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Polyphosphate

The impact of polyphosphate on growth and nutrient uptake of *Pelargonium x hortorum* 'Mårbacka' and *Petunia x hybrida* 'Origami Watermelon'.

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Polyfosfat

Effekten av polyfosfat på tillväxt och näringsupptag hos *Pelargonium x hortorum* 'Mårbacka' och *Petunia x hybrida* 'Origami Watermelon'.

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Abstract

Phosphorus (P) is an essential macronutrient for plants. For example, it is required for the transfer of energy, in the photosynthesis and as a component of the phospholipids in the cell membrane. Phosphorus can exist in several different forms, depending on the soil's pH-value. The plant can only absorb the primary ($H_2PO_4^-$) and the secondary (HPO_4^{2-}) forms, named orthophosphate (OP). Polyphosphate (PP) is a general term for several linked water soluble P molecules. The molecules are linked to each other by oxygen atoms, which determine the molecule's chemical properties and stability. PP are described as good sources of P fertilizers because of their water solubility and high concentration of P, which are said to increase the plant growth capacity by generating a better root system and growth. A more vigorous root system will generate a faster, stronger growth and earlier flower development. Condensed PP are also of interest as a micronutrient carrier.

The main question in this study is: can PP contribute to a better growth of plants than OP as a P-source? This paper provides a literature review and results from a greenhouse experiment with the aim to examine PP effect on growth and nutrient uptake. The Greenhouse experiment consisted of irrigation with a solution composed of 66 % PP and 34% as OP. The control was a solution containing 100 % OP applied as monokaliumphosphate KH_2PO_4. Model plants used were P*elargonium x hortorum* 'Mårbacka' and *Petunia x hybrida '*Origami Watermelon'. The trial continued for six weeks in controlled climate conditions desirable for optimum growth. Factors that were measured during growth and harvest were plant height, branching, leaf and flower development, and root development. Also, nutrient uptake was studied by leaf sample analysis. The results showed no significant difference between PP and control either on growth or on nutrient uptake.

The literature aimed to treat and compile the existing information that is published on PP impact of growth and the underlying mechanism behind. PP efficiency is relatively unexplored. There is literature that demonstrates both a better P utilization with PP compared with OP but also studies showing no increased effectiveness in compare to OP. The effectiveness of PP as P source depends on the soil's chemical reactions and environmental factors such as time, substrate, pH, and temperature and the plant's growth stage.

Content

Vocabulary

P – Phosphate

OP – Orthophosphate

PP – Polyphosphate

Hydrolysis – A chemical process when molecules are cut into two different parts by the addition of water molecules.

Oligomers - Composition of x number of molecules.

Sorption – A chemical process by which one substance get attached to another.

Chelated – A metal that is attached to an anion with more than one site.

Leaf analysis - Measurement of essential nutrient concentration of plant tissue by laboratory analysis.

Introduction

Phosphorus (P) is an essential macronutrient for plants and an essential fertilizer for successful crop production [Syers et al. 2008]. Phosphorus can exist in several different forms, depending on the soil's pH-value [Syers et al. 2008]. The plant can only absorb the primary ($H_2PO_4^-$) and the secondary (HPO_4^{2-}) form, named orthophosphate (OP) [Syers et al. 2008]. But the dynamics of P in the soil is complicated and plant availability of the P applied varies [Dick, 1985].

Phosphorus is not readily mobile in the soil, which means that roots must reach out to the P bound to the soil particles [Beegle, 2015]. The plant's P uptake highly depends on its root architecture and available nutrients in the soil solution [Syers et al. 2008]. Besides the genetic traits of the root, external factors in the root zone environment such as soil texture, acidity, degree of compaction and the atmospheric composition of the soil influence the uptake [Beegle, 2015].

Polyphosphate (PP) is a general term for several linked water soluble P molecules [McBeath, 2006]. Addition of PP in the nutrient solution is said to increase the plant quality [Van Schie, 2014]. Polyphosphate is claimed to give a stronger, more vigorous root system and generates a faster growth, stronger plants and earlier flowering [Van Schie, 2014].

Aim

The aim of this work is to investigate if the presence of PP affects plant growth and root development and furthermore, to study the plant P utilization and uptake of macro- and micronutrients in the presence of PP.

Specific questions that should be answered are:

- What effect has the availability of PP on plant growth?
- What effect has the availability of PP on plant root growth?
- How does the availability of PP influence plant uptake of micro and macro nutrients?

Limitations

There are several factors affecting the growth of plants, but this study will only take into account P, PP and associated factors that affect plant growth, root development and nutrient uptake.

Background

Phosphorus

Phosphorus (P) is an essential macronutrient for plants and represents approximately 0,12% of the earth's crust [McBeath, 2006]. Phosphorus is essential for all forms of life because of its genetic role [Uchida, 2000]. It is required for the transfer of energy, in the photosynthesis, in the phospholipids of the cell membrane and are a part of the DNA and RNA constructions [Uchida, 2000]. The highest concentrations of P are in the seed and P is required in high quantity during cell division and for metabolism [Uchida, 2000]. The development of root, flower, fruit and seed are therefore highly dependent on the quantity of P [Uchida, 2000].

There are three major P fractions in soils; 1) organic P, which may account for up to 50% of the total P; 2) insoluble inorganic fraction and 3) a very small, highly variable, soluble fraction that can be absorbed by plants [Dick, 1985]. The distribution of available P is determined by the number of dissolved minerals, amount and stage of breakdown of organic remains, soil pH, mineralization of organic P and the activity of microorganisms will also affect the availability [Havlin et al.1999].

Phosphorus exists in several different forms, depending on the soil's pH [Syers et al. 2008]. The plant can only adsorb the primary ($H_2PO_4^-$) and the secondary (HPO_4^{2-}) orthophosphate (OP) forms as P sources [Syers et al. 2008]. At pH below 7.2 the $H_2PO_4^-$ dominates and at pH above 7.2 HPO_4^{2-} is the dominating form [McBeath, 2006]. At pH 7.2, the concentrations of the two forms are equal in the soil solution and at this level the maximum of plant available P occur [Thomason, 2002]. The primary form of OP is absorbed more effective than the secondary OP form in plants [Spectrum Analytic Inc, 2015; Menzies, 2009].

Movement of phosphorus in soil

Nutrient becomes available to the roots through mass flow by the roots absorption [Barber et al. 1963]. Mass flow can supply the roots with much of the plants needs for nutrients such as nitrogen (N), magnesium (Mg) and calcium (Ca) [Barber et al, 1963]. But in some soils mass flow does not supply enough of the necessary P since P is strongly fixed in the soil [Barber et al. 1963]. P will then primarily be transferred by diffusion which, compared to mass flow, is a very slow process [Barber et al. 1963].

During diffusion, ions move along a concentration gradient towards the root, from a higher to a lower concentration [Syers et al. 2008]. When P ions are absorbed by the roots from the inner solution, the concentration of P ions is reduced [Syers et al. 2008]. This results in a concentration gradient driving the diffusion of nutrients towards the roots again [Barber et al.1963].

Root structure

Different plants have different requirements and adaptability to different environmental conditions which generates a significant morphological diversity among the roots' structures [Harper et al. 1991]. The study of the root structure, its shape and development can give an evolutionary answer to what happens when certain resources are lacking [Harper et al. 1991]. Many plants have wide root systems, a feature possibly related to the time when they had to acquire nutrients from soils with very low concentrations of plant-available nutrients [Syers et al. 2008].

Plant development, health and productivity are directly depending on the root architecture [Lynch, 1995]. All factors affecting root growth negatively, also affects the root's ability to absorb P [Spectrum Analytic Inc, 2015]. A plant's root system is responsible for the attaching of the plant in the stratum, in an upright position, affecting its resistance to environmental factors such as wind and water [Kramer & Boyer, 1995].

