Plant growth, leaf photosynthesis, and nutrient-use efficiency of citrus rootstocks decrease with phosphite supply

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Abstract

Some formulations of phosphite (Phi) have been recommended as a source of P nutrition for several crops including citrus even though there are known negative effects of Phi on plant growth. Changes in plant growth and metabolism after Phi application should be reflected in altered nutrient-use efficiency and leaf photosynthesis. We carried out a greenhouse study using seedlings of two contrasting citrus (*Citrus spp.*) rootstocks, Carrizo citrange (CC) and Smooth Flat Seville (SFS), growing in either aerated hydroponic culture or sterilized native sandy soil. Plants were subjected to four P treatments: No P (control, P_0); 0.5 mM P_i (PO_4-P); 0.25 mM P_i + 0.25 mM Phi (P_i + Phi), or 0.5 mM Phi (Phi). Photosynthetic characteristics, concentrations of total P (P_t) and soluble PO_4-P or PO_3-P in leaves and roots, and plant growth were evaluated after 80–83 d P treatments. Overall, the P_i plants had the highest P_t (total P) and total plant dry weight while the P_0 plants had the lowest P_t but highest total root length and root-to-shoot ratio. Leaf chlorophyll (SPAD readings) and net assimilation of CO_2 (A_{CO2}) of the P_0 and Phi plants were similarly lower than those of P_i and P_i + Phi plants. Growth responses of the P_i + Phi treatment were intermediate between the P_i and Phi treatments. Although Phi increased P_t and soluble-PO_4-P concentration in leaves and roots above the P_0 treatment, this did not translate into increased plant growth. In fact, the Phi treatment had some phytotoxic symptoms, impaired P- and N-utilization efficiency for biomass production as well as lower nutrient-use efficiency in the photosynthetic process. Thus, these two rootstocks could not use Phi as a nutritional source of P.

Key words: phosphate / hydroponic / anions / nitrogen / root growth

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1 Introduction

Some formulations of phosphite (Phi) products can be effective fungicides to combat *Phytophthora* root rot (*Guest* and *Grant*, 1991; *Barret* et al., 2003), and their use can increase growth or yield of several agricultural commodities including pineapple, avocado, green pepper (*Pegg* et al., 1985; *Rohrbach* and *Schenck*, 1985; *Förster* et al., 1998), and citrus (*Orbovic* et al., 2008). Additional positive effects of Phi have been described in orange trees where foliar sprays of Phi during winter were used to increase flowering, juice soluble solids, and fruit yield (*Albrigo*, 1999). The use of two applications of K-phosphite increased the number of commercially valuable large-size citrus fruit and total soluble solids in juice compared to nonsprayed control fruit (*Lovatt*, 1999). The mechanism by which fruit size was increased was explained in terms of properly timed improved P nutrition. *Orbovic* et al. (2008) concluded that citrus growers could apply Phi to soil or to leaves, either alone or in combination with PO_4-P (P_i), because of its dual action as an antifungal agent and as an indirect source of P. In this case, the positive effects of Phi on leaf P were attributed to the potential oxidation of Phi to P_i in sandy soil. Several Phi formulations have been registered and recommended as P fertilizers, either as soil or foliar applications, for a number of crops including citrus. However, the mechanisms of how Phi affects P nutrition and plant growth are not consistent and merit further study especially for root rot–susceptible crops (such as citrus) with a high probability of Phi application (*Thao* and *Yamakawa*, 2009).

Studies have shown negative effects of Phi on plant growth and metabolism of many species (*Carswell* et al., 1996, 1997; *Förster* et al., 1998; *Wells* et al., 2000; *Ticconi* et al., 2001; *Singh* et al., 2003; *Thao* et al., 2008a, b; *Ratjen* and *Gerendás*, 2009), concluding that Phi has little or no nutritional value. We hypothesized that any negative effects of Phi on plant growth and metabolism should be reflected in altered nutrient-use efficiency and reduced photosynthesis. Moreover, since citrus rootstock cultivars differentially tolerate P deficiency (*Graham* et al., 1997; *Mattos Jr.* et al., 2006), it is possible that rootstocks differ in their response to Phi supply.

The objectives of this study were to determine the effects of P_i and Phi availability on growth, mineral nutrition, and photo-

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synthetic characteristics of two contrasting citrus rootstock cultivars. We also tested the hypothesis that Phi alone or in combination with P_i affects growth and nutrient physiology differently in hydroponic and sand culture.

2 Materials and methods

2.1 Plant material and growth conditions

Plants were grown in either hydroponic solution or sand culture in an unshaded greenhouse, under natural photoperiod with average day/night temperatures of 38°C/24°C, maximum photosynthetically active radiation (PAR) of 1200 μmol m^{-2} s^{-1} and relative humidity from 40% to 100%, from May to August, 2009. Uniform 3-month-old seedlings of Carrizo citrange (CC, *Citrus sinensis* [L.] Osb. × *Poncirus trifoliata* [L.] Raf.) and Smooth Flat Seville (SFS, *Citrus aurantium* putative hybrid) rootstocks were purchased from a local nursery. As a rootstock, CC typically produces large high-yielding trees that have intermediate tolerance to *Phytophthora* root rot whereas SFS produces intermediate-sized trees that have a high tolerance to *Phytophthora* (*Castle* et al., 2006). Seedlings were bare-rooted and either planted in black well-drained 0.55 L pots filled with sandy soil (described below) or supported in identical undrained pots containing 0.40 L of ¼-strength Sarruge's modified nutrient solution (*Sarruge*, 1975) without P (= diluted basic nutrient solution, ¼BNS) for establishment. Each solution pot was equipped with a small tube extending to the bottom through which air was continuously bubbled for aeration. The full-strength BNS contained, in mM, 13.0 N (8.0% as NH_4^+), 5.0 Ca, 3.0 K, 1.25 Mg, 1.25 S and, in μM, 41.60 B, 46.70 Fe, 8.20 Mn, 3.50 Zn, 1.0 Cu, 1.25 Mo.

One week after transplant, ¼BNS was replaced by full-strength BNS with different P composition to establish four treatments: P_0 = no P (control); P_i = 0.5 mM P_i (phosphate, PO_4-P); P_i + Phi = 0.25 mM P_i + 0.25 mM Phi (phosphite, PO_3-P), and Phi = 0.5 mM Phi. The total P concentration in the nutrient solution of the P_i was based on recommendations for fertigation of potted citrus seedlings (*Bataglia* et al., 2008) and our previous experiments (unpublished data) in which 0.5 mM P_i supply was sufficient to support good growth. Technical grade KH_2PO_4 and KH_2PO_3 were used as P_i and Phi sources, respectively. Solution pH was adjusted to 5.8–6.0 using 0.1 M KOH, and hydroponic solutions were replaced every 7 d.

