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Conventional and novel uses of phosphite in horticulture: potentialities and challenges

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Uso convenzionale e innovativo del fosfito in orticoltura: potenzialità e sfide

Riassunto. Il fosfito (Phi; H_2PO_3- o HPO_3^{-2}) o l'acido fosforico coniugato (H_3PO_3), una forma ridotta del fosfato inorganico (Pi; H_2PO_4- or HPO_4^{2}-), suscita un grande interesse come fitofarmaco in ortoflorofrutticoltura. Infatti, tale molecola può controllare batteri, funghi e nematodi fitopatogeni sia direttamente che indirettamente mediante induzione di resistenza nella pianta. Phi può controllare e/o indurre resistenza verso batteri patogeni quali *Erwinia amylovora* e *E. carotovora*, così come i generi di oomiceti *Peronospora, Plasmopara, Phytophthora* e *Pythium*, i generi di funghi *Alternaria, Rhizoctonia* e *Macrophomina*, e i nematodi *Meloidogyne javanica, Pratylenchus brachyurus, Heterodera avenae* e *Meloidogyne marylandi*. Di recente il fosfito viene proposto per le sue proprietà biostimolanti che si manifestano con incrementi di produttività e miglioramenti della qualità dei prodotti delle colture soprattutto in condizioni di stress abiotico. Nei sistemi agricoli convenzionali Phi non sembra avere un effetto diretto sulla nutrizione della pianta e non dovrebbe essere considerato come un vero e proprio fertilizzante. Tra l'altro è stato evidenziato che gli effetti benefici del fosfito si manifestano principalmente su colture che si trovano in condizioni di buona nutrizione fosfatica. La realizzazione di piante transgeniche in grado di utilizzare il fosfito come fonte di fosforo apre scenari futuri di utilizzo del fosfito come fertilizzante fosfatico.

Parole chiave: fosfito, acido fosforoso, biostimolante, fosforo, fitofarmaco.

Introduction

Phosphite (Phi) has largely been used as a pesticide, supplemental fertilizer, and biostimulant in agriculture. As a plans biostimulant, Phi may improve nutrient uptake and assimilation, abiotic stress tolerance and yield quality. Additionally, Phi may promote root growth, yield and nutritional value in a number of horticultural crops. Moreover, Phi has extensively been used in controlling pathogenic bacteria, fungi and nematodes. Phosphite has also been used as an alternative fertilizer, albeit its contribution to P nutrition is limited and it has been the subject of controversy in the technical and scientific world. In conventional agricultural systems, phosphate (Pi) is the sole P-containing nutrient important for optimal plant growth and development.

Phosphorus (P) is a critical plant macronutrient, making up about 0.2% of a plant's dry weight biomass. It is a key component of essential molecules such as nucleic acids (DNA and RNA), phosphoproteins, phospholipids, sugar phosphates, enzymes and energy rich compounds such as adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP) (Manna *et al.*, 2016). Consequently, P plays a pivotal role in vital plant cell processes including genetic heredity, photosynthesis, respiration and energy transfer. Because P also participates in phosphorylation and dephosphorylation of target proteins, it is a crucial component of almost every signal transduction pathway and controls diverse cellular functions for proper plant growth and development, triggering responses to environmental stimuli and stress factors as well (Gómez-Merino and Trejo-Téllez, 2015).

Plants absorb and metabolize P as inorganic phosphate (Pi). To ensure functional metabolic reactions,

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Pi homeostasis must be kept between 5 to 20 mM in the cytoplasm. Plants absorb P only in its soluble inorganic form of Pi, $H_2PO_4^-$ or HPO_4^{2-}, which occur in the soil between 0.1 to 1 µM (Malboobi *et al.*, 2012; 2014). Because of such a huge difference between P source and demand, this macronutrient becomes critical for plant metabolism. In fact, P ranks as the second most vital element for plant growth and development, just after nitrogen (N), and hence it is considered a major constraint in agriculture and food production.