Different root systems have different strategies to take up P from the soil [Föhse et al. 1988]. Some plants increase uptake rates per unit of root and others increase the size of their root system [Föhse et al. 1988]. The different strategies can vary within plant species, depending on hybrid and variety [Syers et al. 2008]. The plants strategy for root growth depends on plant genetics and soil properties [Spectrum Analytic Inc, 2015]. External factors also affect root growth and its function. Factors include soil properties such as volume, structure, stoniness, moisture retention and the soil atmosphere [Syers et al. 2008].

Phenotypes show different growth strategies regarding where the growth takes place, and where new lateral roots develops is influenced by a stimulus from the environment [Hodge et al. 2009]. One ecotype of a plant species may increase root growth rate at a certain stimulus, while another ecotype lack response to this stimulus [Gifford et al. 2013].

A plant's root architecture varies with its depth ability, elongation and density of lateral roots, root hairs and how the root system is branched in the soil [Hodge et al. 2009]. The primary root which is the first portion of the root that starts growing, develop from meristematic tissue [Hodge et al. 2009]. When the primary root grows with a low P availability there is a decrease in the primary cellular growth within the root system, affecting the root elongation zone [Hodge et al. 2009].

Phosphorus uptake and efficacy

P efficiency may be defined as the ability of a plant to produce 80% of its maximum yield at a certain level of P [Föhse et al. 1983]. In general, the nutrient uptake depends on two factors, the soil's supply of nutrients in an available form as well as the plant's uptake of the available nutrient [Beegle, 2015].

Different plant species differ in their ability to reach optimum growth and maximum yield [Föhse et al. 1988]. Cultivars within the same species can differ in their capacity for active P uptake and these differences are genetically controlled [Syers et al. 2008].

There are two ways in which different P efficiencies can be explained [Föhse et al. 1988].

1. (Internal) The efficiency to produce yield (The amount of P needed in the plant to produce one unit of dry substance) [Loneragan & Asher, 1966].

2. (External) The uptake efficiency (The ability of the root system to take up P from soil and accumulate it in the shoots. This depends on the amount of root per unit of shoot, the roots capability to absorb P and the roots state of growth) [Loneragan & Asher, 1966].

Phosphorus efficiency is related not only to the amount of available P in soil but it also depends on the plant characteristics, as root-shoot ratio and absorption rate per unit of root (influx) [Föhse et al. 1988]. The difference in external uptake depends on the plants internal P need for optimum growth [Föhse et al. 1988]. There are species with a low efficiency (low influx) and low root-shoot ratios and there are species with a medium to high efficiency (high influx) and high root-shoot ratios [Föhse et al. 1988].

External P uptake and root growth are related and the relation between the factors are important for the ability of different plants for P uptake [Wissuwa, 2003]. Low P mobility results in that the plant's uptake mainly depends on the root's exploitation of the soil [Richardson et al. 2009]. Genotypes with a higher external P-uptake efficiency are likely to show a more complex root growth, because the extra P taken up that will allow further biomass accumulation, producing a better root growth [Wissuwa, 2003].

The growth of the root is controlled by where the uptake of nutrients is located depending on the plants growing strategy [Lynch, 1995]. With an uneven distribution of nutrients, plants develop their roots in areas with higher concentrations of nutrient [Lynch, 1995]. If there is an increased nutrient concentration around the whole root surface, plants seem to be more open to changes in the soils structure, than if it is a change only nearby certain parts of the root surface [Lynch, 1995]. In this case, the plant can itself optimize

the nutrition situation by regulating its root growth to the current situation [Lynch, 1995].

Phosphorus deficiency

Phosphorus is needed during the primary stages of cell division [Uchida, 2000]. At an early stage of P deficiency, the symptoms are usually not prominent [Uchida, 2000]. As P is relatively mobile in the plant, P can be transferred to younger leaves causing symptoms on older leaves [Uchida, 2000]. Severe P deficiency will result in stunted growth and weaker plants with a limited root system, late maturity and reduced fruit and seed development [Uchida, 2000].

Symptoms of P-deficiency vary between species [Hue et al. 2000]. Fruit trees create shorter, fewer shoots and deformed seeds and fruits [Hue et al. 2000].

The size of a plant's root system is an important characteristic to tolerate P deficiency [Wissuwa, 2003]. The diffusion rates of P in the soil solution are generally low because P easily binds to soil particles and becomes unavailable for roots [Barber et al. 1963]. To maintain a high root growth with a low level of P the plant needs expanding its roots to explore a larger soil volume [Föhse et al. 1991]. The root's architecture and the amount of root hairs control the P uptake and the ability to manage P deficiency [Wissuwa, 2003].

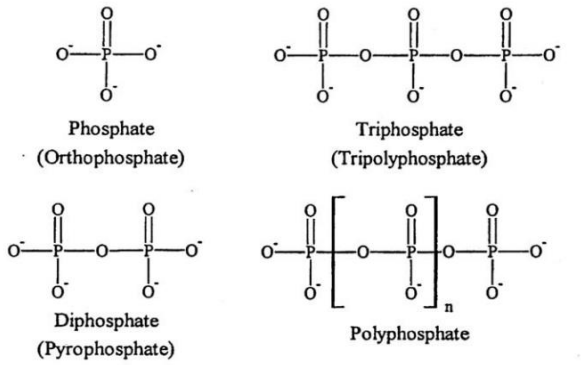
pH

The proportion of easily available P is at its maximum in the soil solution at a pH between 6.5-7.5 [Yara, 2016]. The pH will affect the availability and the interactions with other ions related to available P [Dubus & Becquer, 2001] Acid soil (low pH) has effects on plant growth and the amount of free aluminium (Al) and iron (Fe) in the soil solution [Syers et al. 2008]. During low pH, P react to form strong bonds and minerals with Al and Fe [UHM, 2016]. At a pH above 7 (alkaline soil) P react to form strong bonds and minerals with Ca [UHM, 2016]. A change of pH, generally cause a decrease in P concentration [Föhse et al, 1988]. By adding a fertilizer with a reaction that stabilizes the pH, P accessibility can be improved [Murphy et al. 1981].

Polyphosphate

Inorganic polyphosphate (PP) is a general term for several linked water soluble P acid molecules ($PO^{3}_{4}^{-}$), where the number of molecules determines the designation of the P molecule [McBeath, 2006]. The molecules are linked by oxygen atoms to each other to form either linear chains (linear PP), cyclic arrangements (metaphosphates) or branched structures (ultraphosphates) [Niemeyer, 1999]. The form of the chains determines the molecule's chemical properties and stability [Niemeyer, 1999]. For linear PP, stability is reduced as the chain length increases [Rashchi & Finch, 2000]. Due to their chemical structure, arranged into chains or rings, PP are expected to be less susceptible to precipitation- or fixation reactions in soils [Philen & Lehr, 1967].

Fig 1. PP-forms [Rashchi & Finch, 2000]



Hydrolysis

Polyphosphate cannot be taken up by plants as a P source directly, it must first be hydrolysed into simpler forms of OP [Busman, 1984]. The hydrolysis reaction of PP added to soils is highly depending on the complex interactions of several chemical and environmental factors affecting the rate and effect of the hydrolysis [Dick, 1985].

The hydrolysis occurs when the polymer chains of PP are broken down into simple P molecules in the presence of active enzymes (phosphatases) produced by microorganisms in the soil and by the plant roots [McBeath, 2006]. The hydrolysis of PP in soil is affected by chemical and biochemical reactions (e.g. root activity) [Dick & Tabatabai, 1986]. An optimum pH for phosphatases in soils varies from pH 11 for alkaline phosphatase to 6.5 for acid phosphatase [Eivazi & Tabatabai, 1977]. The hydrolysis can occur at some level without the presence of enzymes (chemical hydrolysis), depending on the soil's biological activity, moisture content, pH and temperature [Hons et al. 1986].

The efficiency and speed of hydrolysis is affected by the properties of soil; temperature, soil fixation and formation of soluble or insoluble complexes with cations affect the concentration of PP [Chang & RacZ, 1977].

