Journal of Soil Science and Plant Nutrition, 2013, 13(3), 612-620

The effects of phosphite on strawberry yield and fruit quality

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Abstract

Phosphite ($H_2PO_3^-$; Phi) has been shown to increase fruit quality and activate plant defense mechanisms in plants when provided in a nutrient state with sufficient phosphorous. In this study, five solutions containing different percentages of Phi (0, 20, 30, 40 and 50%) in Steiner's solution were evaluated during the flowering and fructification stages; the Steiner' s nutrient solution was kept al 50% during the flowering stage and at 75% from the beginning of the fructification stage on. The objective was to determine the effects of phosphite on total P concentration in leaves, yield, pH, electrical conductivity (EC), anthocyanin concentration, and fruit size of strawberries (cv. Festival). The experiments were performed in a tunnel-type greenhouse using drip irrigation and volcanic rock (volcanic gravel) as substrate. In the fruit development phase, the concentration of P in the leaves was proportional to the level of Phi used. Although no significant differences were observed when compared to the control, the addition of 20% Phi slightly improved yield and fruit size. The highest pH, EC and anthocyanin concentration were identified in the fruit of plants treated with 30% Phi. Our findings suggest that supplying Phi at 30% or less in the nutrient solution does not significantly affect yield but does affect fruit quality and activates plant defense mechanisms by producing a higher concentration of anthocyanins.

Keywords: *Fragaria x ananassa* Duch, anthocyanins, electrical conductivity of fruit, pH of fruit, fruit size

1. Introduction

Phosphite ($H_2PO_3^-$) is an isostere of the phosphate anion ($H_2PO_4^-$) in which one of the oxygen atoms bonded to the P atom is replaced by hydrogen (Ouimette and Coffey, 1990). Due to the structural similarity of these anions and the kinetic properties of plant phosphate transporters, phosphite is transported by high-affinity phosphate transporters (D'arcy-Lameta and Bompeix, 1991). Although phosphite can be transported into the interior of the cell, the ion is not involved in P metabolism (ATP production, photosynthesis or respiration), and the similarity between phosphate and phosphite seems to be related only to P assimilation. Phosphite is not converted to phosphate inside the plant and does not participate in any biochemical pathways (Varadarajan *et al*., 2002), but it does disrupt the phosphorylation of proteins during phosphate deficiency. In *Arabidopsis*, phosphite suppresses the activity of nucleolytic enzymes, the expression of acid phosphatases, and the genetic carriers of phosphate (Ticconi *et al*., 2001). Phosphite has also been shown

to have a fungicidal effect on oomycetes (Orovic *et al.*, 2008), particularly on the genus *Phytophthora* (Lobato *et al.,* 2008; Rebollar-Alviter *et al*., 2010).

The reported effects of phosphite on plant growth and yield have been contradictory. In species such as *Allium cepa* and *Brassica nigra*, negative effects were reported (Sukarno *et al*., 1993), but these effects were attenuated by administering phosphate (Varadarajan *et al*., 2002). However, Moor *et al.* (2009) found that fertilizing with phosphite did not affect strawberry growth or yield compared with traditional phosphate fertilization, although, it did increase the quality of the fruits by activating the synthesis of ascorbic acid and anthocyanins. On the other hand, Rickard (2000) reported that foliar phosphite increased the yield and quality of several cultivars. Furthermore, applying phosphite as a source of phosphorous had detrimental effects on plants suffering from phosphorous deficiency (Mcdonald *et al*., 2001; Singh *et al*., 2003; Lee *et al*., 2005; Schroetter *et al*., 2006). On the other hand, applying phosphite to plant roots in the presence of sufficient phosphorous was synergistic, promoted the absorption of phosphorous into tomato plants (Bertsch *et al*., 2009), and suppressed the negative effects of phosphite (Varadarajan *et al*., 2002). Thus, the effects of phosphite on plants depend strongly on the phosphorous state of the plant (Thao and Yamakawa, 2009).

Based on previous studies, a nutrient solution containing sufficient phosphorous in the form of phosphate was used for strawberry (cv. Festival). We evaluated the effects of different percentages of phosphite added to the nutrient solution on the concentration of total P in leaves and the activation of the antioxidant system, which determines the concentration of anthocyanins, yield, pH, electrical conductivity (EC), and strawberry fruit size.