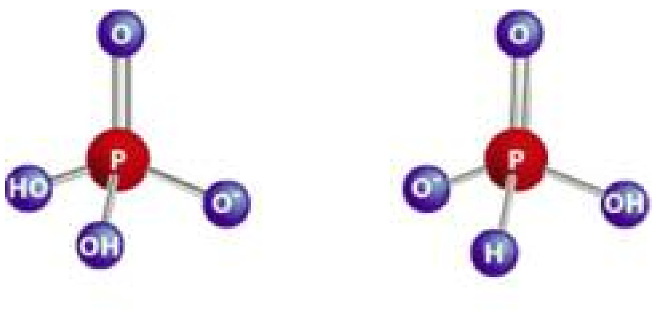
Whilst P is the 11th most abundant element in the Earth´s crust (4×10^{15} metric tons), only a small part of it (20-50%) is available for plants in the form of Pi (Schröder *et al.*, 2010). Such a limitation negatively affects plant productivity, and therefore P-fertilizers are applied. Nevertheless, nearly 80% of P fertilizers applied to crops is lost because of precipitation and adsorption to mineral surfaces, or conversion to organic forms; in very sandy and organic soils, P-leaching can also take place (Lehmann and Schroth, 2003; Manna *et al.*, 2016). Due to the low efficiency of P use, P fertilizers are excessively applied in crop fields. This inefficient and uneconomical P utilization not only raises agricultural costs, but also generates eutrophication of water bodies. Modern agriculture depends on Pi derived from phosphate rock, which is a non-renewable resource and peak P production is expected to occur between 2030 and 2040, while current global reserves may be depleted in 50-100 years (Cordell *et al.*, 2009). In addition, crop plants have to compete with weeds for space, light, water and nutrients, and herbicides have become less effective in controlling weeds (Manna *et al.*, 2016). Consequently, better insight is needed into the availability of this finite resource and the environmental repercussions related to its use. Novel agricultural practices and technologies as well as innovative approaches to sustainable use can attenuate environmental impacts and enhance the long-term supply of this vital plant nutrient (Syers, 2011). Thereafter, phosphite (Phi), a reduced form of Pi, might be an alternative to address the above concerns to a considerable extent. In this review, we outline recent advances in research concerning the use of Phi as a pesticide, an inducer of plant resistance against pathogens, and a biostimulant that improves yield, harvest quality and responses to environmental stressors. In addition, we explore the recent development of Phi-mediated fertilization, weed control and selectable marker platforms useful in plant genetic transformation approaches with a wide spectrum of applications in horticulture.

Chemical properties of Phi and its metabolism in plants

Orthophosphate or inorganic P_i is the most oxidized form of P found in nature. In the P_i molecule, four oxygen (O) atoms are bounded with a single P atom (fig. 1). At neutral pH in the soil solution, the Pi ion is present as a mixture of $H_2PO_4^-$ and HPO_4^{2-}. In nature P_i is the sole P-containing nutrient important for optimal plant growth and development, while the form H_2PO_4- is how P_i is normally metabolized in plant cells (McDonald *et al.*, 2001). Nonetheless, over the past three decades, Phi has increasingly been used to improve the yield of many crop species, and just recently, novel approaches based on genetic engineering have developed transgenic plants capable of using Phi as an alternative fertilizer and herbicide (López-Arredondo and Herrera-Estrella, 2012; Manna *et al.*, 2016).

Phosphite (H_2PO_3-) is an isostere of the P_i anion, in which hydrogen replaces one of the oxygen atoms bound to the P atom (Varadarajan *et al.*, 2002). Phosphite may also be referred to as phosphorous acid or phosphonate, though the term phosphonate is used to mean a wide range of compounds containing carbon-phosphorus bonds like fosetyl-Al (McDonald *et al.*, 2001; Metcalf and van der Donk, 2009). Fosetyl-Al was indeed one of the first trademarks patented in the United States, and when the corresponding patent expired, several companies formulated a series of Phi-containing products with other ions (i.e. Ca, Cu, K, and Na, among others).

Chemically, Pi and Phi are similar, though the lack of an O atom in Phi significantly changes the nature



Pi ($H_2PO_4^-$) **Phi ($H_2PO_3^-$)**

Fig. 1 - Chemical structures of phosphate (Pi; H_2PO_4-) and phosphite (Phi; H_2PO_3-). The Pi ion has one more oxygen (O) atom than the Phi one. Commercial formulations containing Phi have ions combined with other elements such as iron (I), potassium (K) and aluminum (Al), among others.