Polyphosphate reactions in the soil

Temperature is the most important environmental factor influencing the rate of PP hydrolysis in the soil [Hons et al. 1986]. At a given temperature, the overall rate of hydrolysis is a result of a complex interaction of many soil factors and of the PP structure [Van Wazer et al. 1955]. Minimum hydrolysis has been observed at 5°C and maximum at 45°C [Ahmad & Kelso, 2001]. The hydrolysis rate of the PP increased linearly with increasing temperature from 5-35°C [Hons at el. 1986]. An increase of the temperature will increase the enzymatic and microbial activities which will affect the hydrolysis positively [Ahmad & Kelso, 2001]. Colder temperatures decrease the rate of hydrolysis of PP [Engelstad & Allen, 1971].

The soil pH also affects the hydrolysis reaction since the pH is affecting the soil's enzymatic activity [Hons et al. 1986; Dick & Tabatabai, 1987]. By decreased pH the metal solubility will increase and thereby lower the sorption to linear molecules such as PP [Dick & Tabatabai, 1986]. This will make PP more susceptible for hydrolysis reactions [Dick & Tabatabai, 1986].

Another important factor that affects the hydrolysis rates of PP is the oxygen content of soils [Hons et al. 1986]. When soils are flooded, a change occur in the microbiological, physical and chemical processes due to a relative lack of oxygen and the activity of aerobic organisms is replaced by anaerobic [Patrick & Mahapatra, 1968]. These organisms cause a change of the soil environment by using oxidized soil components which decrease the hydrolysis of PP [Patrick & Mahapatra, 1968].

Polyphosphate fertilizer

Each plant needs a specific level of nutrients and nutrient composition for an optimum growth [Lynch, 1995]. The presence of PP in fertilizers is profitable in that it will sequester some micronutrients slowly (e.g Zn & Mn) and avoid their precipitation when present in liquid fertilizer solutions [Busman, 1984].

PP fertilizers are an analytical challenge as it contains chemically different forms of P compared to fertilizers where P occurs entirely as OP [McBeath, 2006]. Increased temperature and decreased pH level has been shown to have a negative effect in soluble PP fertilizers due to instabilities in the hydrolysis reaction [McBeath, 2006].

Polyphosphate-based fertilizer commonly contains 50-55% PP, 30-40% OP and the remaining amount are present as other complex forms of P [Hashimoto & Lehr, 1973]. PP - fertilizers are thermodynamically unstable and the proportion of each form of P does not remain constant due to the hydrolysis reaction [McBeath, 2006].

Materials & Methods

Plant materials

Two different model plants were used in the experiment;
Pelargonium x hortorum 'Mårbacka' and *Petunia x hybrida* 'Origami Watermelon'. Seven weeks-old *Pelargonium* and four weeks-old *Petunia* cuttings were bought from commercial producers. The experiments contained a total of 30 petunia and 44 pelargonium plants.

Experimental setup

In order to investigate the question of how PP effects growth and nutrient uptake, an experiment was made with two P supply treatments, one with 66% PP (34% OP) and one with 100% OP (Control). For each treatment, 22 (*Pelargonium*) respectively 15 (*Petunia*) replicates were used in order to record plant growth and the concentration of nutrients (table 1).

Cuttings of *Pelargonium* 'Mårbacka' and *Petunia* 'Origami Watermelon' were transplanted into 12-cm round plastic pots. The pots were filled with commercial S-soil (Hasselfors Garden, Örebro, Sweden). The S-soil was selected to give a good oxygen supply to the roots during growth. S-soil is peat-based, with a low level of minerals in the substrate (pH 6.0). The amount of mineral fertilizers in the potting soil was calculated to supply the plant with nutrient for three - four days after the start of the experiment. The pots were filled with substrate to the pot´s edge and weighed for an equal amount of substrate in all pots. All *Pelargonium* plants were repotted (after 17 days of growth) to 14-cm round plastic pots.

The PP treatment consisted of 66% PP and the remaining 34 % was OP. The control consisted of 100% OP, applied as monokaliumphosphate KH_2PO_4.

Table 1. Experimental setup

	Pelargonium				*Petunia*			
Treatment	PP 66% PP, 34% OP		Control 100% OP		PP 66% PP, 34% OP		Control 100% OP	
Use Solution (stock solution A+B)	Vegetative phase	Generative phase	Vegetative phase	Generative phase	Vegetative phase	Generative phase	Vegetative phase	Generative phase
Numbers of Replicates	22		22		15		15	

Nutrient solution

Nutrient solution was made separately for PP- treatment and control, consisting of two stock solutions, A and B, mixed separately to avoid precipitation and reaction between the minerals. Fertilizer solution was kept in plastic tank with a volume of 50 l (Table 2).

Table 2. Use solution recipe (PP and Control) for the vegetative respectively generative phase. The different salts in tank A and B were dissolved in 2 L water per tank and then diluted in the use solution to 50l. During the vegetative phase Micro-Mix (Rexolin APN) was replaced with Sonneveld macro-mix (5ml) (see recipe below and appendix). In the generative phase, Rexolin APN was used according to the original recipe. **Super FK* contains the unique polyphosphate.

	Control		*PP*	
2:100 L tank	Vegetative phase	Generative phase	Vegetative phase	Generative phase
	Stock Solution A: 2 L 1% adding in use solution			
Calcium nitrate	174 g	98 g	174 g	98 g
Potassium nitrate	126 g	72g	99,8 g	45,8 g
	Stock Solution B: 2 L 1% adding in use solution			
Nitric Acid 53%	-	-	23,2 mL	23,2 mL
*Super FK***	-	-	58,6 mL	58,6 mL
Monopotassium phosphate	9,9 g	9,08 g	3,72g	2,82kg
Potassium sulfate	-	11,76 g	-	11,76 g
*Micro mix**	2,4 g (5ml)*	2,4 g	2,4 g (5ml)*	2,4 g
Magnesium sulfate	58 g	40 g	58 g	40 g
EC - based on 1% dilution	2,2 mS	1,6 mS	2,2 mS	1,6 mS
pH	5,9	5,9	5,9	5,9
	Acid tank C: (regulate pH)			
Nitric acid 53%	ca 18 ml	ca 18 ml	ca 18 ml	ca 18 ml
EC	2,2	1,6	2,2	1,6

Greenhouse conditions

The experiments were conducted in a glasshouse at SLU, Alnarp. The greenhouse conditions were constant during the experiment. The temperature in the glasshouse was set at 20°C during the day and 18°C at night. Additional light was given at 400W with high pressure sodium (HPS) lamps between 7 a.m. and 11 p.m, total16 h additional light throughout the growth period. The relative humidity was set to 70%.

Irrigation

All of the pots were arranged in a completely randomized design and were re-randomized weekly during the experiment. Irrigation started five days after planting (6/4) and was then executed depending on solar intensity, growth and model plant as necessary to prevent water stress and dry substrates, normally 2-4 days, 1 dl - 4 dl/session. The vegetative period was irrigated with vegetative use solution through day 16 of totaly 29 days. Remaining days, the plants were watered with the use solution for generative phase. Irrigation with the use solution was made a total of 8 times during the trial period. To ensure equal nutrient supply for plant growth, the same amount of nutrient solution was distributed at each irrigation time to all plants. Irrigation was made only by the use solution, manually, using a measuring cup.

During each irrigation the electrical conductivity (EC) and pH was measured and balanced if necessary through dilution or addition of nutrient solution (EC), and addition of appropriate acids (nitric acid 53%) (pH). pH was measured with a SevenGo Pro sg8 and EC was measured with an EcoScan con5.

Plant evaluation

All plants were measured individually at planting by height, number of leaves, number of leaf- and flower buds. During the growth period the following factors were measured, at a total of 9 times: height, leaf numbers, leaf buds, flower buds, flowers, wilted flowers (*Petunia*) and branching (*Petunia*) (table 3). Final measurements were made by the factors mentioned above as well as root structure, root dry weight and a leaf analysis was done to measure the plants nutrient concentration.

