those of (00) plants in Exp I, supporting the widely held view that VAM roots are more efficient than nonmycorrhizal roots at P accumulation in low nutrient soils (Fig. 2c). The effectiveness of (00) roots in Exp II reflects the increased availability of P due to addition of supplemental KH<sub>2</sub>PO<sub>4</sub> to these plants only (Fig. 2, b and d).

The P contributed by individual VAM half-root systems appeared greater in (0+) than (++) plants (Fig. 2, e and f). Data of Exp I (Fig. 2, a and c) suggested that only 14% of the P in (0+) plants would have been taken up by the nonmycorrhizal half-root system if it functioned only as well g<sup>-1</sup> dry wt as its counterpart in (00) plants. Other experiments have shown that P was not distributed evenly across root halves of (0+) citrus seedlings. At the level of nutrient addition used in these experiments (1.55 mg P week<sup>-1</sup>), P concentrations in nonmycorrhizal root halves can be less than half those of mycorrhizal halves (0.16 versus 0.33% dry weight; data not shown). Photosynthate transport to (0) halves of (0+) plants also was similar to that of (00) plants. Therefore, we expect minimal alteration of the P uptake and associated physiology of nonmycorrhizal root halves by the

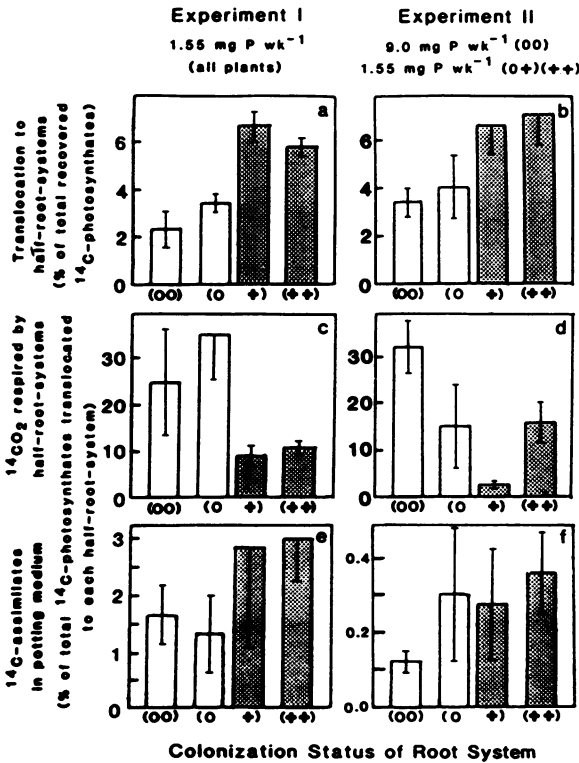


FIG. 1. Photosynthate allocation and components of sink strength in mycorrhizal (+) and nonmycorrhizal (0) halves of split-root systems in (00), (0+), and (++) citrange seedlings after pulse-chase labeling for 10 min + 2 h. Total photosynthate translocated to each half-root system (a and b), and percentage of this released as  $^{14}\text{CO}_2$  (c and d) and recovered in the growth medium (e and f). The growth medium included exudates and extraradical VAM hyphae. Potting media used were: calcined clay and coarse sand in Exp I and a more nutrient-retentive medium grain sand in Exp II. Data for (00) and (++) plants represent means of both half-root systems and hence, statistical comparisons should be restricted to (00) vs (++) and (0) versus (+) root systems on (0+) plants. Exp I, mean of seven  $\pm$  SEM; Exp II, mean of four  $\pm$  SEM.

presence of mycorrhizal halves in (0+) citrus seedlings, and we assume that the majority of P in leaves had been taken up by the mycorrhizal half. Calculations based upon this reasoning would represent upper limits to the accumulation of P in leaves due to uptake by the mycorrhizal half of (0+) plants (Fig. 2, e and f). A similar comparison can be made in Exp II, since nonmycorrhizal half-root systems of (0+) plants were not given supplemental P. These calculations show that net P acquisition in leaves of (0+) plants per mycorrhizal half would lie between 0.75 and 1.25 mg P g $^{-1}$  dry root weight (Fig. 2, c and e, respectively) in Exp I, and 1.5 to 3.0 mg P g $^{-1}$  dry root weight in Exp II (Fig. 2, d and f, respectively).