The sand culture used a native Candler sand soil, hyperthermic, uncoated Typic Quartzipsamments, sand: 970 g kg^{-1}, pH = 5.8, OM < 1%, with Mehlich-extractable P < 10.0 mg kg^{-1}. The sand was collected from a central Florida area under native vegetation adjacent to a nearby citrus grove from 0.10–0.25 m depth. The soil was steamed in metal containers for 8 h to kill any microorganisms that could interact with Phi and influence plant responses. The sterilized sand was air-dried, sieved, and 0.6 kg was used to fill each pot. Pots were thoroughly irrigated after transplanting, and plants were fertigated with the ¼BNS without P for 1 week after which this nutrient solution was replaced by full-strength BNS with the same four P treatments used in the hydroponic culture. The P treatments were applied until leaching occurred, 3–4 times per week; the volume of solution ranged from 40 to 65 mL for each application. Every 15–20 d, deionized water was applied for leaching to avoid any salt accumulation in the root zone. Nutrient concentration as well as volume and frequency of fertigation were based on weekly N demand of similar citrus seedlings (35–45 mg per plant per week; *Orbovic* et al., 2008). At the beginning of the P treatments (May 7, 2009), shoot tips were carefully tagged with a loose wire loop in order to evaluate subsequent new-stem and new-leaf growth. The experimental design was a complete factorial design with 2 citrus rootstock cultivars × 4 P treatments × 2 growth media with 6 replicate plants and was conducted for 83 d. The hydroponic and sterilized sand-media treatments used in this experiment were selected to minimize Phi oxidation to phosphate. The fertigated native sand media was intended to allow normal soil–root and soil–phosphorus interactions involved in P acquisition.

2.2 Net gas exchange, chlorophyll fluorescence, and SPAD readings

Net assimilation of CO_2 (A_{CO2}), leaf transpiration rate (E_{lf}), stomatal conductance (g_s), internal CO_2 concentration (C_i), and photosynthetic water-use efficiency (WUE = A_{CO2} E_{lf}^{-1}) were measured after 80–81 d of P treatments using fully expanded leaves that were developed after beginning P treatments. Net gas exchange was measured on a single leaf of each plant with a portable photosynthesis system (LI6200; LI-COR Inc. Lincoln, NB, USA) using a 0.25 L cuvette. During gas-exchange measurements, PAR exceeded 800 μmol m^{-2} s^{-1}, leaf temperature was 30°C–34°C, and relative humidity varied from 40% to 50%. Chlorophyll-fluorescence characteristics were measured with a pulse-modulated fluorometer (model OSI-FI, Optic-Sciences, Hudson, NH, USA) using the same leaves. Fluorescence was measured in light-exposed leaves and also in dark-adapted leaves that had been covered with light-exclusion clips for a minimum 20 min. Maximum quantum efficiency of photosystem II (F_v/F_m) was determined as $F_v/F_m = (F_m - F_o) / F_m$; where F_m and F_o were maximum and minimum fluorescence of dark-adapted leaves, respectively. Quantum yield (Y) was measured as $Y = (F'_m - F') / F'_m$, where F'_m and F' were the maximal and steady-state fluorescence yield in the light, respectively. This parameter measures the proportion of the light absorbed by chlorophyll associated with the photochemistry in photosystem II (*Pérez-Pérez* et al., 2007). Leaf greenness (chlorophyll index) was evaluated on the same leaves used for gas exchange and fluorescence using a SPAD-502 (Minolta Corp., Ramsey, NJ, USA) after 81 d of P treatments. Photosynthetic P-use efficiency (PPUE) and photosynthetic N-use efficiency (PNUE) were also calculated as A_{CO2} (μmol m^{-2} s^{-1}) per total P or per total leaf N concentration expressed on area basis (mg m^{-2} or g m^{-2}, respectively).

2.3 Growth parameters

Plants were harvested after 83 d of P treatments and separated into stems and leaves, developed before and after P

treatments (old and new leaves, respectively), woody roots (> 3.0 mm in diameter), and fibrous roots (< 3.0 mm in diameter). Total leaf area (cm^2) per plant was measured using a portable leaf-area meter (LI-3000; LI-COR). Fibrous roots were spread on a 2 cm grid pattern for measurement of root length (m $plant^{-1}$) using the line-intercept method (*Tennant*, 1975). These roots represented an estimate of the young thin root fraction that was probably responsible for the majority of water and nutrient absorption (*Korner* and *Renhardt*, 1987). The specific root length of fibrous roots (m g^{-1}) was calculated for each plant. Tissues were rinsed with deionized water, oven-dried at 60°C for at least 72 h, their dry weight (DW) determined, and ground to a fine powder using mortar and pestle.

2.4 Phosphate and phosphite extraction and quantification using capillary electrophoresis

About a 0.1 g subsample of the ground new leaves and the entire root system were extracted in 60 mL centrifuge tube with 6–8 mL of ultrapure water by hand-shaking every 10 min at room temperature for 2 h. The suspensions were centrifuged at 17 136 *g* for 15 min at 15°C. The supernatant was collected and filtered through a 0.45 μm nylon syringe filter. Concentrations of P_i and Phi in the filtrate were immediately analyzed using capillary electrophoresis (GPA 100, Groton Biosystem, Boxborough, MA) with a conductivity detector. Standard calibration curves were developed from dilutions of stock solutions of KH_2PO_4 and KH_2PO_3.

2.5 Phosphorus and nitrogen concentration

The total P (P_t, g kg^{-1}) in new leaves and roots and total N in new leaves (N_t, g kg^{-1}) were determined by means of an inductively coupled argon plasma–emission spectrophotometer (6500 Series ThermoScientific, Waltham, MA, USA) and total N analyzer (LECO Truspec, St. Joseph, MI, USA). A nutrient-utilization efficiency index was calculated as PUE or NUE as the ratio of new leaves or entire root DW (g^2) per P or N content (mg), respectively, as proposed by *Siddiqi* and *Glass* (1981). This index (g^2 mg^{-1}) takes into account absolute biomass increase, which is an important parameter to quantify responses of plants to nutrient sources and/or nutrition rates (*Siddiqi* and *Glass* 1981; *Good* et al., 2004).