By harvest, four plants from *Pelargonium* were excluded both for the treatment and control because of strong famine (unknown reason). All of Petunia plants were used for plant evaluation.

Plants were harvested 66 (*Pelargonium*) resp. 63 (*Petunia*) days after planting. After the last measurement the roots were cut off at the base and carefully washed free from the substrate in cold water. Extreme caution was committed not to damage the most tenuous roots. When the roots were free from the

substrate, root length was measured by yardstick [cm]. The roots were then oven-dried at 70°C for 48 hours before weighing for dry biomass determination.

Table 3. Overview of plant evaluation

Height	Growth from the edge of the pot to the highest leaf.
Leaf number	Fully developed leaves > 5mm
Leaf bud	Visible leaf buds < 5mm closed buds
Flower bud	Closed flower buds (no visible colour)
Flower	From bud burst (visible colour) to full-blown flower
Wilted flower	Wilted flowers
Branch	Branches from the main stem
Root length (wet)	Length from root base – along to root tip [cm]
Root weight (dry)	[g]
Leaf analysis	Concentration of micro- and macronutrients % (macro) and mg/kg (micro)

Analytical method

Five *Pelargoniu*m plants for PP and Control respectively were randomly assigned for leaf analysis. Due to *Petunia's* small leaf area and low dry weight and a need of 5 g dry weight/sample needed for analysis, the plants were randomised into five groups consisting of three plants each to be pooled to one sample. For leaf analysis, 80% of the plants upper leaves were collected (remaining 20 % leaves were of bad condition due to natural aging with yellowing and dry leaves) and placed in paper bags to be oven-driede at 70°C for 48 hours. The dried leaf material was analysed by Yara Research Centre (Hanninghof, Yara International).

Statistics method

Growth data were analysed with Excel 2016. The differences in growth and nutrient concentration were compared with paired t-test (Excel 2016). Differences were considered significant at $P<0.05$.

Sources of error

After two weeks of growth, *Pelargonium* plants suffered significantly from iron deficiency. The visible effects were noticeable for 30% of the plants at both the control and treatment. The symptoms were typical for iron deficiency where younger leaves become chlorotic between the veins, while the veins remain dark. No visible signs were seen on *Petunia*. After observation Sonneveld* (appendix) micro mix was added. Two dl ready mixed solution was distributed to the pelargonium plants every second day, on three occasions. A week after observation (three weeks of growth) irrigation started with the generative use solution, according to original recipes containing Rexolin APN in stock solution B (table 2). The symptoms of iron deficiency gradually disappeared. After two weeks, there were only a few leaves with symptoms. Iron deficiency may have developed in connection with a less stable chelated DTPA for iron.

Results

In this section the results from the greenhouse are presented. The results section is divided into two parts. Part 1, growth of the above-ground parts: leaves, buds and flowers. Part 2 includes root growth and nutrient uptake. The results will be presented separately, for the two model plants in order.

The analysis is based upon the t-test (Excel 2016). The statistical significance level is illustrated by * with a P value < 0.05. No statistical significance is illustrated with a blank field. The impact from other factors is not evaluated in this test.

Part 1: Growth; leaves, buds and flowers

Pelargonium x hortorum 'Mårbacka'

Table 4. Summary of the final size (measuring during harvest of plant)

X	observations	PP (Mean value +/- SD)	Control (Mean value +/- SD)	p - value	significance
Height [cm]	18	5,87 ± 1,91	5,31 ± 1,52	0,35	
Quantity leaf	18	28,11 ± 7,17	29,28 ± 5,96	0,60	
Quantity gemma	18	3,17 ± 1,54	3,56 ± 3,00	0,64	
Quantity bud	18	2,06 ± 1,11	1,5 ± 0,86	0,10	
Quantity flower	18	1,06 ± 0,64	0,64 ± 0,86	0,06	

No significant differences were observed between the PP and control.

Growth for all factors with associated comments for both growth and final size is show below.

Fig 2. Growth as a foctor of time, $\bar{x}$ [cm].

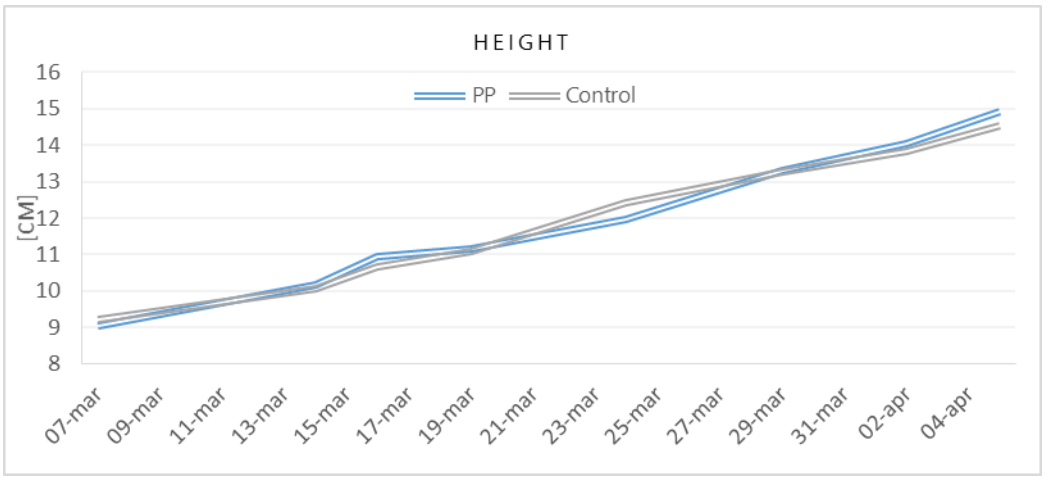
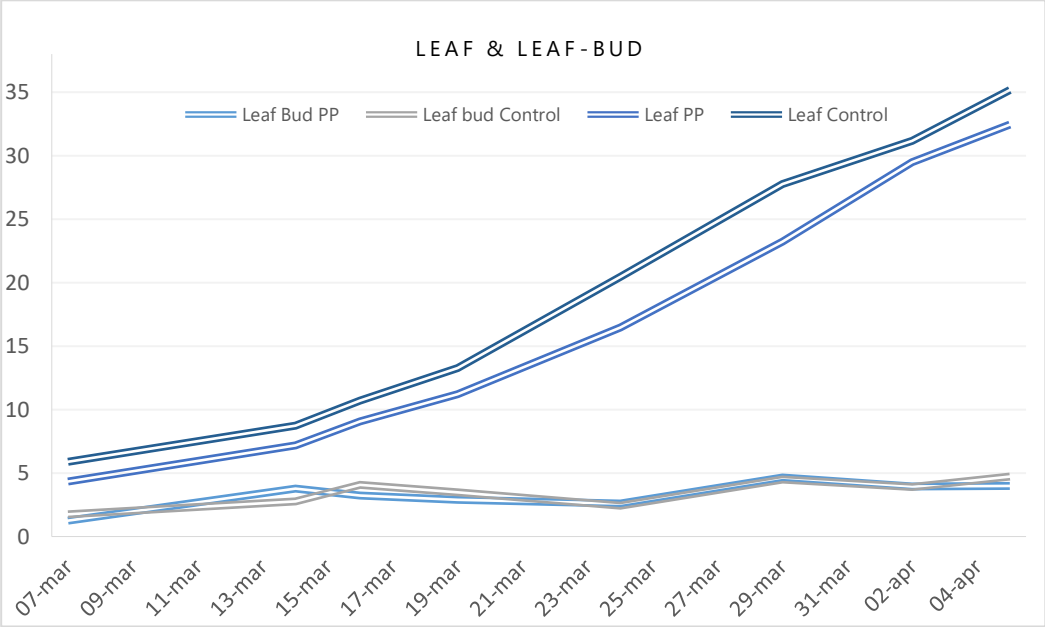


Table 5. Summary of growth height, weekly [cm].

