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Availability of phosphorus in greenhouse cropping systems with tomatoes - influence of soil and citric acid -

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Abstract
Analyses of plant sap from organic greenhouse tomato crops show that the levels of phosphorus (P) are frequently low, despite the fact that soil analyses indicate P status in the soil to be good. In the present study, two soils (A and B) with a pH just over 6 and a high total content of N and P were investigated with respect to release of P and uptake in a tomato crop. The fertilisers primarily used on soil A was silage and bloodmeal, and on soil B Vinasse and blood meal. An incubation experiment showed that the release of P per unit time was greater from soil A than soil B. This difference between the two soils may be due to lower soil organic matter concentration, with less P sorbed by Al and Fe on soil A, and a fertiliser regime which favours a high concentration of dissolved organic carbon in this soil. Tomato plants grown for 10 weeks in the soils had greater DW production, total uptake of P and concentration of P in the leaves on soil A than on soil B. Addition of citric acid to the soils in order to mobilise P increased plant yield and uptake of N and P on soil A. Despite the increased growth, the concentration of P in plant leaves increased and the N concentration was unchanged. On soil B addition of citric acid decreased Zn uptake in the tomato plants despite good availability, resulting in a lower concentration of this micronutrient in the leaves. The plants on soil A maintained their concentrations of all micronutrients analysed, but the ratio of P to micronutrients increased. Thus for commercial organic tomato growers, adding citric acid with the irrigation water offers a possibility to increase P availability.

Keywords: Phosphorus, Nitrogen, Micronutrients, Tomato crop, *Solanum lycopersicum* Mill., Citric acid

Introduction

For the past ten years, a participatory research group in central Sweden has been working on issues relating to organic production of greenhouse tomatoes. An important issue for the growers in the group is to have a fertilisation strategy that prevents nutrient deficiency developing in the crop. Analyses of plant sap from existing commercial crops show that the levels of phosphorus (P) are frequently low (Magnusson *et al*., 2010), despite the fact that soil analyses indicate P status in the soil to be very good.

Phosphorus can be easily fixed in soils, primarily by sorption to mineral surfaces and soil organic matter (SOM) and by ionic association between P and iron (Fe), aluminium (Al) and calcium (Ca). A study by Ohno *et al.* (2007) showed that soils with high levels of SOM tended to have less P in solution than soils with lower levels of organic matter. The higher SOM levels were associated with higher levels of oxalate-extractable Fe and Al, and therefore higher P sorption capacity and lower sorption site occupancy. Zhang *et al.* (2005) suggested that the correlation between SOM and P sorption is the result of P sorption by Al and Fe associated with organic matter.

When soils are deficient in P, plants exude low-molecular-weight organic acids (LOAs) which can mobilise P. Therefore, a strategy to increase P availability can be to add LOAs to the growing medium. LOAs increase P availability primarily by desorption and solubilisation mechanisms. Desorption of P by addition of LOA decreases in the order tricarboxylic acid (i.e. citric) > dicarboxylic acid (i.e. malic, tartaric, oxalic) > monocarboxylic acid (i.e. acetic, formic, lactic) (Bolan *et al*., 1994; Oburger *et al*., 2011). Solubilisation is achieved by formation of organic-metal complexes (chelate) between LOAs and ions associated with P (Bolan *et al*., 1994; Hariprasad & Niranjana, 2009). The complex formation of LOAs depends on the stability constant for metal ions, which in turn depends on the number of -COOH ions and the solubility factor (pKa), with e.g. a tricarboxylic acid such as citric acid being more effective than dicarboxylic and monocarboxylic acids.

Another factor that may influence P availability to plants is fertilisation regime. Studies by Ohono & Crannell (1996) and Ohono *et al.* (2005) have shown that use of organic amendments such as animal and green manure can increase soil P availability, animal manure probably having a more long-term effect than green manure. In contrast, a very stable organic material such as sphagnum peat moss has little effect on P accumulation in plants, according to Barker (2012).