2.6 Statistical analysis

Data were analyzed using a factorial analysis of variance (ANOVA; SAS version 9.1; SAS Institute, Cary, NC). For significant three-way interactions, analyses of rootstocks × P treatments were run within each growth-media experiment. If no significant three-way interaction was observed, the statistical analysis was performed using averaged values across growth media. The rootstock effects were compared using the F test. Duncan's multiple range test was used to compare P treatments at $p < 5\%$. Linear and quadratic regressions with simple correlation analysis were used to describe relationships between selected variables.

3 Results

3.1 Plant growth

The effects of P treatments on plant-growth parameters of both rootstocks were independent of the growth media as there were no three-way interactions (Tab. 1). Thus, analyses of growth values were averaged across the two media to determine effects of P_i or Phi treatments on rootstocks. Overall, hydroponically grown roots were thinner, 10.10 m g^{-1} *vs.* 6.73 m g^{-1} ($p < 5\%$) than sand-grown roots and hydroponically grown plants allocated relatively less growth to roots than to shoots as supported by a lower root-to-shoot ratio in

Table 1: Growth of Carrizo citrange (CC) and Smooth Flat Seville (SFS) citrus rootstock seedlings after 83 d phosphate (P_i) and/or phosphite (Phi) treatments. Results from plants grown hydroponically and in sand media were combined. P-treatments comparison: means followed by different lowercase letters within columns ($n = 96$ or 48) are significantly different by the Duncan's multiple range test ($p < 5\%$). Rootstocks comparison: means followed by different uppercase letters across paired columns ($n = 24$, comparison within each P treatment) or small letter in the columns ($n = 96$, comparison across P-treatments average) are significantly different by the F test ($p < 5\%$).

P treatments/ rootstocks	TPDW[$]	WRDW	FRDW	TRL	SFRL	R : S ratio	NLN		TLA	
	rootstocks average						CC	SFS	CC	SFS
	/ g $plant^{-1}$			/ m $plant^{-1}$	/ m g^{-1}	/ g g^{-1}	/ leaves $plant^{-1}$		/ cm^2 $plant^{-1}$	
P_0	**1.98 c**[§]	**0.38 b**	**0.57 a**	**5.10 a**	**10.90 a**	**0.80 a**	9.3 cA	5.8cB	73.6 cA	82.2 cA
P_i	**4.49 a**	**0.65 a**	**0.47 b**	**4.20 b**	**7.51 b**	**0.47 b**	18.8 aA	16.8 aB	165.2 aB	285.7 aA
P_i + Phi	**3.51 b**	**0.59 a**	**0.41 b**	**2.74 c**	**6.80 b**	**0.48 b**	15.6 bA	13.6 bB	128.9 bB	209.3 bA
Phi	**1.57 c**	**0.28 c**	**0.22 c**	**1.74 d**	**8.52 b**	**0.40 b**	6.6 cA	4.4 cB	51.4 cB	62.9 cA
	P-treatments average									
CC	**2.80 a**	**0.51 a**	**0.41 a**	**3.80 a**	**9.46 a**	**0.54 a**				
SFS	**2.97 a**	**0.43 b**	**0.43 a**	**3.10 b**	**7.38 b**	**0.51 a**				

§ Bold font represents data without significant interactions between P treatments and citrus rootstocks.
$ TPDW: total-plant dry weight; WRDW: woody-root dry weight; FRDW: fibrous-root dry weight; TRL: total root length; SFRL: specific fibrous-root length; R : S: root-to-shoot ratio; NLN: new-leaf number; TLA: total leaf area

hydroponically than in sand-grown plants, 0.48 *vs.* 0.57 ($p <$ 5%). Since rootstock growth responses to P treatments were similar in both hydroponic and sand culture, analyses of growth values were averaged across the two media to determine the effects of P_i or Phi treatments on rootstocks.

After 83 d P treatments, plant growth was greatest in the P_i treatment as measured by total-plant (TP) DW, woody-root (WR) DW, new-leaf number (NLN), and total leaf area (TLA) (Tab. 1). The TPDW, WRDW, and total root length (TRL) of Phi plants were 57%–65% less than in the P_i treatment. In addition, Phi plants had dark appearing roots and misshapen new leaves with chlorotic and necrotic spots. In general, growth responses to the P_i + Phi treatment were intermediate between the P_i and Phi treatments as P_i + Phi plants had 22% less TPDW and 35% less TRL than the P_i plants.

The lack of significant interaction between rootstocks and P treatments for growth parameters indicated that the growth of these rootstocks responded in a similar way to Phi supply even though CC had greater WRDW, TRL, and SFRL than SFS (Tab. 1). However, SFS produced more TLA and leaf DW (data not shown) than CC, probably because SFS has entire leaves whereas CC has smaller thinner trifoliate leaves, but the two rootstocks had similar root-to-shoot ratios. Low P increased growth allocation to roots as the P_0 plants had the greatest fibrous root (FR) DW, TRL, specific fibrous-root length (SFRL), and root-to-shoot ratio at the end of the experiment.

3.2 Phosphorus and nitrogen nutrition

There were significant three-way interactions among rootstocks × P treatments × growth media for P_t concentration, accumulated P_t (mg plant^{-1}) and also for soluble PO_4-P and PO_3-P concentrations in the new leaves and roots (Tabs. 2 and 3). The analysis of variance within each growth medium revealed that P_0 leaves of both rootstocks were P-deficient (< 1.0 g kg^{-1} P_t) (*Bataglia* et al., 2008) and roots had < 0.8 g kg^{-1} P_t (Tab. 2). Concentration of P_t in Phi leaves was increased above the deficient level, however, there was a negative correlation between foliar P_t concentration and TPDW in Phi plants (Fig. 1a). Although Phi SFS had a higher concentration of foliar P_t in hydroponic media in relation to P_i, Phi CC plants had reduced P_t concentration compared to P_i plants (Tab. 2). TPDW was positively related with accumulated P_t in the new leaves (Fig. 1b).

Similar to P_t, P_0 plants had the lowest values of soluble PO_4-P in new leaves and roots (< 0.6 g kg^{-1}) whereas the highest PO_4-P occurred in those that received 0.5 mM P_i (> 1.2 g kg^{-1}; Tab. 3). Furthermore, PO_4-P concentrations in P_0 plant tissue were higher in SFS than in CC with the excep-

Table 2: Concentration and accumulation of total P (P_t) in new leaves and roots of Carrizo citrange (CC) and Smooth Flat Seville (SFS) citrus rootstock seedlings grown in hydroponic or sand media for 83 d on phosphate (P_i) and/or phosphite (Phi) treatments. P-treatments comparison: means followed by different lowercase letters within columns (n = 96 or 48) are significantly different by the Duncan's multiple range test ($p < 5\%$). Rootstocks comparison: means followed by different uppercase letters across paired columns (n = 24, comparison within each P treatment) or lowercase letters with in the columns (n = 96, comparison across P-treatments average) are significantly different by the F test ($p < 5\%$).