Measure day	observations	PP (Mean value +/- SD)	Control (Mean value +/- SD)	p – value	significance
7-march	18	8,84 ± 1,35	9,34 ± 1,33	0,39	
14-march	18	10,18 ± 1,68	10,06 ±1,49	0,83	
16-march	18	10,94 ± 1,55	10,67 ±1,32	0,59	
19-march	18	11,15 ±1,54	11,07 ±1,36	0,88	
24-march	18	11,95 ± 1,61	12,43 ±1,44	0,37	
29-march	18	13,30 ± 1,77	13,27 ±1,41	0,95	
2-april	18	14,04 ± 1,91	13,84 ±1,44	0,73	
5-april	18	14,91 ±1,13	14,52 ±1,66	0,56	

No significant differences were observed between the PP and the control ($p<0.05$). The treatment with PP appears to produce the same growth in height as the control both during the growth period and for final height. No significant variation was observed among the plants, all the plants in both treatment and control had a steady growth (SD, table 4).

Fig 3. Growth quantity x^{-} for leaf and leaf bud as a factor of time.



Tabel 6. Summary of growth quantity of leaf, weekly.

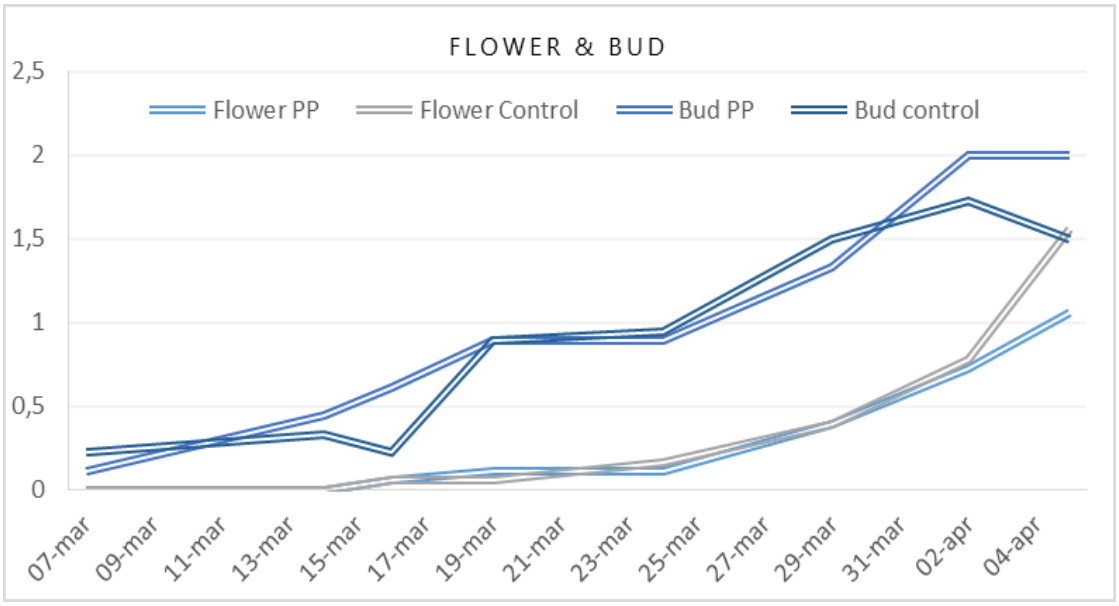
Measure day	observations	PP (Mean value +/- SD)	Control (Mean value +/- SD)	p -value	Significance
7-march	18	4,3 ± 1,2	5,9 ± 1,99	0,01	*
14-march	18	7,2 ± 1,6	8,7 ± 2,86	0,06	
16-march	18	9,1 ± 2	10,7 ± 3,21	0,08	
19-march	18	11,2 ± 2,7	13,3 ± 3,26	0,06	
24-march	18	17,4 ± 3,6	19,4± 4,21	0,06	
29-march	18	23,2 ± 4,4	27,8 ± 5,29	0,01	*
2-april	18	29,5 ± 6,6	31,2 ± 6,01	0,44	
5-april	18	32,4 ± 6,8	35,2 ± 6,87	0,25	

Tabel 7. Summary of growth quantity of gemma, weekly.

Measure day	observations	PP (Mean value +/- SD)	Control (Mean value +/- SD)	p -value	significance
7-march	18	0,88 ± 0,68	1,67 ± 1,26	0,43	
14-march	18	3,55 ± 2,43	2 ± 2,31	0,06	
16-march	18	2,5 ± 1,95	3,17 ± 2,32	0,37	
19-march	18	2,88 ± 1,66	3,5 ± 1,46	0,26	
24-march	18	2,6 ± 1,21	2,44 ± 1,66	0,68	
29-march	18	4,65 ± 1,94	4,5 ± 1,92	0,83	
2-april	18	3,94 ± 1,35	3,89 ± 1,97	0,92	
5-april	18	4 ± 1,632	4,72 ± 2,42	0,32	

No significant difference was observed generally between development of the treatments regarding leaf buds/quantity of leaf during the growth period or final size ($p < 0.05$). During measure 7th of march (first measure) and 29th of march the control had significantly more leaves. Bud and leaf grew in relation to each other and no visual signs appeared that PP had a significant effect on leaf size or colour. No significant variation was observed among the plants, all the plants in both treatment and control had steady growth.

Fig 4. Growth quantity $\bar{x}$ for flower and flower bud as a factor of time.



Tabel 8. Summary of growth quantity flower, weekly.

Measure day	observations	PP (Mean value +/- SD)	Control (Mean value +/- SD)	p -value	significance
7-march	18	-	-	-	
14-march	18	-	-	-	
16-march	18	0,55 ± 0,23	0,56 ± 0,23	1,00	
19-march	18	0,11 ± 0,31	0,06 ± 0,23	0,56	
24-march	18	0,11 ± 0,31	0,12 ± 0,37	0,64	
29-march	18	0,39 ± 0,59	0,39 ± 0,59	1,00	
2-april	18	0,72 ± 0,65	0,78 ± 0,63	0,80	
5-april	18	1,06 ± 0,64	0,64 ± 0,86	0,06	

Tabel 9. Summary of growth quantity flower bud, weekly.

Measure day	observations	PP (Mean value +/- SD)	Control (Mean value +/- SD)	p -value	significance
7-march	18	0,11 ± 0,31	0,22 ± 0,42	0,39	
14-march	18	0,44 ± 0,68	0,33 ± 0,58	0,61	
16-march	18	0,61 ± 0,56	0,22 ± 0,53	0,06	
19-march	18	0,88 ± 0,66	0,89 ± 0,66	1,00	
24-march	18	0,88 ± 0,45	0,94 ± 0,52	0,74	
29-march	18	1,33 ± 0,75	1,5 ± 0,69	0,50	
2-april	18	2 ± 0,82	1,72 ± 0,65	0,28	
5-april	18	2 ± 1	1,5 ± 0,83	0,12	

No significant difference was observed between PP and the control for the number of flower buds and flowers ($p<0.05$). But measure day 16th march (table 9, flower bud) and 5th april (table 8, quantity flowers) it is near a significant difference where PP indicate to have a positive effect on the development of buds and flowers.