2. Materials and Methods

2.1. Experimental conditions

The study was performed in a tunnel-type greenhouse located at 19° 29′ N, 98° 53′ W, at an altitude of 2,250 m. The diurnal temperature averaged 24 °C, while the nocturnal temperature averaged 11 °C. The luminosity averaged 530 mmol m^{-2} s^{-1}.

2.2. Plant material

Strawberry plants (*Fragaria x ananassa* Duch., cv. Festival) were established in red volcanic rock previously sifted to a particle size of 3 to 5 mm in diameter and placed in 30 x 30 cm black polyethylene bags. The distance between the plants was 30 cm and 1 m between rows.

2.3. Treatments and experimental design

Five nutrient solutions with optimum levels of macro- and micronutrients that differed only in the percentage of phosphite ($H_2PO_3^-$) were evaluated. These nutrient solutions were formulated using a modification of the Universal Nutrient Solution of Steiner (1984), where the concentrations for 100% of mol_c m^{-3} are 10.56 NO_3^-, 1.44 NH_4^+, 1.0 $H_2PO_4^-$; 7.0 SO_4^{2-}, 7.0 K^+, 9.0 Ca_2^+, and 4.0 Mg^{2+}. The solutions were complemented with a mixture of micronutrients having the following concentrations (mg L^{-1}): 1.6 Mn, 0.11 Cu, 0.865 B, 0.023 Zn, 0.048 Mo, and 5.0 F, where the Mn, Cu and Zn were supplied in the form of sulfates, B as H_3BO_3, Mo as H_2MoO_4, and Fe as Fe-EDTA according to Steiner and van Winden (1970). The concentrations of phosphite evaluated in the nutrient solution were 0, 20, 30, 40 and 50% relative to the total P in the nutrient solution (phosphate + phosphite). The phosphite was supplied as phosphonic acid (H_3PO_3), and the pH was maintained between 5.5 and 5.8 to ensure phosphite availability (Hanrahan *et al*., 2007). The addition of phosphite was performed during 48 days throughout the flowering phase, using a 50% Steiner's nutrient solution; and during 135 days in the course of the fruiting phase, using a 75% solution. A generalized randomized block design (RGB) was used in which each of the five blocks had the treatment applied three times (15 replicates per treatment). The experimental unit was a 30 x 30 cm black polyethylene bag containing one strawberry plant.

2.4. Evaluated variables

Leaf P concentration

The plant P concentration was determined by digesting dry leaf tissue with a mixture of perchloric and nitric acid (Alcántar and Sandoval, 1999). Two sampling dates were carried out: the first one 30 days after treatments, and the second one 131 days after treatments, corresponding to the flowering and fruit development stages, respectively. The extracts were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (VARIAN™, Liberty II, Mulgrave, Victoria, Australia).

Yield and fruit size

Over a four month period (from day 55 through day 183 after Phi treatments application), the total yield was recorded by weighing the fruits from each plant, and individual fruit size was assessed by measuring the fruit length and diameter using Vernier calipers.

Biochemical parameters of fruit quality

The pH and EC were determined from the fruit pulp 122 days after treatments, which was obtained by blending 10 g of fresh fruit with 50 mL of distilled water and then inserting a potentiometer (Conductronic, PC18, Puebla, Mexico). The concentration of anthocyanins in the fruit (at day 126 after treatments) was determined using the methods described by Mancinelli *et al.* (1975), using a 95% methanol extract and 1.5 N HCl in a 85:15 (v/v) proportion and a spectrophotometer (Espectronic 20, Bausch & Lomb, USA) at 530 nm.

2.5. Statistical analyses

First of all, preliminary data analyses were carried out by using the Shapiro-Wilk and Kolmororov-Smirnov tests in order to determine a normal distribution of data; besides, the Levene, O'Brien and Bartlet tests were performed to verify the homogeneity of variance. Subsequently, an analysis of variance (Proc ANOVA) was performed and the means (fixed effects) were compared by a Tukey-test (α=0.05). The analyses were brought about using the Statistical Analysis Systems software, ver. 9.3 (SAS Institute Inc., 2011).