Fig. 1 - Strutture chimiche del fosfato (Pi; H_2PO_4-) e fosfito (Phi; H_2PO_3-). Lo ione Pi ha un atomo di ossigeno (O) in più del Phi. I formulate commerciali che contengono Phi presentano ioni combinati con altri elementi quali ferro, potassio e alluminio.

and reactivity of the resultant molecule. Both P_i and Phi display tetrahedral molecular geometry, but because of the structural difference, the charge distribution is distinct in each molecule. Thus, both the shape of the molecule and the charge distribution seem to influence the binding of P_i and Phi to their interacting molecules, and most enzymes involved with phosphoryl transfer reactions readily discriminate between Phi and P_i (Plaxton, 1998). Nonetheless, some plant and yeast proteins (i.e. membrane P_i transporters and proteins involved in the P_i-sensing-machinery) appear to recognize Phi as P_i (McDonald *et al*., 2001), and Phi is known to interfere with many of the Pi starvation responses in these organisms (Varadarajan *et al*., 2002), through the modulation of signal transduction pathways responsible for the detection of, and response to internal P_i levels (Jost *et al*., 2015; Plaxton and Carswell, 1999).

The three O atoms in the Phi molecule give this anion increased mobility in plant tissues through both the xylem and the phloem, so that it can be successfully applied throughout the plant (fig. 2). Because of its higher solubility, Phi is more rapidly absorbed and translocated within the plant than P_i (Ratjen and Gerendas, 2009; Jost *et al*., 2015).

Conversely, commercial P-fertilizers are usually solid, have low solubility in water, react strongly with the soil matrix and are more prone to be adsorbed to soil particles than Phi. These facts render P_i largely immobile in the soil and only a small fraction of it is available to the plant, eroding over time within the soil solution. Importantly, commercial Phi-containing products have higher concentrations of P (39%) than traditional P_i-based fertilizer (32% P). Moreover, solubility of Phi-salts is higher than that of their analogous P_i-salts, making leaf and root Phi-uptake more efficient. Importantly, Phi triggers hormesis, which is a biphasic dose response characterized by a low dose stimulation or beneficial effect and a high dose inhibitory or toxic effect (Mattson, 2008). Therefore, the application of Phi-containing compounds must be tightly regulated, since excessive dosages of Phi can cause toxicity or detrimental effects to plants (Gómez-Merino and Trejo-Téllez, 2015).

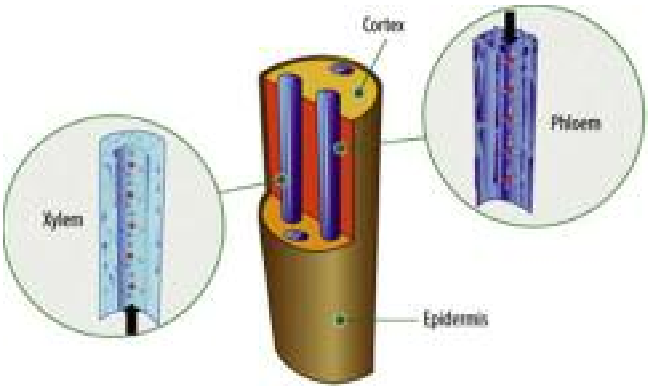


Fig. 2 - Phosphite is highly mobile within the plant in a systemic manner. If applied to the roots, Phi is mobilized through the xylem. If sprayed on the leaves, the ion is also absorbed and transported through the phloem.

Fig. 2 - Il fosfito è molto mobile all'interno della pianta in maniera sistemica. Se somministrato alle radici Phi è mobilizzato attraverso lo xilema. Se spruzzato sulle foglie lo ione è assorbito e trasportato attraverso il floema.

Both P_i and Phi are acquired by plant cells via P_i transporters (Varadarajan *et al*., 2002; Jost *et al*., 2015), though these proteins are primarily involved in P_i uptake (Guest and Grant, 1991; Ullrich-Eberius *et al*., 1981), and their role in Phi acquisition is secondary (d'Arcy-Lameta and Bompeix, 1991; Danova-Alt *et al*., 2008; Jost *et al*., 2015). Importantly, P_i transporters (named Pht) are distributed throughout the plant and consequently P_i and Phi can be taken up by leaves through foliar sprays or by the roots as a soil application, and through irrigation water, nutrient solution or growth medium. Since Phi is highly water soluble (Jost *et al*., 2015), it is more rapidly absorbed and translocated within the plant than P_i (Ratjen and Gerendas, 2009). Furthermore, because in nature plants lack the mechanisms to metabolize Phi, it remains relatively stable and is not significantly oxidized within the plant cells, and thus its effects are usually long lasting. Nevertheless, when Phi is applied to the soil, it comes into contact with microorganisms, which mediate the oxidation of Phi to P_i. Thus, by this indirect method, Phi can become available to the plant as a P nutrient after microbial oxidative reactions, albeit this conversion takes months.