Petunia x hybrida 'Origami Watermelon'

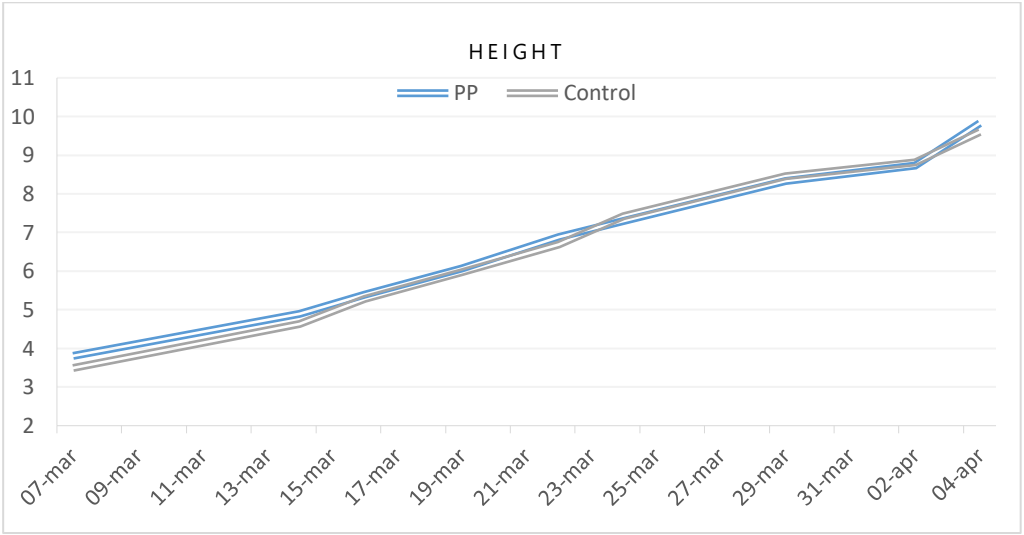
Table 10. Summary of the final size. (Measuring during harvest of plant).

x	observations	PP (Mean value +/- SD)	Control (Mean value +/- SD)	p - value	Significance
Height [cm]	15	5,99 ± 0,89	6,11 ± 1,17	0,78	
Branching	15	12,87 ± 2,83	13,60 ± 1,62	0,41	
Quantity flower bud	15	4,13 ± 1,31	4,47 ± 1,67	0,56	
Quantity flower	15	12,27 ± 3,07	13,33 ± 4,60	0,48	
Quantity wilted flower	15	4,20 ± 1,94	4,20 ± 2,40	1	

No significant differences were observed between the PP and the control.

Growth for all factors with associated comments for both growth and final size is shown below.

Fig 5. Growth as a factor of time $\bar{x}$ [cm].

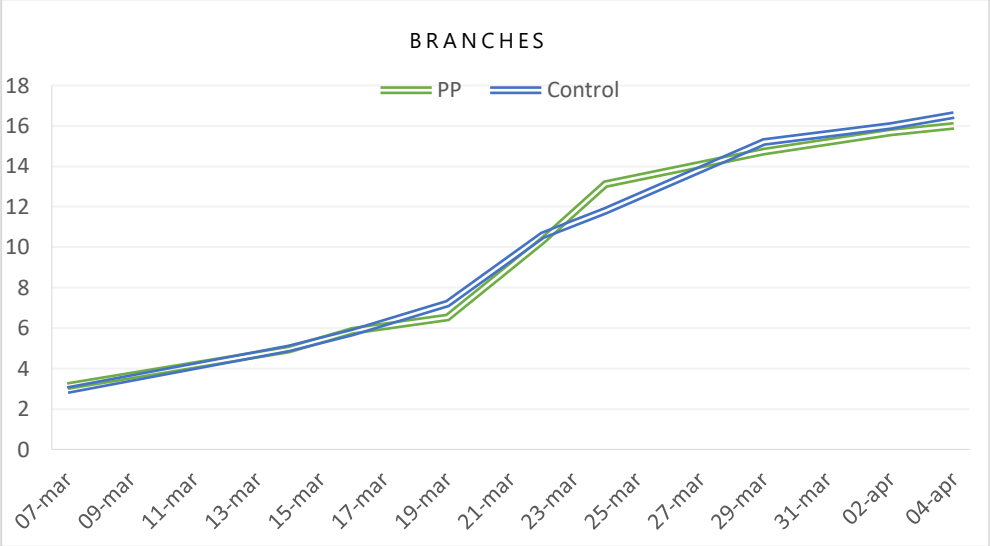


Tabel 11. Summary of growth height, weekly [cm].

Measure day	observations	PP (Mean value +/- SD)	Control (Mean value +/- SD)	p - value	significance
4-march	15	3,80 ± 0,89	3,49 ± 1,93	0,99	
14-march	15	4,9 ± 1,09	4,63 ± 1,66	0,99	
16-march	15	5,39 ± 1,08	5,29 ± 2,28	0,99	
19-march	15	6,06 ± 1,08	5,60 ± 2,37	1	
22-march	15	6,88 ± 1,03	6,70 ± 2,52	1	
24-march	15	7,3 ± 1,04	7,43 ± 2,75	0,99	
29-march	15	8,33 ± 0,90	8,45 ± 3,07	0,99	
2-april	15	8,73 ± 0,67	8,81 ± 3,44	0,99	
4-april	15	9,83 ± 0,91	9,6 ± 3,83	0,99	

No differences were observed between the PP and the control ($p<0.05$) Treatment with PP appears to produce the same growth in height as control both during the growth period and for final size.

Fig 6. Growth total number of branches $\bar{x}$ as a factor of time.

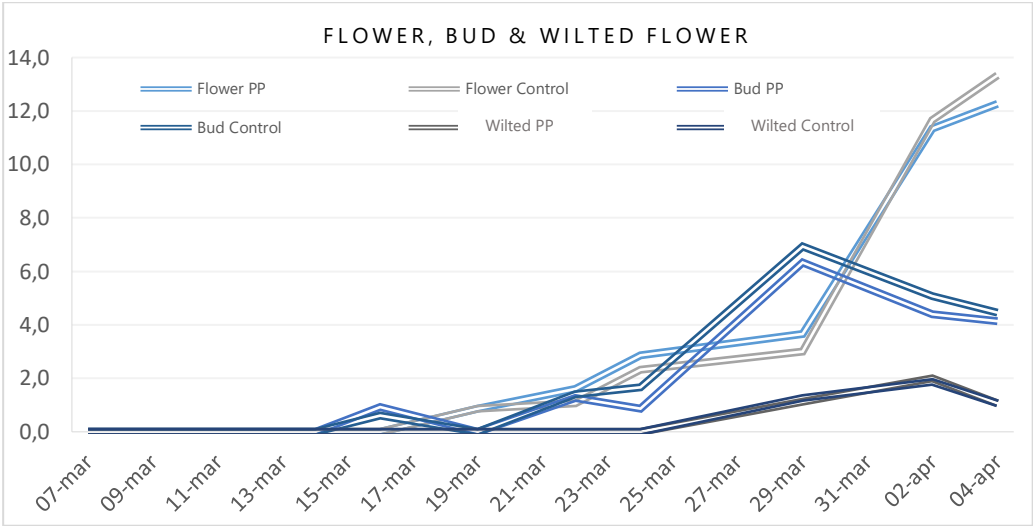


Tabel 12. Summary of growth branching, weekly.

Measure day	observations	PP (Mean value +/- SD)	Control (Mean value +/- SD)	p - value	Significance
4-march	15	3.13 ± 0,62	2,93 ± 0,44	0,33	
14-march	15	4,94 ± 1,44	5 ± 1,46	0,90	
16-march	15	5,87 ± 1,36	5,8 ± 0,98	0,88	
19-march	15	6,53 ± 1,31	7,2 ± 2,07	0,31	
22-march	15	10,33 ± 1,53	10,6 ± 2,39	0,73	
24-march	15	13,33 ± 1,93	11,8 ± 2,45	0,12	
29-march	15	17,73 ± 2,38	15,2 ± 1,89	0,57	
2-april	15	15,66 ± 2,33	16 ± 2,03	0,69	
4-april	15	16 ± 2,71	16,53 ± 1,75	0,54	

No differences were observed between the PP and the control ($p<0.05$)

Fig 7. Growth quantity x^{-} for flower, flower bud and wilted flower as a factor of time.



Tabel 13 Summary of growth quantity flower, weekly.