Availability of P also affects the availability of other plant nutrients. Phosphorus deficiency can give rise to nitrogen (N) deficiency through disruption of protein synthesis in the plant (Jeschke *et al.*, 1997; de Groot *et al*., 2003). Formation of organic-metal complexes (chelates) increases the plant availability of micronutrients such as Fe, zinc (Zn), manganese (Mn) and copper (Cu), but at the same time high P levels can cause deficiency of micronutrients through antagonism during uptake in the plant (Epstein & Bloom, 2005).

The aim of the present study was to examine the effects of adding citric acid to the growing medium on P availability to a greenhouse-grown tomato crop. The soils and greenhouse-grown tomato plants were investigated with respect to:

- Release and uptake of P from soils with different chemical composition and fertilisation strategy
- The effects of adding citric acid with the irrigation water applied to the soils on: i) yield, and ii) uptake and concentrations of P, N, Fe, Mn, Zn and Cu.

Materials and methods

The study was based on two soils (A and B) taken from sites where organic tomato production had been carried out for a long time. The fertiliser used for soil A was based on farmyard manure, silage and bloodmeal, while the fertiliser used for soil B was based on Vinasse, a by-product from the yeast industry that is high in N and K and low in P (Ekoväx, Slättegård), Kalimagnesia (a potassium-magnesium fertiliser) and blood meal.

The experiments on soils A and B were carried out in 2008 (year 1) and 2009 (year 2). In year 1, an incubation experiment was carried out without plants and a greenhouse experiment with tomatoes. In year 2, another greenhouse experiment was carried out on tomatoes, but in this case citric acid was added with the irrigation water in one of the two treatments per soil.

Characterisation of greenhouse soils

Soil samples were taken in four beds at the two commercial sites at fixed points 0.2 m from the plants (surface soil was scraped away before sampling) to a depth of 0.3 m, with 10 sampling per bed. Each bed represented one block in the experiments. Soil A consisted of 17% clay, 9% silt, 66% sand and 16% gravel and had an organic matter content of 16% =124%. Soil B consisted of 13% clay, 28% silt, 57% sand 2% gravel and had an organic matter content of 23%. The high levels of soil organic matter (SOM) content reflect previous organic matter amendments.

The total content of N and P was high in both soils (Table I). However, the concentration of N, P, Mn and Zn was higher in soil B (Mn and Zn were nearly twofold higher than in soil A), while the concentration of Cu and Fe was higher in soil A. The pH of both soils was just over 6, which was maintained throughout the experiment.

Table I. Chemical composition (two-year means) of greenhouse soils A and B, which were taken from sites of commercial organic tomato production in Sweden.

Soil	N	P	Fe	Zn	Mn	Cu	pH
		%			$mgkg^{-1}$		H_2O
A	0.62	0.26	1.23	62.4	272	26.3	6.2
B	1.08	0.40	0.85	118,0	538	19.2	6.4

The results of P fractionation according to Hedley *et al*. (1982) with some modifications (Otabbong & Persson, 1994) are shown in Table II. The pH in both soils was below the threshold at which Al and Fe dominate P sorption reactions (pH 8) (Tisdale *et al*., 1999). The P sorption capacity is often assumed to equal the sum of oxalate-extractable Al and Fe in low pH and non-calcareous soils (Zhang *et al*., 2005). In the present study oxalate-P was 1 501 mg kg^{-1} DM soil in soil A and 2 252 mg kg^{-1} DM in soil B. In soil A, the concentration of organically bound P was 1 106 mg kg^{-1} DM soil (~38%), while in soil B the corresponding value was 1 828 mg (~45%).

Table II. Soil P disposition in soil samples from greenhouse soils A and B, which were taken from sites of commercial organic tomato production in Sweden. The fertilisation regime was based on farmyard manure, silage and bloodmeal on soil A and Vinasse, Kalimagnesia and bloodmeal on soil B.

