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Phosphorus nutrition on mycorrhizal colonization, photosynthesis, growth and nutrient composition of *Citrus aurantium**

C. R. JOHNSON
Department of Ornamental Horticulture, University of Florida, Gainesville, Fl 32611, USA

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Summary Roots of sour orange (*Citrus aurantium* L.) seedlings were inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus, *Glomus intraradices* Schenck and Smith or provided an inoculum filtrate (non-VAM plants) and fertilized with weekly applications of 0.0, 6.25, 25.0 or 100.0 mg P solution per 1200 ml container. Colonization was lower after the first 9 weeks with increased P, but P caused little inhibition of colonization after 26 weeks. Leaf tissue levels of P were higher with VAM colonization and high P fertilization. Photosynthetic rates were correlated with P content in leaf tissue of control plants, but no correlation was observed for VAM infected seedlings. Results suggested that factors in addition to improved P nutrition influence photosynthetic rates of VAM plants.

Introduction

Growth benefits by vesicular-arbuscular mycorrhizal (VAM) fungi have been described on numerous host plants[5,17] and are often attributed to improved P nutrition[5,17,20]. High P levels in leaf tissue of VAM host plants has been suggested as a primary reason for high photosynthetic rates[11,14] and subsequent growth response. However, additional factors may affect photosynthetic function of VAM plants. For example, Bevege *et al.*[3] observed VAM fungi may function as a metabolic sink causing basipetal mobilization of photosynthates to roots, thus providing stimulus for greater photosynthetic activity[4]. In addition, enhanced levels of cytokinins[1] and gibberellins[2] were found in association with VAM infection. Increase of such hormones, especially cytokinins, could elevate photosynthetic rates by stomatal opening[10], influencing ion transport[25], and regulating chlorophyll levels[19,23].

This study examines the role of P nutrition and VAM colonization on photosynthesis and growth of sour orange seedlings.

Materials and methods

Sour orange seedlings (*Citrus aurantium* L.) were transplanted at the four leaf stage into 1200 ml plastic containers filled with 1 Canadian peat : 1 fired montmorillinite clay (v/v) medium

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having 7 mg kg^{-1} available P (bicarbonate-solution analysis). Medium was amended with dolomitic limestone, superphosphate (8.7% P) and STEM (Soluble Trace Element Mix manufactured by W. R. Grace and Co., Cambridge MA, USA) at 4.20, 0.25 and 0.25 kg M^{-3}, respectively. Half the plants were inoculated with the mycorrhizal fungus *Glomus intraradices* Schenck and Smith using a 10 g mixture of chlamydospores (150 spores g^{-1} soil), hyphae and colonized roots. An inoculum filtrate was applied to roots of non-VAM plants. All plants were fertilized weekly with 40 mg N per 1200 ml container from 25-0-25 (17.8% NH_4^+, 7.2% NO_3^-; 21% K) solution. One of four P treatments (0.0, 6.25, 25.0, or 100.0 mg P per 1200 ml container as H_3PO_4) were included in solutions. The pH was adjusted to 5.5 using 2 *N* KOH or 4 *N* H_2SO_4 and K levels maintained at constant level in all treatments.

Plants were grown in a glasshouse with maximum daylight irradiance of 1000 μmol m^{-2} s^{-1} at 400–700 nm and temperatures maintained at 28°C day and 24°C night. A randomized block design was used with treatments replicated 12 times and single plants as experimental units. Half the plants were harvested for analyses 9 weeks after inoculating. The experiment was terminated after 26 weeks. Root colonization by *G. intraradices* was determined 9 and 26 weeks after inoculation using root clearing and procedures described by Phillips and Hayman[18] and a gridline intersect method[8].

Carbon dioxide fluxes were measured on the most recently expanded leaves using a 65 cm^3 plexiglass cuvette in an open flow system through an Anarad Model AR-600 R (Santa Barbara, CA, USA) differential infrared gas analyzer. Photosynthetic photon flux density at leaf surface was 1000 μmol m^{-2} s^{-1} provided by a high pressure sodium vapor lamp (Sylvania LU 400). Heat radiation from the light source was dissipated by circulating a copper sulfate-water solution through a water bath positioned between light source and cuvette. Temperatures in the cuvette were maintained at 28.0 ± 1.2°C.

Shoot and root dry weights and concentrations of P in tissues of the most recently developed leaves were determined after 9 and 26 weeks. The vanadate-molybdate-yellow procedure was used for P analysis.

Results

Percent VAM colonization was inhibited at the highest P levels, 9 weeks after inoculation, but there were no differences 26 weeks after inoculation (Table 1). Roots of plants subjected to higher P fertilization had more sporulation in cortical cells of roots (Fig. 1).