Media	P treatments/ rootstocks	P_t						
		new leaves		roots		new leaves		roots
		CC	SFS	CC	SFS	CC	SFS	rootstocks average
		/ g kg^{-1}				/ mg plant^{-1}		
Hydroponic	P_0	0.71 cA	0.67 cA	0.62 cA	0.79 bA	0.22 bA	0.27 bA	**0.60 c**§
	P_i	2.36 aA	2.14 bA	3.82 aA	4.60 aA	2.43 aB	5.21 aA	**4.61 a**
	P_i + Phi	2.34 aA	1.86 bB	4.19 aA	4.66 aA	2.51 aA	3.70 aA	**4.89 a**
	Phi	1.58 bB	2.91 aA	2.96 bB	5.02 aA	0.36 bB	0.54 bA	**1.86 b**
								P-treatments average
	CC							**2.65 b**
	SFS							**3.33 a**
		CC	SFS	rootstocks average				
Sand	P_0	0.94 dA	0.90 cA	**0.75 b**		**0.37 c**		**0.60 c**
	P_i	2.70 aA	2.00 aB	**1.40 a**		**3.05 a**		**1.81 a**
	P_i + Phi	1.77 bA	1.95 aA	**1.40 a**		**1.05 b**		**1.26 b**
	Phi	1.35 cA	1.64 bA	**1.37 a**		**0.51 c**		**0.72 c**
				P-treatments average				
	CC			**1.12 b**		**1.06 b**		**1.08 a**
	SFS			**1.32 a**		**1.41 a**		**1.12 a**

§ Bold font represents data without significant interactions between P treatments and citrus rootstocks.

Table 3: Concentration of water-soluble phosphate (PO_4-P) and phosphite (PO_3-P) in new leaves and roots of Carrizo citrange (CC) and Smooth Flat Seville (SFS) citrus rootstocks seedlings grown in hydroponic or sand media for 83 d on phosphate (P_i) and/or phosphite (Phi) treatments. P-treatments comparison: means followed by different lowercase letters within columns (n = 96 or 48) are significantly different by the Duncan's multiple range test (p < 5%). Rootstocks comparison: means followed by different uppercase letters across paired columns (n = 24, comparison within each P treatment) or lowercase letter within columns (n = 96, comparison across P-treatments average) are significantly different by the F test (p < 5%). nd: not determined.

Media	P treatments/ rootstocks	PO_4-P				PO_3-P			
		new leaves		roots		new leaves		roots	
		CC	SFS	CC	SFS	rootstocks average			
		/ g kg^{-1}							
Hydroponic	P_0	0.24 dB	0.47 bA	0.20 cB	0.28 cA	**nd**		**nd**	
	P_i	1.33 aA	1.48 aA	1.52 aB	3.01 aA	**nd**		**nd**	
	P_i + Phi	0.90 bB	1.34 aA	1.56 aA	1.98 bA	**0.19 a**		**0.18 b**	
	Phi	0.44 cB	1.48 aA	0.74 bB	2.00 bA	**0.24 a**		**0.50 a**	
						P-treatments average			
	CC					**0.20 a**		**0.34 b**	
	SFS					**0.23 a**		**0.53 a**	
		CC	SFS	rootstocks average		CC	SFS	CC	SFS
Sand	P_0	0.30 cB	0.54 cA	**0.35 c**		nd	nd	nd	nd
	P_i	1.90 aA	1.29 aB	**0.81 a**		nd	nd	nd	nd
	P_i + Phi	0.71 bB	1.19 aA	**0.48 b**		0.16 bB	0.23 aA	0.30 bA	0.27 bA
	Phi	0.73 bB	0.90 bA	**0.51 b**		0.30 aA	0.21 aB	0.48 aB	0.83 aA
				P-treatments average					
	CC			**1.05 a**					
	SFS			**1.08 a**					

§ Bold font represents data without significant interactions between P treatments and citrus rootstocks.

tion of root concentration in sand media. There were highly significant correlations between PO_4-P and P_t concentrations in new leaves (r = 0.78; p < 0.01%; n = 96) and roots (r = 0.86; p < 0.01%; n = 96). The addition of 0.5 mM Phi in the nutrient solution increased PO_4-P concentration in the leaf tissue above that of the P_0 treatment, but the total amount of PO_4-P accumulated (mg plant^{-1}) in roots and new leaves did not differ (data not shown). Concentrations of PO_3-P were much smaller than of PO_4-P, and increases of PO_3-P in new leaves were less than in roots. For instance, up to three times more PO_3-P was found in roots than in leaf tissue in Phi SFS grown in sand (Tab. 3).

Phosphorus-utilization efficiency for biomass production (PUE) was 53%–85% lower in Phi new leaves than in P_i plants (Tab. 4). PUE was the lowest in roots (< 0.3 g^2 mg^{-1}) of Phi plants but highest in the P_0 treatment. PUE for SFS leaves of P_i and P_i + Phi treatments were higher than PUE for CC leaves while the opposite result occurred for roots. Although all levels of leaf N_t were above the sufficiency range

Table 4: Phosphorus-utilization efficiency for biomass production (PUE) of new leaves and roots, total nitrogen concentration (N_t), and nitrogen-utilization efficiency for biomass production (NUE) of leaves of Carrizo citrange (CC) and Smooth Flat Seville (SFS) citrus rootstocks seedlings after 83 d of phosphate (P_i) and/or phosphite (Phi) treatments. Results from plants grown hydroponically and in sand media were combined. Means followed by different lowercase letters within columns (n = 48) and uppercase letters across paired columns (n = 24) are significantly different by the Duncan's multiple range test and F test (p < 5%), respectively.