	Soluble P	Al-P		Fe-P		Ca-P	Mineral P	Total P
	Total	Total	Organic	Total	Organic	Total	Total	
Soil	mg kg^{-1} DM soil							
A	151	948	761	553	345	277	964	2893
B	86	1072	930	1180	898	441	1282	4061

Incubation experiment

The release of P was monitored for 12 weeks in a dark climate chamber kept at a temperature of 20 °C. The soil samples (300 g DM of soil A and 200 g DM of soil B) were placed in 1-L pots without lids. Soil A contained 0.9 g total P and soil B 0.8 g total P. Distilled water was then added corresponding to the equivalent of 40% of water-holding capacity (WHC). Additional distilled water was added once a week to bring the weight back to the original level. The experiment included four replicates per soil and samples were taken at the start and then every two weeks (in week 0, 2, 4, 6, 8, 10 and 12). On each sampling occasion all soil in the pots was removed and frozen.

Greenhouse experiments

The soil from the cultures was collected and placed in boxes (0.4 m x 0.4 m x 0.5 m) with drainage holes in the base. Distilled water was then added corresponding to 50% (year 1) and 40% (year 2) of WHC. The amounts of fresh (FW) and dry (DW) soil, N and P added to the boxes with the soil are shown in Table III.

Table III. Total amounts of plant fresh (FW) and dry (DW) weight produced on soil A and B and of N and P added with the soil in greenhouse experiments on tomatoes in years 1 and 2. Soils A and B were taken from sites of commercial organic tomato production in Sweden.

	FW soil (kg)		DW soil (kg)		N (g)		P (g)	
Soil	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
A	30	35	20.1	25.2	127	156	54	66
B	32	30	17.6	16.8	178	193	74	64

Six-week-old tomato plants of the variety Mecano with two tops, grafted on Maxifort, were planted on 12 March in year 1. In year 2, single-top plants of the variety Maranello, grafted on Beaufore, were planted on 20 March. The experiments, which were surrounded by border plants to even out climate differences within the greenhouse, had a completely randomised block design with four replicates, the treatments being randomly placed in each block. The temperature in the greenhouse was kept at ~19 °C during the day and ~16 °C at night. The plants were pollinated with the aid of bees. No fertilisers were added during the experiment.

In year 2, citric acid was added with the irrigation water (12 g per 25 L water) in a mixture with a pH of 3.6. This pH value lies within the range normally found in the rhizosphere (Oburger *et al.*, 2011).

The trials were terminated 10 weeks after planting, just before the first harvest, i.e. at a time when the plants are most heavily loaded and nutrient deficiency can easily develop. Yields of FW and DW were recorded for each plant. Leaf samples (three per apex) were taken from every plant; over the first truss, centrally and the upper 20 cm long leaf. The stem sample consisted of three 10 cm long pieces including a truss (without fruit), which were taken from the plant base, centre and above the topmost flowering truss. The oldest fruit in each fruit-bearing truss were pooled to one bulk sample. All plant material was dried at 80 °C and milled before chemical analysis.

Chemical analyses

Soil texture was characterised by the pipette sedimentation procedure (Ljung, 1987). pH in air-dried soil was determined in water extracts, 2:1 by volume and organic matter concentration by loss on ignition, corrected for H_2O in the clay fraction. Before chemical characterisation, the soil was dried at 35 °C and sieved (2 mm mesh). The total content of N in soil and plants was analysed on a LECO analyser (CN 2000 USA) according to the Dumas method (Bremner & Hauk, 1982). Analyses of other plant nutrients were performed using an inductively coupled plasma emission spectrometer (Perkin Elmer Optima 3000 DV) after wet digestion with nitric acid (HNO_3) in the greenhouse experiment, while available P in the soil in the incubation experiment was analysed after extraction with acetic acid solution, pH 3.4 (Spurway method). Total nutrient contents were calculated based on DW (105 ºC).

Statistical analyses

In the incubation experiment the variables acetic acid-extractable P and week were analysed statistically using a fitted linear model comprising the factor soil type. In the greenhouse experiments the analyses aimed to assess the effects of the factor soil type and in year two addition of citric acid on the variables tomato yield and nutrient uptake using a fitted linear model. When significant effects were found, Tukey´s method was used to compare treatment means. Means were considered significantly different at the 0.05 level. All statistical analyses were performed using JMP (9).