Shoot dry weight of VAM colonized sour orange plants 9 weeks after inoculation was substantially greater than non-VAM plants and was increased at higher P levels for both plant groups (Fig. 2). However, VAM fungi benefited growth at only the 0.0 and 6.25 mg P levels after 26 weeks. Root dry weights showed treatment responses similar to shoots, except non-VAM plants had greater root development at 26 weeks with high P fertilization.

Leaf tissue levels of P at 9 and 26 weeks were increased with higher P rates and tissue levels of P were higher with VAM colonization except for the 100.0 mg P treatment (Table 2).

Photosynthesis (mg CO_2 m^{-2} s^{-1}) after 9 weeks increased from lowest to highest P levels for non-VAM plants, while the highest photosynthetic rates for VAM plants were observed for those receiving 25.0 mg P (Table 3). Photosynthesis was greater for VAM compared to

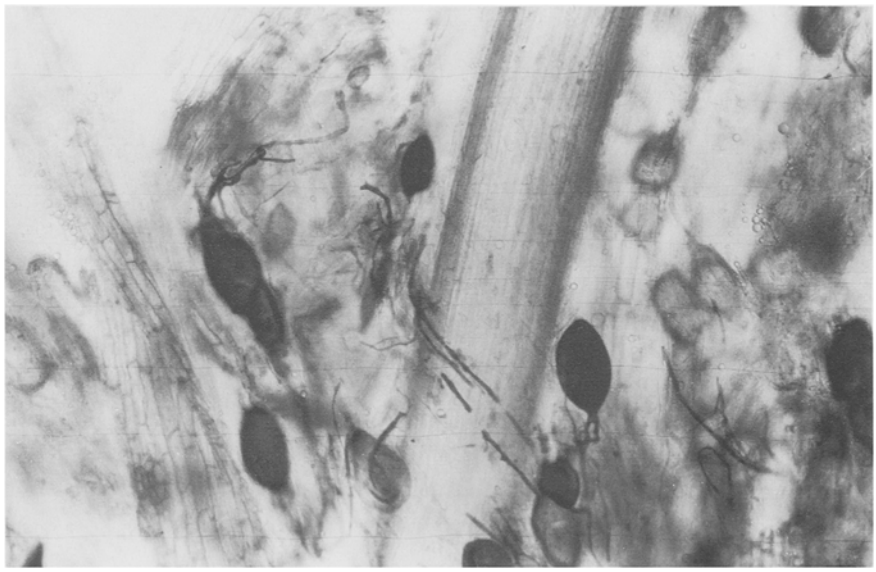

Fig. 1. Root containing numerous chlamydospores of *Glomus intraradices*, ×800. Root segments displaying increased formation of chlamydospores with higher P fertilization.

Table 1. Influence of phosphorus fertilization on percentage root colonization and sporulation of sour orange inoculated with *Glomus intraradices* determined 9 and/or 26 weeks after inoculation

P levels (mg)*	Percentage colonization		Percentage root segments sporulating
	9 weeks	26 weeks	26 weeks
0.00	41 a+	58 a	12 c
6.25	38 a	54 a	10 c
25.00	35 ab	54 a	18 b
100.00	28 b	50 a	30 a

+ Values in columns followed by the same letter are not significantly different at the 0.05 level according to Duncan's new multiple range test.
* P levels represent mg weekly/1200 ml container.

non-VAM plants except for the 100.0 mg P treatment. Photosynthetic rates of non-VAM plants after 26 weeks were lowest with 0.0 mg P, and increased with higher P application levels. Lowest photosynthetic rates of VAM colonized plants were at the 0.0 mg P treatment, but photosynthesis did not increase with higher P levels as in non-VAM plants treatment. Photosynthesis at 26 weeks was greater in VAM plants at all P levels compared to non-VAM plants.

Photosynthetic rates were linearly correlated with P leaf tissue levels for control plants at both 9 and 26 weeks (Fig. 3). However,