P treatments	PUE				N_t		NUE	
	new leaves		roots					
	CC	SFS	CC	SFS	CC	SFS	CC	SFS
	/ g^2 mg^{-1}				/ g kg^{-1}		/ g^2 mg^{-1}	
P_0	0.44 aA	0.55 bA	1.40 aA	1.05 aB	43.3 aA	34.4 aB	0.009 cA	0.012 cA
P_i	0.40 aB	1.03 aA	0.70 bA	0.59 bB	35.8 bA	26.7 cB	0.028 aB	0.084 aA
P_i + Phi	0.36 aB	0.71 bA	0.53 bA	0.39 bcB	37.5 bA	28.7 bcB	0.021bB	0.056 bA
Phi	0.19 bA	0.15 cA	0.29 bA	0.24 cA	30.1 cA	31.5 abA	0.009 cA	0.009 cA

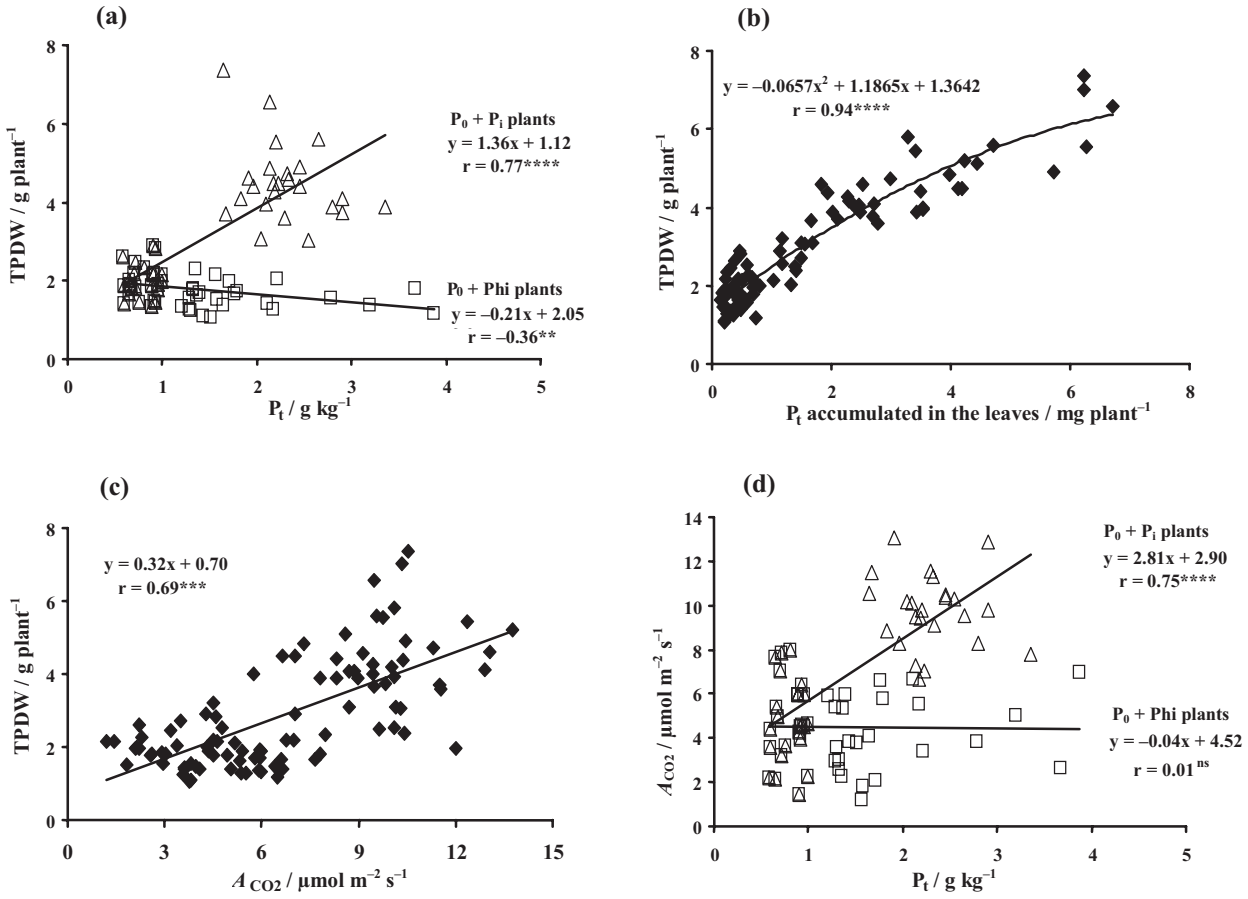

Figure 1: a) Relationships between total-plant dry weight (TPDW) and total leaf P (P_t) from either P_0 + P_i (triangles, n = 48) or P_0 + Phi plants (squares, n = 48) in Carrizo citrange (CC) and Smooth Flat Seville (SFS) citrus rootstock seedlings combined; b) TPDW *vs.* P_t accumulated in the leaves across all treatments (n = 96); c) TPDW *vs.* net assimilation of CO_2 (A_{CO2}) across all treatments (n = 96), and d) net assimilation of CO_2 (A_{CO2}) and total leaf P (P_t) from either P_0 + P_i (triangles, n = 48) or P_0 + Phi plants (squares, n = 48) in CC and SFS citrus rootstock seedlings combined. ns: nonsignificant (p > 5%); $^{**}p$ < 1%; $^{***}p$ < 0.1%; $^{****}p$ < 0.01%.

(26 g kg^{-1}, *Bataglia* et al., 2008), concentration of N_t in leaves was higher in P_0 than in P_i plants. Furthermore, NUE was lower in P_0 and Phi plants than in the two P_i treatments for both rootstocks (Tab.4).

3.3 SPAD readings, net gas exchange, and chlorophyll fluorescence

The P_0 and Phi plants had leaf chlorophyll readings up to 15% below that of the P_i treatment (Tab. 5). Overall, SPAD values were positively correlated with TPDW (r = 0.47; p < 0.01%; n = 96) and foliar P_t (r = 0.49; p < 0.01%; n = 96). A_{CO2} and g_s were by about 50% lower in both P_0 and the Phi plants compared to the P_i plants (Tab. 5). There were higher values of C_i in the P_0 and Phi treatments compared to P_i treatment, and C_i was higher in SFS leaves than in CC leaves. Moreover, the Phi treatment resulted in the lowest measured E_{lf}. Leaf WUE was reduced in P_0 plants of both rootstocks as compared to P_i treatment; leaf WUE was greatest in the P_i CC. Overall, A_{CO2} rates were strongly correlated with total plant growth as largest plants had the highest A_{CO2} (Fig. 1c). A_{CO2} was positively related to P_t concentration in the leaves when data from P_0 and P_i were combined, however, increased P_t did not enhance A_{CO2} when the P_0 and Phi data were plotted together (Fig. 1d).