3. Results and Discussion

3.1. Leaf P concentration

In Figure 1, the total P concentration in the leaves of plants during flowering and fruit development are shown. Higher concentrations of P were measured during fruit development, which was independent of the treatment and exhibited a positive correlation with the concentration of phosphite supplied. During the flowering phase, no trend in the total leaf P concentration was observed that could be attributed to the treatments. Importantly, during the flowering stage, two aspects must be pointed out. Firstly, the interval of P considered as sufficient for strawberry leaves ranges from 2.5 to 4 g kg^{-1} DM (Hancock, 1999). Although differences among treatments concerning P concentrations in leaves during the flowering stage are observed, data obtained in our experiment are considered among those intervals cited above. This indicates that Phi applications did not produce any abnormality in P concentration in leaves (i. e. out of the range described). Accordingly, Ávila *et al.* (2013) evaluated different dosages of Phi in *Phaseolus vulgaris* and found that the tissue P concentration and the total P accumulation in shoots and roots of Pi-sufficient plants were not significantly affected by Phi treatments applied in nutrient solution. Interestingly, the treatment using the highest level of Phi (50%) brought about the highest P concentration in leaves. This response may be attributed to the fact that under this Pi:Phi ratio (50:50) in the nutrient solution, a Pi deficiency may occur, which triggers a higher accumulation of P in leaves. Similarly, in *P. vulgaris* Pi-starved plants at the highest Phi level (512 µM Phi) there was a substantial increase in P concentration, corresponding to 7.2-fold in shoot and 11.7-fold in root (Ávila *et al.*, 2013).

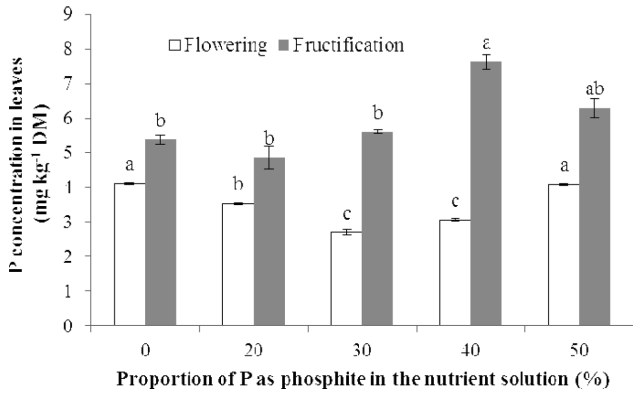

Figure 1. Concentration of P in strawberry leaves (cv. Festival) treated with five different concentrations of P as phosphite in the nutrient solution during two phenological phases. Means with different letters are significantly different among treatments. Error bars indicate ± SD ($p<0.0001$ for P concentration during the flowering stage; $p=0.0101$ for P concentration during the fructification stage; $n=5$).

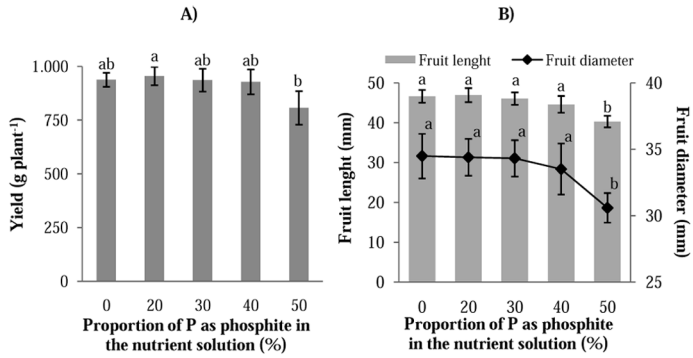

Figure 2. Strawberry (cv. Festival) yield totaled over four months (from 51 to 170 days after treatment) (A) and fruit size (B), during treatment with five different concentrations of phosphorous in the form of phosphite in the nutrient solution. Means with different letters are significantly different among treatments. Error bars indicate ± SD [($p=0.0179$ for yield; n=10); ($p<0.0001$ for length and diameter of fruit; $n=32$)].