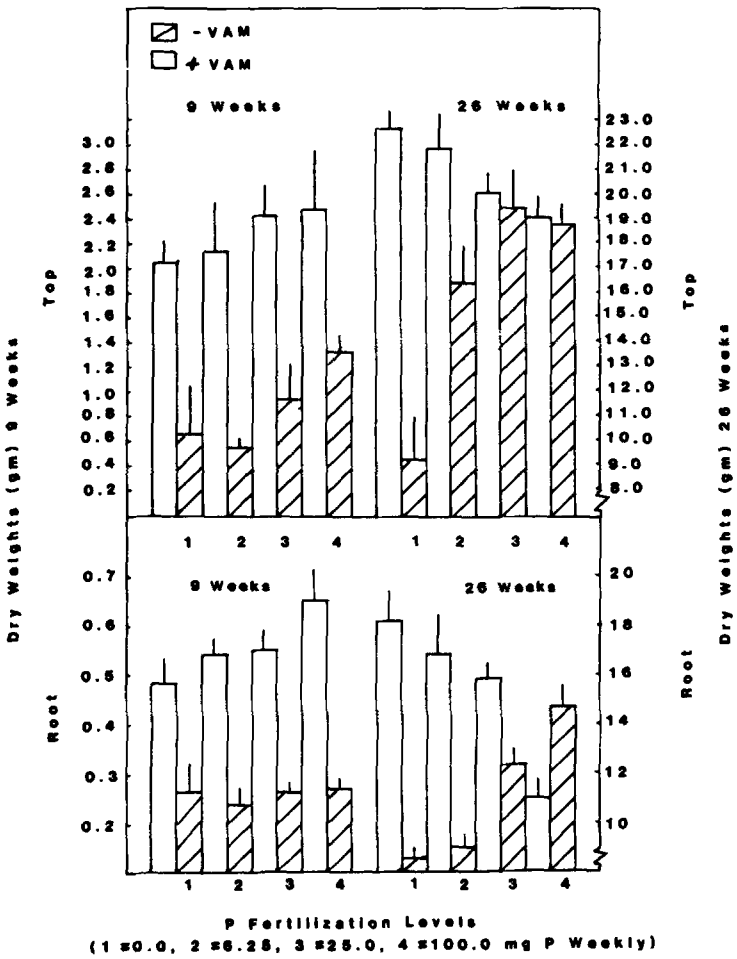

Fig. 2. Comparison of top and root dry weights of vesicular-arbuscular mycorrhizal (+ VAM) *vs* control (– VAM) sour orange plants grown under four fertilization levels determined 9 and 26 weeks after inoculation. Vertical bars represent standard deviation of means.

there was no correlation between levels of P in the leaf tissue and photosynthesis for VAM plants.

Discussion

Inhibition of colonization with high P nutrition at 9 weeks may have been due to a reduction in root membrane-mediated exudation of host metabolites[7]. Such limitation of exudates during initial stages of mycorrhizal establishment could restrict supply of necessary substrates for extensive colonization. Observations of P inhibition on VAM development have been made on citrus[15] and numerous other

Table 2. Influence of phosphorus fertilization and VA mycorrhizae (VAM) on P levels in sour orange leaves determined 9 and 26 weeks after inoculation

P levels (mg)+	Weeks	P (% dry wt) – VAM	+ VAM
0.00	9	** 0.07 c	0.20 c
6.25	9	** 0.09 c	0.25 b
25.00	9	* 0.21 b	0.25 b
100.00	9	NS 0.26 a	0.28 a
0.00	26	** 0.09 c	0.13 c
6.25	26	** 0.12 bc	0.17 b
25.00	26	* 0.16 b	0.19 b
100.00	26	NS 0.22 a	0.24 a

+ P levels represent mg weekly/1200 ml container.

*, **, NS Significant difference at 5%, 1% level, and not significantly different respectively between non-VAM and VAM colonized plants based on a paired t-test.

+ Values in columns followed by the same letter are not significantly different at the 5% level according to Duncan's new multiple range test.