Phi also reduced maximum quantum yield of dark-acclimated leaves (F_v/F_m) and quantum yield (Y) of CC when compared to P_i treatment which did not differ from P_i + Phi plants. SFS had consistently lower F_v/F_m and Y values than CC in the P_0 and P_i treatments, and F_v/F_m and Y of P_0 plants were lower than in P_i plants. The PPUE or PNUE for A_{CO2} in Phi treatment were 50%–63.0% lower than those of P_i (Tab. 6). The P_0 treatment resulted in either similar or more efficient use of the limited P in the photosynthetic process compared to P_i plants, whereas PNUE of P_0 plants was as low as Phi plants.

4 Discussion

Although hydroponically grown plants had a lower root-to-shoot ratio than sand-grown plants, growth and physiological responses to P treatments were similar in both hydroponic and sand culture. Thus, we could not support our original hypothesis that responses of these citrus rootstocks to Phi would vary with growth media. This may have been related to the fact that the sand was initially autoclaved which would

Table 5: Effects of 80 d phosphate (P_i) and/or phosphite (Phi) treatments on SPAD chlorophyll index readings, net assimilation of CO_2 (A_{CO2}), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), leaf transpiration (E_{lf}), photosynthetic water-use efficiency (WUE = A_{CO2} E_{leaf}^{-1}), maximum quantum yield of dark-acclimated leaves (F_v/F_m) and quantum yield (Y) of leaves Carrizo citrange (CC) and Smooth Flat Seville (SFS) citrus rootstocks seedlings. Results from plants grown hydroponically or in sand media were combined. P-treatments comparison: means followed by different lowercase letters within columns (n = 96 or 48) are significantly different by the Duncan's multiple range test ($p <$ 5%). Rootstocks comparison: means followed by different uppercase letters across paired columns (n = 24, comparison within each P treatment) or lowercase letters within columns (n = 96, comparison across P-treatments average) are significantly different by the F test ($p <$ 5%).

P treatments/ rootstocks	SPAD	A_{CO2}	g_s	C_i	E_{lf}	WUE		F_v/F_m		Y	
	rootstocks average					CC	SFS	CC	SFS	CC	SFS
		/ μmol m^{-2} s^{-1}	/ mol m^{-2} s^{-1}	/ ppm	/ μmol m^{-2} h^{-1}	/ μmol mmol^{-1}					
P_0	**60.3 b**§	**4.89 c**	**0.04 b**	**197.4 a**	**1.6 ab**	3.49 bA	3.16 bA	0.72 cA	0.59 bB	0.53 bA	0.39 cB
P_i	**71.5 a**	**9.80 a**	**0.07 a**	**137.1 c**	**2.0 a**	5.13 aA	4.37 aB	0.83 aA	0.80 aB	0.70 aA	0.65 aB
P_i + Phi	**69.8 a**	**8.19 b**	**0.06 a**	**146.8 bc**	**2.0 a**	4.00 bA	4.32 aA	0.81 aA	0.79 aA	0.66 aA	0.64 abA
Phi	**58.6 b**	**4.37 c**	**0.04 b**	**173.6 ab**	**1.2 b**	3.49 bB	4.55 aA	0.77 bA	0.76 aA	0.53 bA	0.57 bA
	P-treatments average										
CC	**69.1**	**7.04 a**	**0.051 a**	**151.9 b**	**1.8 a**						
SFS	**61.1**	**6.57 a**	**0.053 a**	**175.6 a**	**1.7 a**						

§ Bold font represents data without significant interactions between P treatments and citrus rootstocks.

have negated any potential of soil bacteria to oxidize Phi to P_i. The high fertilizer-application rate, other than the low P in the P_0 treatment, also would have facilitated a high nutrient-uptake rate. Thus, both hydroponic and sterilized sand media likely maximized plant–Phi interactions in the rhizosphere. In addition, we did not confirm the hypothesis that the citrus rootstock varieties, CC and SFS, would respond differently to Phi supply to their roots, since their growth responses to Phi were similar (Tab. 1). Even when Phi was mixed with P_i in the P_i + Phi treatment, growth of both citrus rootstocks was less than the P_i treatment and there was remarkable reduction in root development in the Phi plants of both rootstocks (Tab. 1). This supported previous observations on the potential of Phi to compromise root growth (*Thao* et al., 2008a, b) and water uptake in low-P_i komatsuna plants supplied with Phi (*Thao* and *Yamakawa*, 2010). The inhibition of *Arabidopsis* growth by Phi was considered to be a consequence of competitive inhibition of P_i assimilation and an inability of the plants to readily utilize Phi *via* oxidation to P_i (*Ticconi* et al., 2001). If Phi is not converted into P_i then Phi is unable to enter P bio-chemical pathways (*MacDonald* et al., 2001; *Varadarajan* et al., 2002).

Both citrus rootstocks absorbed Phi from the nutrient solution since soluble PO_3-P was detected in the new leaves and roots (Tab. 3), as was observed previously for citrus seedlings (*Orbovic* et al., 2008) and other crops (*Thao* et al., 2008b; *Ratjen* and *Gerendás,* 2009). Although both P_i and Phi may be translocated (*Orbovic* et al., 2008), the higher PO_3-P found in roots than in leaf tissue of Phi SFS grown in sand does not support the idea that Phi is readily translocated from roots to shoots. There is a structural similarity between P_i and Phi that allows both forms of P to be taken up *via* membrane P_i transporters (*Varadarajan* et al., 2002), but there may be an impaired uptake of P_i after application of Phi (*Thao* and *Yamakawa*, 2010), contributing to the negative effects of Phi on growth (*Carswell* et al., 1997). Although Phi plants had increased PO_4-P and P_t concentration in the leaves above the levels in P_0 plants, this could have been a concentrating effect from reduced plant growth (Tab. 1,

Table 6: Photosynthetic phosphorus-use efficiency (PPUE) and photosynthetic nitrogen-use efficiency (PNUE) of Carrizo citrange (CC) and Smooth Flat Seville (SFS) citrus rootstocks seedlings subjected to phosphate (P_i) and/or phosphite (Phi) treatments. Results from plants grown hydroponically and in sand media were combined. Means followed by different lowercase letters within columns (n = 48) and uppercase letters across paired columns (n = 24) are significantly different by the Duncan's multiple range test and F test ($p <$ 5%).