Results

Soil P content and tomato crop growth and nutrient uptake, year 1

The content of acetic acid-extractable P in the incubation experiment was greater in soil A than soil B ($p=0.003$), as can be seen from the slope of the line in Figure 1. In other words, release of P per unit time was greater from soil A than soil B.

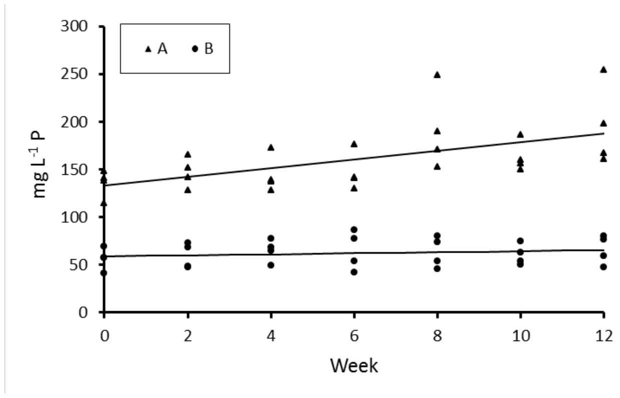

Figure 1. Content of acetic acid-extractable P (mg L^{-1} P) in soil A and soil B during the 12-week incubation experiment without plants.

In the greenhouse experiment, DW production at the end of the 10-week experimental period was greater on soil A than on soil B, as were the total uptake of P and the concentration of P in the leaves (Table IV). However, yield of tomatoes was unaffected by soil.

Table IV. Yield of total biomass (DW) and fruits (FW), total P uptake in the plant and concentration of P in the leaves at the end of a 10-week greenhouse experiment with tomatoes grown on soils A and B, which were taken from sites of commercial organic tomato production in Sweden.

	Plant biomass			Tomato fruit
	DW	P		FW
Soil	g $plant^{-1}$	g $plant^{-1}$	% in leaf	kg $plant^{-1}$
A	391a	1.7a	0.49a	2.3a
B	315b	0.8b	0.20b	2.3a

Means within columns followed by different letters are significantly different at $P<0.05$

Effects of adding citric acid with the irrigation water

On soil A, addition of citric acid increased the yield of the tomato plants in terms of total DW and fruit yield (FW), and also increased uptake of N and P (Table V). Despite the increased growth, the concentration of both N and P in the plant leaves was maintained at the same level or even increased. No such differences were observed on soil B. The concentration of N and P in the leaves was low overall compared with published values (Bergmann, 1992), except when citric acid was added to soil A.

Table V. Effect of adding citric acid to the irrigation water on yield of total biomass (DW) and fruits (FW), total P uptake in the plant and concentration of P in the leaves at the end of a 10-week greenhouse experiment with tomatoes. Soils A and B were taken from sites of commercial organic tomato production in Sweden.

		Plant					Tomato fruit
		DW	N		P		FW
Soil	Treatment	g $plant^{-1}$	g $plant^{-1}$	% in leaf	g $plant^{-1}$	% in leaf	kg $plant^{-1}$
A	Control	381c	10.2b	3.6a	1.2b	0.3b	3.1c
	Citric acid	506a	12.9a	3.6a	2.1a	0.5a	4.3a
B	Control	400bc	8.5b	2.7b	0.9c	0.1c	3.4bc
	Citric acid	457ab	8.5b	2.2b	1.0bc	0.1c	4.0ab

Means within columns followed by different letters are significantly different at $P<0.05$

As can be seen in Table VI, the concentration of Fe and Zn in the leaves was adequate (Bergmann, 1992) on both soils, while the concentration of Mn was low on soil A and that of Cu was low on soil B. Addition of citric acid with the irrigation water decreased Zn uptake in the tomato plants on soil B, while it increased Cu uptake in the plants on soil A. The concentration of Zn and Mn in the leaves decreased on soil B.