The highest total P content in leaves was observed in plants treated with high concentrations of phosphite (40 and 50% of the total P) during fruit development (Figure 1). Schink and Friedrich (2000) reported that due to the low redox potential of phosphite oxidation to phosphate, the plants do not utilize phosphite as a source of P. Nevertheless, it has been found that phosphite treatments increase the total P concentration in lettuce sprouts and roots, and in Japanese spinach (*Brassica rapa* var. Komatsuna) (Thao and Yamakawa, 2009).

3.2. Yield and fruit size

Yield was significantly affected (p=0.0292) by the treatments. The highest yield was obtained by supplying 20% of the total P as phosphite (955.63 g plant^{-1}). However, there were no significant differences between this treatment and the control or with phosphite treatments of 30% and 40%. Plants treated with 50% P as phosphite in the nutrient solution had 15.54% yield reduction in comparison to those receiving 20% (Figure 2A).

Watanabe (2005) reported beneficial effects of phosphite on the development of cucumber and the performance of Satsuma oranges. However, Schroetter *et al.* (2006) ahowed negative effects of foliar phosphite application on performance. Furthermore, these effects were more severe when plants were grown in P deficient soils. Similarly, Ratjen and Gerendás (2009) found that applying phosphite to soil as a P source for zucchini cultivation caused phytotoxicity, which inhibited the formation of flowers and fruits. In this study, strawberry performance decreased with increasing concentrations of phosphite in the nutrient solution from 30% P as Phi on, though the performance between treatments was not significantly different from the control (Figure 2A). These results are consistent with those reported by Moor *et al.* (2009), who found that fertilizing strawberry (cv. Polka) with phosphite does not increase yield compared to traditional phosphate fertilization. Moreover, Estrada-Ortiz *et al.* (2011) reported differential effects of phosphite on strawberry based on the phenological stage and found the fruiting stage to be more sensitive than the flowering stage. The addition of 30% of the total phosphorus as phosphite stimulated plant metabolism and increased the concentrations of chlorophyll a and b, total amino acids, and proteins in the leaves.

Fruit length was reduced in plants receiving 50% P as phosphite in the nutrient solution (p=0.001), and the same trend was observed for fruit diameter (Figure 2B). The remaining phosphite treatments exhibited no significant differences in fruit size compared to the control. A 50:50 ratio of phosphite:phosphate in the nutrient solution significantly reduced the size of the fruit (Figure 2B). Negative effects of Phi increasing levels on plant yield are due to a significant reduction of the availability of P as phosphate for plants. Although Phi can be uptaken and transported by plants, the ion is not involved in P metabolism and even more, the Phi is not converted into phosphate inside plants (Varadarajan *et al*., 2002).

3.3 Biochemical parameters of fruit quality

Plants treated with the nutrient solution containing 30% P as phosphite produced fruit with the highest pH (3.41) (p=0.0009), which was similar to plants receiving control, 20% and 40% phosphite treatments. The treatment with the highest phosphite concentration in the nutrient solution (50%) had the most acidic fruits (pH=3.30) (Figure 3A).

Similarly, the EC also exhibited significant differences among treatments (p=0.0426). The highest EC value for fruit pulp (1.04 dS m^{-1}) was obtained using the 30% phosphite nutrient solution, while the lowest value (0.97 dS m^{-1}) was obtained using the 40% P as phosphate solution (Figure 3A). The fruit EC values showed low variation among the treatments, while the pH of the fruit was highly influenced by the phosphite concentration in the nutrient solution.