P treatments	PPUE		PNUE	
	CC	SFS	CC	SFS
	/ μmol CO_2 s^{-1} (mg P)$^{-1}$		/ μmol CO_2 s^{-1} (g N)$^{-1}$	
P_0	0.10 aA	0.06 aB	1.78 cA	1.35 bA
P_i	0.06 bA	0.05 aA	3.97 aA	4.07 aA
P_i + Phi	0.05 bA	0.06 aA	2.97 bA	4.25 aA
Phi	0.03 cA	0.02 bA	1.47 cA	1.59 bA

Fig. 1a) since P_t accumulation per plant was not increased in new leaves of Phi plants (Tab. 2). Accumulation of P_t in new leaves had much stronger relationship than P_t concentration with plant growth (Fig. 1b) so leaf P_t concentration after Phi application may not be a good indicator of plant P status. The use of leaf P_t may lead to an incorrect interpretation of improved P nutrition in crop plants after application of Phi.

The PUE for growth in roots and new leaves for Phi plants was lower than for P_i plants (Tab. 4). This is an important result because in addition due to the low P_i availability and phytotoxic leaf symptoms associated with Phi, the lower nutrient-utilization efficiency confirmed compromised P functions in Phi plants. This negative effect of Phi was also reflected in reduced NUE (Tab. 4) and PNUE suggesting that even though plants were well-supplied with all other nutrients, Phi negatively affected N utilization for growth and use in the photosynthetic process. Leaf N and chlorophyll concentration are usually correlated (*Bondada* and *Syvertsen*, 2003) but here chlorophyll was reduced despite high leaf N in Phi plants (Tabs. 4 and 5). Low chlorophyll could have contributed to reduced A_{CO2} in these plants since SPAD readings were related to A_{CO2} (r = 0.53; p < 0.01%; n = 96). P_i-deficient plants can have altered N metabolism resulting in less N incorporated into shoot protein (*Rufty* et al., 1990), and low P_i also reduced chlorophyll in tomato plants (*De Groot* et al., 2003).

The low A_{CO2} in P_0 or Phi plants was not explained by the decrease in g_s since C_i was increased (Tab. 4). Thus, the low A_{CO2}, F_v/F_m, and Y in these plants were more limited by direct effects on biochemical processes than by stomatal limitations. In P_i-deficient *Pinus radiata* (*Bown* et al., 2009) and soybean plants (*Freeden* et al., 1990), this nonstomatal limitation on A_{CO2} was related to a reduction in the active site of RuBP carboxylase or to a decrease in the rate of RuBP regeneration. In addition, the reduced growth of P_0 or Phi plants undoubtedly resulted in lower carbohydrate demand from source leaves, which could have led to an additional negative feedback on photosynthesis (*Syvertsen* et al., 2003). Thus, the increased P_t concentration in the leaves of the Phi plants was not reflected in improved A_{CO2} in relation to P_0 plants as occurred in P_i plants (Fig. 1b), resulting in the lowest PPUE for Phi treatment (Tab. 6). Even though P_0 and Phi plants had similarly low A_{CO2}, the higher F_v/F_m and Y in Phi plants suggested that electron transport in PSII in Phi plants was not as limiting to A_{CO2} as in the P_0. Phosphorus-deficient P_0 plants may have been less able to generate and consume ATP and NADPH to support PSII (*Baker*, 2008). Although there was no significant correlation between A_{CO2} with either F_v/F_m (r = 0.17; p = 28%; n = 48) or Y (r = 0.10; p = 49%; n = 48) when data were pooled from P_0 plus Phi plants, there were significant correlations between A_{CO2} and F_v/F_m (r = 0.66; p < 0.01%; n = 48) and Y (r = 0.67; p < 0.01%; n = 48) using data pooled from P_0 plus P_i plants.

Our results clearly show that Phi at these concentrations cannot replace P_i as a source of P for citrus when both are applied either separately or together. Damaging effects of Phi on vegetative growth might have been responsible for the stress-induced increase in citrus flowering following winter applications of Phi (*Albrigo*, 1999). The foliar application of Phi could have inhibited shoot and root growth of orange trees leading to more available carbohydrates and nutrients for flowering and fruit set. Although foliar applications of Phi increased soluble PO_4-P levels in sweet orange seedlings (*Orbovic* et al., 2008), responses of citrus plants to applied Phi (*Albrigo*, 1999; *Lovatt,* 1999) may have been related to phytotoxic properties of Phi rather than its capacity of improving P nutrition.

5 Conclusions

Both growth media had a low potential for oxidation of Phi to P_i and, therefore, would have maximized Phi interactions with CC and SFS rootstock seedlings that responded in a similar way to Phi supply. Although Phi increased concentration of P_t in leaf and root tissues and increased leaf chlorophyll-fluorescence characteristics above P_0 leaves, Phi plants had similarly low plant growth, A_{CO2}, leaf chlorophyll, and nitrogen-use efficiency as P_0 plants. In addition, Phi plants developed phytotoxic symptoms and had lower P-use efficiency than P_0 and P_i plants. Thus, the deleterious effects of Phi on citrus-plant metabolism, growth, and nutrition should be avoided especially when there is no need to use this compound to control *Phytophthora* spp.

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References

Albrigo, L. G. (1999): Effects of foliar applications of urea or Nutriphite on flowering and yields of Valencia orange trees. *Proc. Fla. State Hort. Soc.* 112, 1–4.

Baker, N. R. (2008): Chlorophyll Fluorescence: A probe of photosynthesis in vivo. *Annu Rev. Plant Biol.* 59, 89–113.

Barret, S. R., Shearer, B. L., Hardy, G. E. S. J. (2003): Efficacy of phosphite applied after inoculation on the colonization of *Banksia brownii* stems by *Phytophthora cinnamomi*. *Aust. J. Plant Phathol.* 32, 1–7.

Bataglia, O. C., Furlani, P. R., Ferrarezi, R. S., Medina, C. L. (2008): Padrão nutricional de mudas de citros. Vivecitrus/Conplant, Araraquara.

Bondada, B. R., Syvertsen, J. P. (2003): Leaf chlorophyll, net gas exchange and chloroplast ultrastructure in citrus leaves of different nitrogen status. *Tree Physiol.* 23, 533–559.

Bown, H., Watt, M. S., Mason, E. G., Clinton, P. W., Whitehead, D. (2009): The influence of nitrogen and phosphorus supply and genotype on mesophyll conductance limitations to photosynthesis in *Pinus* radiate. *Tree Physiol.* 29, 857–869.

Castle, W. S., Bowman, K., Graham, J. H., Tucker, D. P. H. (2006): Florida citrus rootstock selection guide. UF/IFAS, SP248, Univ. of Fl, Gainesville.

Carswell, C., Grant, B. R., Theodorou, M. E., Harris, J., Niere, J. O., Plaxton, W. C. (1996): The fungicide phosphonate disrupts the phosphatestarvation response in *Brassica nigra* seedlings. *Plant Physiol.* 110, 105–110.