Table VI. Effect of adding citric acid to the irrigation water on total P uptake in plants and concentration of micronutrients in the leaves at the end of a 10-week greenhouse experiment with tomatoes. Soils A and B were taken from sites of commercial organic tomato production in Sweden.

		Cu		Fe		Mn		Zn	
Soil	Treatment	mg plant^{-1}	ppm in leaf	mg plant^{-1}	ppm in leaf	mg plant^{-1}	ppm in leaf	mg plant^{-1}	ppm in leaf
A	Control	2.0b	6.7a	20.5a	110a	5.5b	30bc	8.9c	40bc
	Citric acid	2.9a	7.2a	25.5a	100a	6.8b	30c	11.7c	30c
B	Control	1.2c	4.1b	23.8a	110a	9.3a	60a	20.4a	90a
	Citric acid	1.1c	3.4b	23.8a	90a	8.5a	40b	17.1b	60b

Means within columns followed by different letters are significantly different at $P<0.05$

The ratio of P to the micronutrients Mn, Fe, Zn and Cu in leaves increased with addition of citric acid to the irrigation water to soil A (data not shown). No such tendency was found for soil B.

Discussion

In the two soils used in this study, SOM and P sorption capacity were higher in soil B than in soil A (Tables II). In addition, P sorbed by Al and Fe associated with SOM was higher in soil B than in soil A (Table II). The release of P in the incubation experiment and the uptake of P in the greenhouse experiment in the same year were lower for soil B than soil A (Figure 1, Table IV). This agrees with previous findings by Ohno *et al.* (2007) that a higher SOM level is associated with higher P sorption capacity and lower sorption site occupancy. Börling *et al.* (2001) and Zhang *et al.* (2005) suggest that this association is the result of P sorbed by Al and Fe associated with SOM.

The greater release of P from soil A than soil B may also be due to differences in fertilisation strategy. The addition of animal manure and silage to soil A probably increased the concentration of dissolved organic carbon (DOC), both directly and via turnover of native SOM, which Ohno *et al*. (2005) found had a linear relationship with water-soluble P concentration.

Addition of citric acid with the irrigation water in the greenhouse experiment in year 2 increased P uptake by the tomato plants on soil A (Table V). The concentration of P in the leaves was rather low (< 0.4%) on both soils, but increased in soil A when citric acid was added. This means that the availability and uptake of P increased at a faster rate than biomass growth when citric acid was added to this soil. The efficiency of LOAs in mobilising P is a function of both the amount of sorption sites and their occupancy and the P concentration in soil solution (Bolan *et al*., 1994; Guppy *et al*., 2005; Oburger *et al*., 2011). In a study with four LOAs and five soils, Oburger *et al*. (2011) observed the greatest effect at a medium to high amount of sorption sites and occupancy. They also found that in a Cambisol soil, P solubility seemed to be controlled by Al and Fe solubility, which in turn was predominantly influenced by the type of LOA. In their case citric acid caused the highest relative solubility.

Addition of citric acid with the irrigation water in the present study also increased total uptake per plant of N on soil A (Tables V and VI). The plants on this soil maintained or increased (Cu) their concentrations of all micronutrients, but the ratio of P to micronutrients increased, i.e. more P was taken up in relation to Fe, Zn, Mn and Cu. This may be partly due to slow release of these micronutrients compared with the rate of P release and partly to increased competition for uptake by the plant (Epstein & Bloom, 2005). On soil B uptake of Zn decreased, despite good availability, resulting in a lower concentration of this micronutrient in the leaves. Similarly, Zhang *et al*. (2012) found that P application depressed Zn concentration in winter wheat, while the effect of P application on Mn, Fe and Cu differed. As the content of DTPA-extractable Zn in soil was not reduced and the decreased Zn concentration could not be explained solely by the 'dilution effect', those authors argued that increased P availability in soil decreased Zn uptake by roots and/or Zn translocation from root to shoot.

Overall, the present study showed that for commercial organic tomato growers, adding citric acid with the irrigation water offers a possibility to increase P availability and thus use this limited resource in a more sustainable way.

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