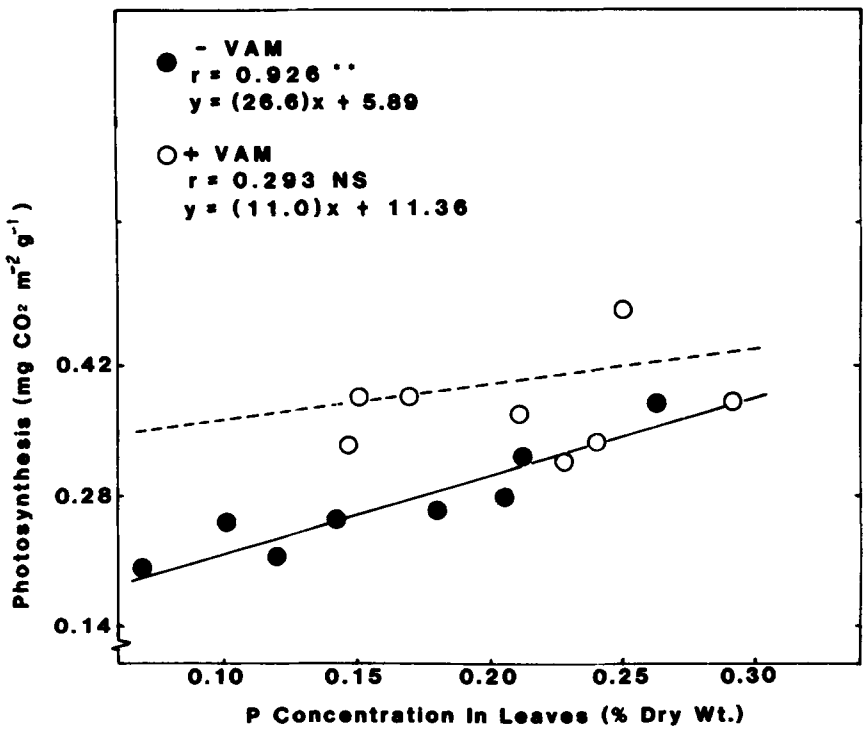

Fig. 3. Correlation between P concentration in leaf tissue and leaf photosynthetic rate of sour orange plants with or without VA mycorrhizal inoculation.

Table 3. Influence of phosphorus fertilization and VA mycorrhizae (VAM) on rate of photosynthesis (mg CO_2 m^{-2} s^{-1}) of sour orange plants determined 9 and 26 weeks after inoculation

	9 weeks		26 weeks	
P levels (mg)[+]	– VAM	+ VAM	– VAM	+ VAM
0.00	** 0.21 d	0.36 b	** 0.19 c	0.34 b
6.25	** 0.29 c	0.38 b	** 0.24 b	0.40 a
25.00	* 0.33 b	0.46 a	** 0.26 ab	0.40 a
100.00	NS 0.39 a	0.40 b	* 0.30 a	0.37 a

[+] P levels represent mg weekly/1200 ml container.
*, **, NS Significant difference at 5%, 1% level and not significantly different respectively between non-VAM and VAM colonized plants based on a paired t-test.
[+] Values in columns followed by the same letter are not significantly different at the 5% level according to Duncan's new multiple range test.

crops[5,17]. Infection levels after 26 weeks, however, were high regardless of P application rates. *Glomus intraradices* is an indigenous VAM fungus found in high P Florida soils and typically sporulates in cortical cells of host root tissues[21]. Increased sporulation in cortical cells with high P nutrition represents a mechanism to reproduce and persist at high levels of infection irregardless of edaphic P concentrations. Increased sporulation with high P fertilization has also been observed on other P-tolerant mycorrhizal fungi[24].

Growth response, expressed as top and root dry weights, of VAM sour orange seedlings was substantial after 9 weeks and may be associated with improved P uptake and higher levels of photosynthesis. Menge *et al.*[15] also noted that sour orange displayed a strong growth response (mycorrhizal dependency) even with elevated levels of fertilization. The absence of beneficial growth response of VAM seedlings at high P fertilization after 26 weeks, could be due to the extensive sporulation and subsequent increased demand for photosynthates by the endophyte.

Higher P levels in leaf tissues as a result of colonization or heavy P applications could be expected to increase rate of photosynthesis[9]. Photosynthetic rates may change through indirect effects of P on ATP/ADP ratios or a direct action on RuBP carboxylase activity[9]. Improvement of photosynthesis by VAM plants is suggested to be the result of higher P tissue levels after infection[11,14]. Data from this research indicate photosynthetic rates are improved with higher leaf tissue levels of P in non-VAM plants, but no correlation with P tissue levels were observed for VAM colonized plants. Improvement of photosynthesis by VAM colonization may be invoked by a number of physiological changes in addition to strictly P related events. Increased respiratory losses[22] from the VAM symbiont could increase sink

demand for photosynthates and provide stimulus for higher photosynthetic rates[4,8]. Koch and Johnson[12] estimated 6–10% of total photosynthates were directly allocated to VAM related events which would include metabolic processes of the symbiont. The effects of growth regulators as mediators of mycorrhizal sink strength must be also considered because their balance is believed to change with symbiotic or parasitic associations[13]. Auxin, gibberellin and cytokinin substances have been found to increase in VAM infected plants[1,2,16] and could therefore effect photosynthate distribution. In addition, increases in some of these hormones could directly elevate photosynthetic rates by stomatal opening[10], enhanced ion transport[25] and regulation leaf chlorophyll levels[19,23].

This research substantiates that increased P leaf tissue levels, achieved through VAM symbiosis or high P fertilization improves growth of *Citrus aurantium* plants. However, P leaf tissue levels are apparently not the limiting factor in elevated photosynthetic rates of VAM colonized *vs* non-VAM plants. Improved photosynthetic rates as a result of VAM symbiosis may be partially explained on basis of increased sink strength for photosynthates and/or biosynthesis of hormones that increase photosynthetic rates.

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