The uptake of Phi is pH dependent and subject to competition by P_i (Ouimette and Coffey, 1990). Once within the plant, Phi shows systemic effects and high chemical stability, displaying great mobility throughout the whole plant. This mobility favors the penetration and transport of the foliar sprays to the rest of the plant (Smillie *et al*., 1989; Brunings *et al*., 2005). Furthermore, mobility of Phi in both xylem and phloem is carried out by Pht proteins, in a similar manner to P_i (Ouimette and Coffey, 1989).

Characterization of some Pht enzymes has been carried out in a number of crop species such as tomato, potato, soybean, rice, barley, and maize (López-Arredondo *et al*., 2014), revealing significant divergence among genotypes. The distinct reported affinities and subcellular localizations of Pht proteins may reflect diverse functional roles such as uptake from the soil as opposed to translocation or remobilization of stored P_i within the plant (Nussaume *et al*., 2011; Ceasar *et al*., 2014).

It has been proven that Phi accumulates in the cytosol and the vacuole (Danova-Alt *et al*., 2008), while the presence of P_i enhances Phi sequestration in the vacuole. Hence, plants with an adequate P_i status can tolerate moderate Phi concentrations without showing detrimental effects (Thao and Yamakawa, 2009). However, Phi displays hormetic effects on plant physiology. Therefore, beneficial responses of Phi on plans would depend not only on the P nutrient status of the plant, but also on the Phi level applied, and the genetic background of the genotypes evaluated. Indeed, Phi accumulates massively in the cytosol and prevents P_i efflux out of the vacuole, while subsequent incorporation of P_i into the cells triggers an extensive transfer of Phi from the cytosol to the vacuole (Pratt *et al*., 2009). The concomitant prevention of Pi efflux from the vacuole may exacerbate P_i-starvation symptoms and lead to accelerated programmed cell death in P_i-starved plants (Singh *et al*., 2003; Jost *et al*., 2015). Therefore, the metabolic state of the cells and the P_i supply had a strong influence on the subcellular localization of Phi (Martinoia *et al*., 2000), which could also affect the mechanisms of interaction between Phi and P_i signaling and possibly the P_i-starvation response under different P-feeding conditions (i.e. sufficiency, starvation, resupply, or preloading) (Danova-Alt *et al*., 2008).

Because of its high mobility within plants (unlike most fungicides), Phi triggers different responses, including defense mechanisms, throughout the whole plant.

Phosphite as pesticide and inductor of plant resistance against pathogens

Experimental and empirical evidence conclusively indicates that Phi functions as an effective pesticide against various species of pathogenic bacteria, oomycetes, fungi and nematodes (Chase, 1993; Smillie *et al*., 1989; Deliopoulos *et al*., 2010; Hofgaard *et al*., 2010; Dias-Arieira *et al*., 2013; Percival and Banks, 2014; Puerari *et al*., 2015). This is because Phi triggers broad-spectrum resistance against pathogens (Jost *et al*., 2015), suggesting that Phi acts as a priming agent of plant defense responses in a number of plant-pathogen interactions (Machinandiarena *et al*., 2012; Massoud *et al*., 2012; Dalio *et al*., 2014).

Phi has been effective in controlling some pathogenic bacteria. In potato, the application of either 1.0 or 0.67% (v/v) potassium Phi inhibited the growth of the bacterium *Streptomyces scabies* by almost 80 and 60%, respectively. However, at lower concentrations, inhibition of bacterial growth was less than 15% (Lobato *et al*., 2010). Furthermore, foliar applications of potassium phosphite to field grown potato crops resulted in post-harvest tubers with a reduced susceptibility to *Erwinia carotovora* infections, suggesting that this compound induced a systemic defense response (Lobato *et al*., 2011). Phosphite applied as a protective and curative treatment significantly reduced blue mold incidence caused by *Penicillium expansum* in wounded and inoculated apple fruit (Amiri and Bompeix, 2011). Blue mold incidence was significantly reduced in fruit treated for 3 min in a solution of Phi at 2 mg mL^{-1} heated to 50 °C after six months of storage at 2 °C. Consequently, Phi may be part of a general blue mold management program. In apple trees, Aćimović *et al*. (2015) demonstrated that with the development of injectable formulations and optimization of doses and injection schedules, the injection of Phi could serve as an effective option for fire blight control caused by *Erwinia amylovora*.