Measure day	observations	PP (Mean value +/- SD)	Control (Mean value +/- SD)	p - value	Significance
4-march	15	-	-	-	
14-march	15	-	-	-	
16-march	15	-	-	-	
19-march	15	0,87 ± 0,96	0,87 ± 0,88	1	
22-march	15	1,6 ± 1,08	1,07 ± 0,77	0,144	
24-march	15	2,87± 1,54	2,33 ± 1,30	0,33	
29-march	15	3,67 ± 2,05	3 ± 2,40	0,435	
2-april	15	11,33 ± 2,75	11,66 ± 4,53	0,8155	
4-april	15	12,27 ± 3,07	13,33 ± 4,60	0,476	

Tabel 14 Summary of growth quantity flower bud, weekly.

Measure day	observations	PP (Mean value +/- SD)	Control (Mean value +/- SD)	p - value	Significance
4-march	15	-	-	-	
14-march	15	-	-	-	
16-march	15	0,93 ± 0,85	0,6 ± 0,95	0,34	
19-march	15	-	-	-	
22-march	15	1,27 ± 0,99	1,4 ± 0,88	0,71	
24-march	15	0,87 ± 1,02	1.67 ± 1,07	0,06	
29-march	15	6,33 ± 2,89	6,93 ±3,32	0,61	
2-april	15	4,4 ± 2,15	5,07 ± 2,32	0,44	
4-april	15	4,13 ± 1,31	4,47 ± 1,67	0,56	

Tabel 15 Summary of growth, quantity wilted flowers, weekly.

Measure day	observations	PP (Mean value +/- SD)	Control (Mean value +/- SD)	p - value	Significance
4-march	15	-	-	-	
14-march	15	-	-	-	
16-march	15	-	-	-	
19-march	15	-	-	-	
22-march	15	-	-	-	
24-march	15	-	-	-	
29-march	15	1,13 ± 0,72	1,07 ± 1,39	0,75	
2-april	15	2 ±1,55	1,87 ± 1,26	0,80	
4-april	15	1,07 ± 0,77	1,07 ± 0,68	1	

No significant difference between PP and control was observed ($p<0.05$) by he number of flower buds, flowers or wilted flowers. We did not see any significant difference of the total production of bud, flower and wilted flowers between the two treatments. Faded flowers was removed as they withered.

Part 2: Root growth and nutrient uptake

Pelargonium x hortorum "Mårbacka"

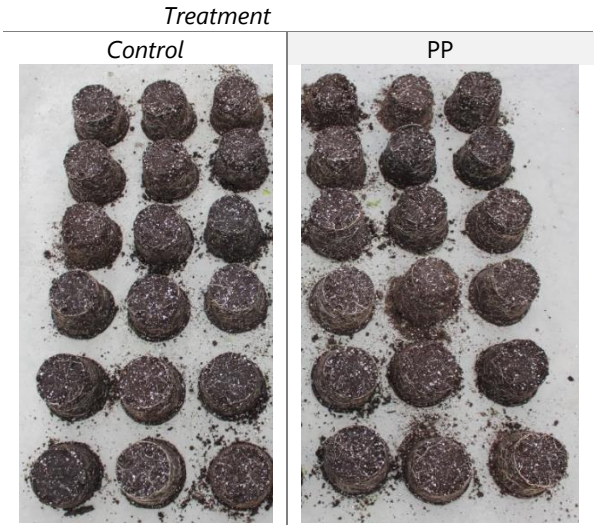
Table 6. Summary of measurements of root structure.

	Treatment				
	Replicates	PP (mean value +/- SD)	Control (mean value +/- SD)	p - value	Significance
Root weight dry [g]	18	0,60 ± 0,17	0,64± 0,12	0,41	
Root length [cm]	18	22,63 ± 7,9	22,26 ± 6,09	0,87	

No significant difference was observed between PP and control the observations ($p<0.05$)

Visual evaluation root system

Fig 8. Visual comparison of the root system.



No visual difference appeared between PP and the control.

*Petunia x hybrida '*Origami Watermelon*'*

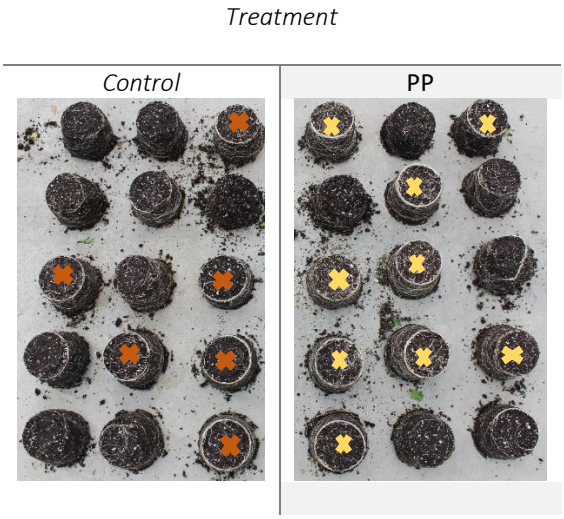
Table 7. Summary of measurements of root structure.

		Treatment			
	Replicates	PP (mean +/- SD)	Control (mean +/- SD)	p - value	significance
Root weight dry [g]	15	0,15 ± 0,07	0,15 ± 0,05	0,98	
Root length, wet [cm]	15	31,60 ± 10,59	29,68 ± 4,46	0,54	

No significant difference was observed between PP and control the observations ($p<0.05$)

Visual evaluation of root system

Fig 9. Visual comparison of the root system. Pots with a more pronounced root growth shows with the X.



There is a small tendency towards more roots with PP compared to the control. There were more visible roots (white root-ring in the top of the root lump) in 9 of the 15 pots in PP treatment comparing with 6 of 15 pots for the control.

Leaf analysis - Nutrient concentration

Pelargonium x hortorum "Mårbacka"

Table 8. Content of nutrients in leaves. Macronutrients in % and micronutrients in mg/kg DW. LOQ - Limit of quantification. Values above the mentioned critical concentration are in the reliable range of the calibration. Values below LOQ can be measured but not quantified.

		Treatment			
	Nutrient (LOQ)	PP (mean value +/- SD)	Contol (mean value +/- SD)	P-value	Significanse
	N (0,15)	3,46 ± 0,08	3,59 ± 0,11	0,85	
	P (0,012)	0,91 ± 0,07	0,64 ± 0,04	0,007	*
	K (0,005)	3,69 ± 0,06	3,51 ± 0,17	0,10	
%	Mg (0,003)	0,30 ± 0,02	0,28 ± 0,018	0,14	
	S (0,003)	0,28 ± 0,01	0,30 ± 0,02	0,15	
	Ca (0,007)	2,77 ± 0,18	2,33 ± 0,27	0,15	
	Na (0,006)	0,08 ± 0,01	0,08 ± 0,02	0,64	
	B (2)	51,10 ± 2,12	44,50 ± 4,51	0,004	*
	Cu (1.5)	4,29± 0,28	4,20 ± 0,26	0,67	
mg/kg	Fe (10)	47,87 ± 2,07	52,33 ± 2,67	0,20	
	Mn (6,5)	31,52 ± 4,46	26,67 ± 5,22	0,19	
	~~Mo (2)~~	~~0,82 ± 0,21~~	~~0,56 ± 0,28~~	~~0,004~~	~~*~~
	Zn (8,7)	39,99 ± 2,26	38,11 ± 1,12	0,07	

Leaf analysis shows significant differences according the amount of concentration P and boron (B) in the presence of PP. This means that addition of PP will give a higher amount of P and B in the plant. Also Zn is close to be significantly different between treatments. For the other elements no significant difference is shown. Values of Mo were below the limit, (LOQ) to be correctly measured. This value will be excluded.

Petunia x hybrida 'Origami Watermelon'

Table 9. Content of nutrients in dry leaves. Macronutrient in % and micronutrients in mg/kg DW. LOQ - Limit of quantification. Values above the mentioned critical concentration are in the reliable range of the calibration. Values below LOQ can be measured but not quantified.