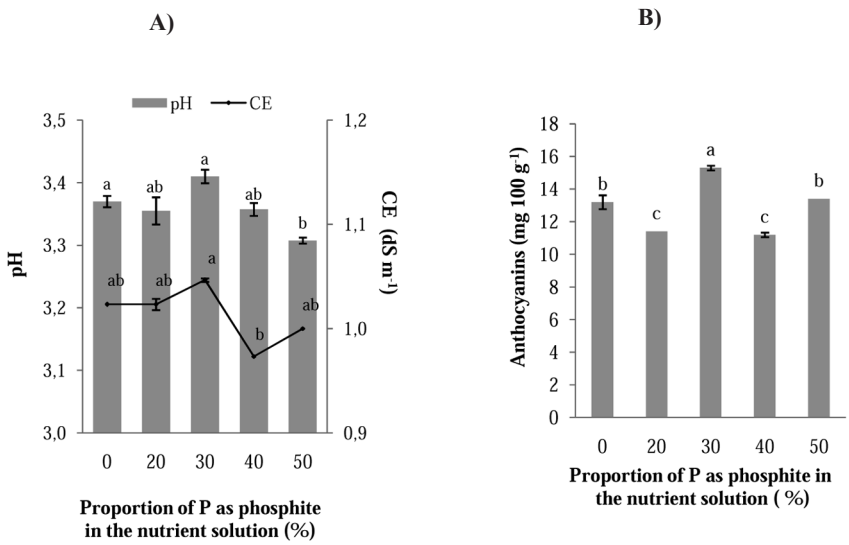

Figure 3. Values of pH and EC (A) and anthocyanin concentrations (B) in strawberry (cv. Festival) fruits treated with five different concentrations of P as phosphite in the nutrient solution. Means with different letters indicate significant differences among treatments. Error bars indicate ± SD [(p=0.0009 for pH; p=0.0426 for EC; n=4); (p=0.0009 for anthocyanin, n=5)].

The salt concentration in the fruit was little affected by the addition of phosphite, except in plants treated with 40% phosphite, which had the lowest EC value. On the other hand, fruit pH was affected more by the treatments (Figure 3A). Pérez de Camacaro *et al.* (2005) found that fruits with a more acidic pH had lower quality and were less attractive for fresh consumption. These results support those of Roudeillac and Trajkovski (2004), who showed that the pH of strawberry fruit should be a minimum of 3.7, regardless of the cultivar, although no specific information is available for the cv. Festival. The anthocyanin concentrations in the fruit were significantly different among treatments (p=0.0009). The treatment with 30% phosphite nutrient solution had the highest concentration of anthocyanins (15.3 mg 100 g^{-1}), indicating that this concentration of phosphite had the greatest influence on the plant immune responses (Figure 3B). Likewise, Estrada-Ortiz *et al.* (2012) report that the addition of 20% P as phosphite improved some features associated with strawberry fruit quality, including total soluble sugars, Brix, and fruit firmness.

The anthocyanin values obtained from the strawberries (cv. Festival) oscillated between 11.2 and 15.3 mg 100 g FW^{-1}, which is near the lower limit of the interval reported by Da Silva Pinto *et al.* (2008) for seven different cultivars of strawberry (12.4 to 44.2 mg 100 g FW^{-1}). The highest concentration of anthocyanins in fruit was found for the 30% phosphite treatment, which indicates that this concentration of phosphite in the nutrient solution promoted the production

of anthocyanins (Figure 3B). Moor *et al*. (2009) also suggested that adding phosphite increases the concentration of anthocyanins in fruit. In Arabidopsis, anthocyanins accumulation was recorded during P deficiency and in the presence of phosphite (Ticconi *et al.*, 2001). The importance of anthocyanins, besides functioning as an antioxidant, is directly related to the color of the strawberry fruit (Yoshida *et al*., 2002). As the concentration of anthocyanins in strawberry fruit increase, the hue angle and luminosity decrease.

4. Conclusions

During the fruit development phase, when using a Phi concentration from 20 to 40% in the nutrient solution, the concentration of P in the leaves was proportional to the level of phosphite applied. When applying P as Phi at concentrations up to 40% in the nutrient solution, no significant effects are observed in comparison to control plants. The pH, EC and anthocyanin concentration in the fruit benefited by supplying 30% phosphite. However, phosphite concentrations equal to or higher than 30% in the nutrient solution negatively affected fruit performance and quality, indicating that the supply of P as phosphite was sufficient. Our findings suggest that supplying 20% phosphite in the nutrient solution improved strawberry (cv. Festival) fruit performance and that supplying 30% phosphite activated defense mechanisms in the plants, which increased the concentration of anthocyanins and improved fruit quality.

Acknowledgements

We thank the National Council of Science and Technology (CONACYT) from Mexico for awarding EEO a Master of Science fellowship and to the Priority Research Line 5 (LPI5) from Colegio de Postgraduados for research facilities and support.

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