Carswell, C., Grant, B. R., Theodorou, M. E., Plaxton, W. C. (1997): Disruption of the phosphate-starvation response of oilseed rape suspension cells by the fungicide phosphonate. *Planta* 203, 67–74.

Förster, H., Adaskaveg, J. E., Kim, D. H., Stanghellini, M. E. (1998): Effect of phosphite on tomato and pepper plants and on susceptibility of peppers to *Phytophthora* root and crown rot in hydroponic culture. *Plant Dis.* 82, 1165–1170.

Freeden, A. L., Raab, T. K., Rao, I. M., Terry, N. (1990): Effects of phosphorus nutrition on photosynthesis in *Glycine max* (L.) Merr. *Planta* 181, 399–405.

Good, A. G., Shrawat, A. K., Muench, D. G. (2004): Can less yield more? Is reducing nutrient input into environment compatible with maintaining crop production? *Trends Plant Sci.* 9, 597–604.

Graham, J. H., Duncan, L. W., Eissenstat, D. M. (1997): Carbohydrate allocation patterns in genotypes as affected by phosphorus nutrition, mycorrhizal colonization and mycorrhizal dependency. *New Phytol.* 135, 335–343.

De Groot, C. C., Boogaard, R., Marcelis, L. F. M., Harbinson, J., Lambers, H. (2003): Contrasting effects of N and P deprivation on the regulation of photosynthesis in tomato plants in relation to feedback limitation. *Plant Soil* 248, 257–268.

Guest, D., Grant, B. (1991): The complex action of phosphonates as antifungal agents. *Biol. Revenues* 66, 159–187.

Korner, C. H., Renhardt, U. (1987): Dry matter portioning and root/length area ratios in herbaceous perennial plants with diverse altitudinal distribution. *Oecologia* 74, 411–418.

Lovatt, C. J. (1999): Timing citrus and avocado foliar nutrient applications to increase fruit set and size. *Hort. Technol.* 9, 607–612.

Mattos Jr., D., Quaggio, J. A., Cantarella, H., Alva, A. K., Graetz, D. A. (2006): Response of young citrus trees on selected rootstocks to nitrogen, phosphorus, and potassium fertilization. *J. Plant Nutr.* 29, 1371–1385.

McDonald, A. E., Grant, G. R., Plaxton, W. C. (2001): Phosphite (phosphorous acid): Its relevance in the environment and agriculture and influence on plant phosphate starvation response. *J. Plant Nutr.* 24, 1505–1519.

Orbovic, V., Syvertsen, J. P., Bright, D., Van Clief, D. L., Graham, J. H. (2008): Growth of citrus seedlings and their susceptibility to *Phytophthora* root rot are affected by PO_3 and PO_4 sources of phosphorus. *J. Plant Nutr.* 31, 774–787.

Pegg, K. G., Whiley, A. W., Saranah, J. B., Glass, R. J. (1985): Control of *Phytophthora* root rot of avocado with phosphorus acid. *Aust. J. Plant Pathol.* 14, 25–29.

Pérez-Pérez, J. G., Syvertsen, J. P., Botía, P., García-Sánches, F. (2007): Leaf water relations and net gas exchange responses of salinized Carrizo citrange seedlings during drought stress and recovery. *Ann. Bot.* 100, 335–345.

Ratjen, A. M., Gerendás, J. (2009): A critical assessment of the suitability of phosphite as a source of phosphorus. *J. Plant Nutr. Soil Sci.* 172, 821–828.

Rohrbach, K. G., Schenck, S. (1985): Control of pineapple heart rot caused by *Phytophthora parasitica* and *P. cinnamoni*, with metalaxyl, fosetyl-Al and phosphorous acid. *Plant Dis.* 69, 320–323.

Rufty Jr., T. W., Mackown, C. T., Israel, D. W. (1990): Phosphorus stress effects on assimilation of nitrate. *Plant Physiol.* 94, 328–333.

Sarruge, J. R. (1975): Soluções Nutritivas. *Summa Phytopatol.* 1, 231–233.

Siddiqi, M. Y., Glass, A. D. M. (1981): Utilization index: A modified approach to the estimation and comparison of nutrient utilization efficiency in plants. *J. Plant Nutr.* 4, 289–302.

Singh, V. K., Wood, S. M., Knowwles, V. L., Plaxton, W. C. (2003): Phosphite accelerates programmed cell death in phosphate-starved oilseed rape (*Brassica napus*) suspension cell culture. *Planta* 218, 233–239.

Syvertsen, J. P., Goni, C., Otero, A. (2003): Fruit load and canopy shading affect leaf photosynthesis and carbohydrate status in 'spring' Navel Orange trees. *Tree Physiol.* 23, 899–906.

Tennant, D. A. (1975): Test of a modified line intersect method of estimating root length. *J. Ecol.* 63, 995–1001.

Thao, H. T. B., Yamakawa, T. (2009): Phosphite (phosphorous acid): fungicide, fertilizer or bio-stimulator? *Soil Sci. Plant Nutr.* 55, 228–234.

Thao, H. T. B., Yamakawa, T. (2010): Phosphate absorption of intact komatsuna plants as influenced by phosphite. *Soil Sci. Plant Nutr.* 56, 133–139.

Thao, H. T. B., Yamakawa, T., Myint, A. K., Sarr, P. S. (2008a): Effects of phosphite, a reduced form of phosphate, on the growth and phosphorus nutrition of spinach (*Spinacia oleracea* L.). *Soil Sci. Plant Nutr.* 54, 761–768.

Thao, H. T. B., Yamakawa, T., Shibata, K., Sarr, P. S., Myint, A. K. (2008b): Growth response of komatsuna (*Brassica rapa* var. peruviridis) to root and foliar applications of phosphite. *Plant Soil* 308, 1–10.

Ticconi, C. A., Delatorre, C. A., Abel, S. (2001): Attenuation of phosphate starvation responses by phosphite in *Arabidopsis*. *Plant Physiol.* 27, 963–972.

Varadarajan, D. K., Karthikeyan, A. S., Matilda, P. D., Raghothama, K. G. (2002): Phosphite, an analog of phosphate, suppresses the coordinated expression of genes under phosphate starvation. *Plant Physiol.* 129, 1232–1240.

Wells, K. L., Dollarhide, J. E., Mundel Jr., R. E. (2000): Effect of phosphite phosphorus on alfalfa growth. *Commun. Soil Sci. Plant Anal.* 31, 2707–2715.