Expressing the effectiveness of P uptake g $^{-1}$  dry weight of root systems does not account for carbon utilized by roots for respiration, exudation, transport to extraradical VAM hyphae, and other processes which result in a loss of carbon which had been partitioned to roots. When these factors were quantified, (00) plants of Exp I appeared to be at least as effective at net P acquisition in leaves relative to C utilized below ground as (0+) and (++) plants since the means of the three groups lie along the same line (mg P = 0.024 [ $\mu\text{g}$  C] - 0.075;  $r^2 = 0.72$ ; Fig. 3). Data of Exp II showed that C translocation below ground increased as the amount of VAM colonized root increased from (0+) to (++), yet total leaf P content remained the same (Fig. 3). The ratio of P accumulation in leaves to C translocated below

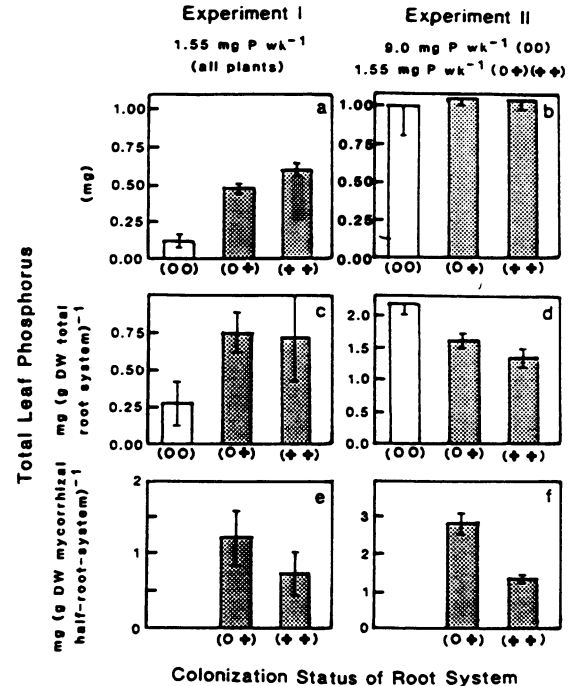


FIG. 2. Phosphorus content of Carrizo citrange leaves and relative efficiency of (00), (0+), and (++) root systems in supplying P to leaves: total mg P in leaves (a and b), mg P (g total root dry wt) $^{-1}$  (c and d), and mg P (g mycorrhizal half-root system) $^{-1}$  (e and f). In e and f, P uptake was assumed to occur through mycorrhizal halves of (0+) root systems because only 14% of total leaf P could be accounted for by nonmycorrhizal roots if P uptake per g root dry weight were equivalent to that observed in Exp I. Potting media used were: calcined clay and coarse sand in Exp I and a more nutrient-retentive medium grain sand in Exp II. Statistics are as in Table II.

ground increased as apparent nutrient availability in potting media increased (Exp I versus Exp II, Fig. 3).

## DISCUSSION

The observation that leaves of plants in Exp II had higher P and lower starch concentrations than those of Exp I was consistent with hypotheses describing P effects on photosynthate partitioning (8). Total P content of leaves may reflect cytoplasmic Pi concentrations under P deficiency conditions (3) such as occurred in Exp I. This could have limited Pi available for effective activity of the triose-P/Pi translocator on the chloroplast membrane and led to buildup of starch in leaves in Exp I. Still, the inverse relationship between leaf P and starch was evident only between experiments where there was a 3-fold difference in leaf P concentrations.

Data presented here show root halves of (0+) plants have metabolic demands similar to their (00) or (++) counterparts. Two methods of calculating the percentage of recently fixed carbon used by mycorrhizal fungi and related host metabolism agree. If (++) and (00) plants are compared, 5.6 to 7.4% more  $^{14}\text{C}$ -assimilates are allocated to (++) roots. If half-root systems of (0+) plants are compared, 3.0 to 3.9% more current photosynthate is estimated to be utilized by the mycorrhizal root half. Twice this, or 6.0 to 7.8% would be estimated for a whole root system. This agreement indicates that differences in shoot vigor and/or leaf P may have had a relatively minor influence on estimates of C utilization by VAM fungi in earlier experiments comparing mycorrhizal and nonmycorrhizal plants (5, 13, 17, 22). Values obtained for Exp I versus Exp II show marked constancy