Phi has been suggested to act as a priming agent of plant defense responses against a number of fungi and oomycetes including the genera *Phytophthora*, *Fusarium* and *Rhizoctonia*, among others (Smillie *et al*., 1989; Förster *et al*., 1998; Machinandiarena *et al*., 2012; Alexandersson *et al*., 2016). In this review we recognize that oomycetes are within the kingdom *Protoctista* rather than the kingdom Fungi. Nonetheless, we will use the terms fungicide, fungistatic and antifungal to include activity against members of either group. Förster *et al*. (1998) reported that incidence of *Phytophthora capsici* was significantly reduced in pepper plants grown hydroponically. Foliar application of Phi reduced late blight infection and tuber blight caused by *Phytophthora infestans* in potato (Andreu *et al*., 2006; Kromann *et al*., 2012). When combining foliar field application with post-harvest application of Phi, Taylor *et al*. (2011) and Miller *et al*. (2006) observed good protection against pink rot caused by *P. erythroseptica* during storage. As well, Lobato *et al*. (2011) and Johnson *et al*. (2010) reported that post-harvest application of phosphite can result in good effect against the spread of potato tuber blight during storage. Furthermore, foliar applications of Phi reduced the susceptibility of tubers to *P. infestans* (Cooke and Little, 2002; Liljeroth *et al*., 2016), while post-harvest application of phosphite was also effective against this oomycete in stored potato tubers (Lobato *et al*., 2008). Combined application of the pesticide mancozeb with Phi resulted in better protection against potato tuber blight than the pesticide alone (Cooke *et al*., 2002). Similarly, Liljeroth *et al*. (2016) demonstrated that the most efficient control against potato late blight under field conditions was obtained with a combination of Phi and the broad spectrum nonsystemic fungicide chlorothalonil (Liljeroth *et al*., 2016).

In ornamental plants such as *Catharanthus roseus*, Banko and Hong (2004) reported that foliar applications of Phi at a concentration of 0.5 mM at three to six day intervals gave protection against *P. nicotianae* similar to foliar applications of *Aliette fungicide* at 3 g L^{-1} applied at 14 day intervals. Furthermore, Shearer and Fairman (2007) observed that application of Phi in a high-volume foliar spray delays and reduces the rate of mortality of four *Banksia* species infected with *Phytophthora cinnamomi*. In species of the genus *Lambertia*, variation within genotypes in efficacy of low-volume aerial Phi spray for control of *Phytophthora cinnamomi* was observed (Shearer and Crane, 2012).

Phosphite reduced the downy mildew damage caused by the fungi *Peronospora manshurica* in soybean (Silva *et al*., 2011). Furthermore, Simonetti *et al*. (2015) reported for the first time the control of *Macrophomina phaseolina* using combined treatment with plant growth promoting rhizobacteria (PGPR) and Phi in soybean grown under greenhouse conditions. Since this fungus is cosmopolitan in distribution and causes dry root rot/stem canker, stalk rot or charcoal rot in over 500 plant species (Khan, 2007), including vegetables, fruits and potatoes, Phi may represent a good control to inhibit its spread in horticultural crops (Shafique *et al*., 2016).

Whereas some Phi-containing products can act properly as fungicides (i.e. potassium Phi), others have been showed to be fungistatic (i.e copper Phi and calcium Phi) (Lobato *et al*., 2010). In any case, Phi has proved to be effective against *P. infestans*, *Fusarium solani* and *Rhizoctonia solani*.

Concerning Phi effect on nematodes, Dias-Arieira *et al*. (2012) observed that potassium phosphite was effective in reducing the population of *Pratylenchus brachyurus* in maize, probably due to the capacity of the Phi to stimulate plant defense mechanisms involving the production of phytoalexins (Dercks and Creasy, 1989). Additionally, manganese Phi was effective against *Meloidogyne javanica* in soybean, reducing the number of eggs per gram of root when applied seven days before the inoculation of nematodes in pest-resistant cultivar MG/BR 46 Conquista (Puerari *et al*., 2013a and b). Similarly, Oka *et al*. (2007) observed that potassium Phi applied to the shoot controlled *Heterodera avenae* and *Meloidogyne marylandi* in wheat and oat, confirming the capability of Phi to stimulate phytoalexin synthesis in treated plants (Dercks and Creasy, 1989). Because nematodes are very common in some vegetables and potatoes, Phi may represent an effective means to control such pathogens in horticulture, which has to be subjected to testing in the future.

Recently, Alexandersson *et al*. (2016) have highlighted the importance of Phi to protect crops against pathogens by activating the plant's own defense, thus emphasizing it as a potential plant resistance inducer (PRI) of paramount significance for novel plant protection approaches. Defense responses triggered by Phi include the accumulation of phytoalexins, while lignification of the cell wall is also common. Importantly, hypersensitive cell death may also take place, thus avoiding the proliferation of infected cells. Lytic enzymes produced by the plant may also contribute to pathogen control.