		Treatment			
	Nutrient (LOQ)	PP (mean value +/- SD)	Control (mean value +/- SD)	p-value	significance
	N (0,15)	-	-	-	
	p (0,012)	0,73 ± 0,03	0,69 ± 0,07	0,28	
	K (0,005)	8,01 ± 0,11	7,90 ± 0,27	0,45	
%	Mg (0,003)	0,43 ± 0,01	0,43 ± 0,02	0,19	
	S (0,003)	0,50 ± 0,01	0,50 ± 0,01	0,92	
	Ca (0,007)	2,72 ± 0,05	2,80 ± 0,16	0,39	
	Na (0,006)	0,01 ± 0,0005	0,01 ± 0001	0,23	
	B (2)	11,74 ± 0,93	10,85 ± 0,70	0,17	
	Cu (1.5)	11,21 ± 0,63	10,55 ± 1,15	0,34	
	Fe (10)	97,42 ± 6,67	92,74 ± 3,11	0,24	
mg/kg	Mn (6,5)	76,31 ± 9,98	81,93 ± 11,23	0,48	
	~~Mo (2)~~	~~1,95 ± 0,76~~	~~1,73 ± 0,36~~	~~0,62~~	
	Zn (8,7)	67,01 ± 7,46	61,65 ± 3,67	0,23	

No significant difference between concentration of nutrients is shown ($p<0,05$). The amount of leaf was not enough to measure the N level. Values of Mo were below the limit of quantification, (LOQ) to be correctly measured. This value will be excluded.

Discussion

The objectives of this study were to evaluate the effect of PP availability for plant growth, root development and plant uptake of micro- and macronutrients.

The greenhouse experiment consisting of the model crops *Petunia x hybrida* 'Origami Watermelon' and *Pelargonium x hortorum* 'Mårbacka' showed overall no significant different effects with PP compared to OP as a P source. The results showed no evidence that PP generated a better or worse plant or root growth compare to OP in terms of a higher P supply to roots and shoots. This is indicating that the effectiveness of a unit of P taken up by plants for increasing the growth is similar between PP- and OP fertilizer.

But, despite the fact that the greenhouse experiment did not show any overall significant difference in growth a couple of values showed a significant difference between the treatments ($p < 0.05$). The leaf analysis of *Pelargonium* showed that the macronutrient P and micronutrients B had higher concentrations in plants irrigated with PP fertilizer, in comparison to plants irrigated with a OP containing fertilizer.

Consequently, PP generated a higher concentration of P in the plants, which is the most desirable property of PP. However, we saw no visible effect on the root growth or leaf/flower quantity due to the increased P concentration on the 18 PP treated *Pelargonium* plants.

A higher P uptake generally generates a larger root growth which in turn generates a higher plant growth with higher biomass [Wissuwa, 2003; Torres-Dorante et al. 2006] including more leaf, flower and stem. The root-shoot ratio, is an important factor highly depending on the P content [Föhse et al. 1988]. A clear difference in the root system and growth should therefore have been a result of the increased P concentration. But as we have no other visible or statistical significant differences, we cannot draw any further conclusion that PP provides a better root- and plant growth than OP.

This may be related to a sufficient level of P in the form of OP in both fertilizers which has been enough for an optimal plant growth. The question is what would have happened if we had used two fertilizer recipes with a lower P content in both treatments (PP and control). Had we seen a difference in growth between the treatments, due to a higher concentration of P in PP than OP?

The variation and evenness of the growth within the various treatments should also be commented. The results show a low variation with an even growth of all plants, regardless of treatment. It has also been suggested that growth and flower development should have been faster with PP in comparison with the OP [Van Schie, 2014]. But we have not been able to see any difference between the PP and the OP treatments. The different growth stages of leaf, bud and flower development has occurred simultaneously no matter the treatment.

What many scientist, however, agree upon [McBeath, 2006; Torres-Dorante et al. 2006] is that the PP efficiency and ability to generate a higher P availability to plants are influenced by ambient factors. It requires optimal conditions for PP to be hydrolysed and become available for the roots. The optimum ratio depends on the interaction between the soils biological and chemical condition such as pH, temperature, water holding capacity, biological activity [Dick, 1985] as well as plant species, its unique root growth and the fertilizer's various interacting components [McBeath, 2006]. Plants can only absorb P from the soil solution if the PP compounds are completely hydrolysed [Busman, 1984].

Polyphosphate is less or more available and effective to plants than OP depending on the soil structure and its environmental factors [McBeath, 2006; Sutton & Larsen, 1964]. Temperature is the most important environmental factor influencing the rate of hydrolysis of PP [Hons et al. 1986]. A higher temperature will generate a faster and more complete hydrolysis of PP [Hons at el.1986]. Applying the PP fertilizer in a warmer climate (greenhouse) should thus provide a more effective result than the application in colder climates (open field). Cool temperatures will decrease the rate of hydrolysis of PP [Engelstad & Allen, 1971].

Furthermore, in favourable conditions, PP binds nutrients easier than OP [Sutton & Larsen, 1964]. We saw a trend in the greenhouse experiment with *Pelargonium* that PP generates higher concentration of P and B in the plant. This knowledge can be used to develop fertilizer efficiency and the knowledge of the reactions and precipitates of desired / undesired minerals in the soil.

Many of the trials that have been done to study the effect of PP have occurred when growing conditions not have been optimal. For example, with different pH and different soil structure, from clay to loams [Hons el al. 1986] and with different temperature [Engelstad & Allen, 1971]. A difference in growth cannot be seen during growth in optimum ambient condition [Dick, 1986; Hons el al. 1986; Engelstad & Allen, 1971]. At an optimum ratio of nutrients, the plants will probably not adapt and take advantage of the extra available resources. To compare P fertilizer–use efficiency, it is important that the growth, even of the fertilized plants, is below its maximum. The effect of PP supply on the root-shoot ratio is, perhaps, only seen when P is needed for additional growth.

My experiment contained different nutrient solutions with different forms of P. The question is whether this is a sufficient difference? Other growth conditions were identical, e.g substrate, temperature and amount of irrigation. The question is what is the optimal amount of P supply to *Pelargonium* and *Petunia*? How much is needed? If there is no need for an increased uptake, will an extra uptake still occur?

Can a change in the relationship between the different fertilizers give us a different effect? Had we seen a different result if we had exposed the plants for any kind of stress? (as is often the case in a more natural growing situation) Had we seen a different result with P - deficiency? drought? or an unfavourable pH?

We know that the effect of fertilizers and hydrolysis of PP is highly dependent on the chemical and biological reactions of the soil. A change in the pH value will change the enzymatic activity in the soil and it will change the ionic composition. A change in soil pH will directly affect P availability and the added fertilizer efficiency.

Conclusion

Polyphosphate is a unique form of fertilizer that is thermodynamically unstable. The PP hydrolysis is essential for the plant's P uptake. The hydrolysis is affected by the interaction between the soil's biological and chemical atmosphere such as pH, temperature, water holding capacity, biological activity, as well as the plant species and its unique root growth and the status of the fertilizers components. A PP-containing fertilizer must be used at the right temperature, applied with the right fertilizer proportion, right time and amount to cause effect.

The greenhouse experiment showed no significantly different effects on root- or shoot growth with PP compared to OP as a P source. A higher concentration of P was found in leaves in the model crop *Pelargonium* with the application of PP. No significant difference was shown in the leaf analysis of Petunia.

Polyphosphate impact is very little documented with few contemporary studies done and much is unexplored. This provides a great opportunity to develop the knowledge of its impact.

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Appendix

Sonneveld and Rexolin APN in compared with micromix content.

	Sonneveld	*Rexolin APN*
	mg/L	mg/l - use solution
Mn	0,27	0,29
Zn	0,26	0,16
B	0,32	0,13
Cu	0,05	0,03
Mo	0,05	0,03
Fe	2,23	0,72