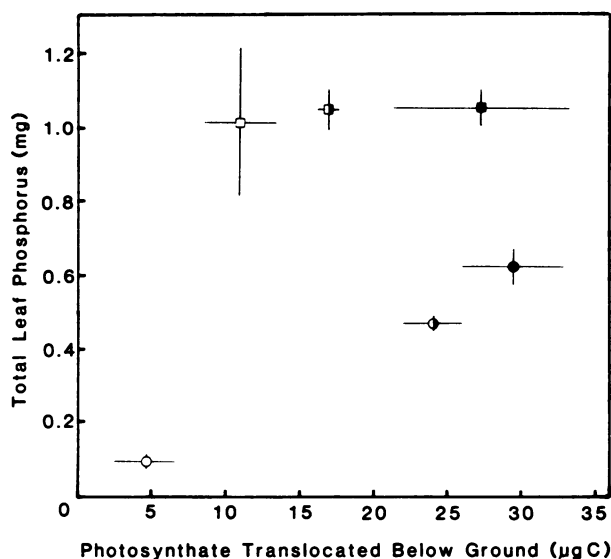


FIG. 3. Phosphorous content of Carrizo citrange leaves versus photosynthate translocated below ground (root exudation + transport to extraradical VAM hyphae + below ground respiration + photosynthate remaining in root tissue). Partitioning of recently assimilated C was calculated from specific activity of  $^{14}\text{CO}_2$ , total  $^{14}\text{C}$  assimilated, and localization of  $^{14}\text{C}$ -assimilates after a 10 min +2 h pulse chase experiment. Statistics, media, and nutrient additions as in Table II. Note that (00) plants in Exp II received supplemental P. Symbols are as follows: ○, ●, ■ = Exp I (00), (0+), (++) respectively; and □, ■, ■ = Exp II (00), (0+), (++), respectively. Least squares regression analysis for Exp I:  $\text{mg P} = 0.024 \mu\text{g C} - 0.075$ ,  $r^2 = 0.72$ ; Exp II:  $\text{mg P} = 0.002 \mu\text{g C} + 1.001$ ,  $r^2 = 0.76$ .

in allocation of C to mycorrhizae over different shoot P concentrations and plant growth responses. Carbon partitioning may thus be a non-nutritional effect of VAM fungi upon host plant physiology.

Nonmycorrhizal roots in the present study respired a greater percentage of current photosynthate translocated to them than did mycorrhizal roots (Fig. 1, c and d). Harris *et al.* (5) studied the respiration of nonmycorrhizal, N and P fertilized *Glycine max* and VAM, nodulated plants. Respiration from VAM fungi, roots, and soil accounted for 44.4 and 32.0% of radiolabeled carbon transported below ground for 6- and 9-week-old plants, respectively (calculated from Tables III and IV in Ref. 5). Nonmycorrhizal roots and soil respired 36.4 and 38.6% of the labeled photosynthate translocated below ground at 6 and 9 weeks, respectively. Therefore, the mycorrhiza respired relatively more of the available photosynthates than nonmycorrhizal roots at 6 weeks but less than nonmycorrhizal roots at 9 weeks when the VAM fungal infection was more developed. The Carrizo citrange plants used here were 8 to 14 weeks old when exposed to  $^{14}\text{CO}_2$ . Lower respiratory activity may be characteristic of mature VAM symbioses (21). However, in two 48-h pulse-chase experiments by Snellgrove *et al.* (22), roots and soil from 11- to 13-week-old mycorrhizal *Allium porrum* respired a mean of 32.25% of the  $^{14}\text{C}$  recovered from below ground fractions while nonmycorrhizal roots and soil respired only 25.7% (calculated from Table II in Ref. 22). Still, some studies show increased respiration in mycorrhizal roots (17) and others do not (21). Longer time periods than the 2 h used here, or 24 h used by Silsbury *et al.* (21) may be necessary to show significant differences in total respiration. This would be particularly true if an extended period were required for transport of  $^{14}\text{C}$ -assimilates within fungal hyphae and/or if these materials were in some way less available for immediate fungal respiration.

A greater proportion of  $^{14}\text{C}$ -photosynthate translocated to root systems was recovered from the rhizosphere and surrounding potting medium in Exp I than in Exp II. This may have been due to greater exudation by roots of plants low in P in Exp I (4) and/or more extraradical mycorrhizal hyphae in Exp I than Exp II. The short-term experiments reported here show no significant association between the increased sink strength of mycorrhizal half-root systems and either respiration of mycorrhizal roots or transport of assimilates to extraradical fungal hyphae (as measured by acid digestion and oxidation of rhizosphere soil). The 3 to 4% of total  $^{14}\text{C}$ -photosynthates allocated to mycorrhizal metabolism in VAM half-root-systems may be present as lipids in intraradical fungal structures. This is particularly important during the growth of spores which are formed inside roots by *G. intraradices*.

We can now estimate the relative carbon cost per leaf P from mycorrhizal and nonmycorrhizal root systems. In the apparently low nutrient-retentive soil of Exp I, mycorrhizal root systems transported significantly more P to leaves than nonmycorrhizal root systems (Fig. 2a) and are more efficient at P uptake on a per root weight basis (Fig. 2c). However, nonmycorrhizal root systems provided as much or more P to the leaves over the life of the plant per current photosynthate translocated below ground, especially when supplemental P was given (Fig. 3) (5).

In low nutrient soil, leaves of (+ +) plants received an additional amount of P from the second mycorrhizal half-root-system when compared to (0+) plants. In the higher nutrient-retentive soil of Exp II, P content in leaves of (0+) and (+ +) plants was equal (Table II). Colonized root halves on (0+) and (+ +) plants in Exp II did not differ significantly in the percentages of their root lengths infected by *G. intraradices*. In addition, (0+) plants translocated less photosynthates to their root systems than (+ +) plants (10.4% vs 13.8%). This difference in total photosynthate partitioning can be ascribed to the demand of mycorrhizal (host + fungus) metabolism in the second VAM root half of (+ +) plants. Since total P in leaves of (0+) and (+ +) plants was equal (Table II), we conclude there is an optimal level of mycorrhizal colonization above which the plant receives no enhanced P uptake (benefit) yet continues to support mycorrhizal metabolism (cost).

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