The effect of Phi on pathogen control depends on application time, cultivar evaluated, location and disease incidence and severity (Cicore *et al*., 2012). Because plants can acquire Phi and translocate it to different organs via both xylem and phloem, this oxyanion can be applied in different ways and the application method depends on the crop-pathogen combination, but foliar application is most common (Kiirika *et al*., 2013). In addition, other techniques such as fertigation, trunk spray, trunk injection, trunk paint, in-furrow as well as root or soil drenches can also be used (Deliopoulos *et al*., 2010; Alexandersson *et al*., 2016).

Although information on the mode of action of Phi is limited and often controversial, it appears that Phi triggers complex processes against pathogens, involving both direct (inhibition of reproduction or slow development rate) and indirect effects (rapid and strong stimulation of plant defense mechanisms) (Smillie *et al*., 1989, Grant *et al*., 1990, Guest and Bompeix, 1990, Guest and Grant, 1991, Jackson *et al*., 2000, Hardy *et al*., 2001, Brunings *et al*., 2005; Daniel and Guest, 2005; Deliopoulos *et al*., 2010). This complexity of mechanisms involved in the prophylactic effects of Phi is thought to have a limited effect on the development of pathogen resistance to Phi (Landschoot and Cook, 2005), albeit Grant *et al*. (1990) reported a naturally occurring *P. cinnamomi* strain resistant to fosetyl-Al. Nonetheless, how the primary recognition of Phi takes place, and which molecular pathways are altered within the plant subsequently to induce the primed state of stimulated defense remain to be elucidated (Schothorst *et al*., 2013; Jost *et al*., 2015).

Phosphite as a biostimulator in horticulture

Phosphite has largely been claimed to elicit biostimulant responses in a number of crop plants of importance in horticulture, resulting in improved yield, fruit quality, and tolerance to abiotic stress factors (Gómez-Merino and Trejo-Téllez, 2015).

Rickard (2000) reported that Phi increased the yield and quality of celery, onion, potato, and pepper. Similarly, soil or foliar applications of Phi improved quality of peaches and oranges when applied as a sole P-source. Results were attributed to a possible conversion of Phi to P_i by microorganisms in the soil or leaves. Nonetheless, since such microbial conversion might take months to be completed, and there is no evidence that Phi can be used directly by plants as a sole source of P nutrition, these responses are uncommon and deserve further studies (Thao and Yamakawa, 2009; Gómez-Merino and Trejo-Téllez, 2015). Indeed, studies on *Brassica nigra* and *Brassica napus* (Carswell *et al.*, 1996; 1997), as well as tomato and pepper (Förster *et al.*, 1998; Varadarajan *et al.*, 2002), demonstrated that Phi is not an appropriate P-source, as plants treated with Phi exhibited significant growth reduction and phytotoxic symptoms. This is consistent with studies performed by Bertsch *et al.* (2009), who found that application of P_i plus Phi (50% as H_3PO_4 and 50% as H_3PO_3) in the nutrient solution to hydroponic lettuce, tomato and banana improved biomass dry weight, foliar area, and P content in the whole plant. Instead, when foliar treatments using 100% P as Phi were applied to those crops, a drastic reduction of plant growth was observed, which was accompanied by evident deleterious effects such as worsened foliage and root deterioration. Recently, Estrada-Ortiz *et al.* (2016) found that Phi has differential effects on lettuce and chard physiology, and positive plant responses (related to P concentrations, total free amino-acids, soluble sugars and chlorophylls) are observed when Phi is used up to 0.25 mM in sufficient P conditions.

In onions, potatoes and tomatoes, Lovatt and Mikkelsen (2006) observed that Phi may increase floral intensity, and fruit yield and quality (e.g. soluble solid content). Such responses were attributed to the effect of Phi on sugar metabolism, internal hormonal and chemical changes, and shikimic acid pathway induction. In potato tubers, Phi increased pectin content in both periderm and cortex tissue (Olivieri *et al.*, 2012). It was also observed that Phi induced defense responses associated with a higher content and activity of polygalacturonase and proteinase inhibitor, while a new isoform of chitinase was detected in the tuber periderm of Phi-treated plants. Lobato *et al.* (2011) found that foliar applications of potassium Phi induced a systemic defense response in potato tubers, including an increase in phytoalexin and chitinase contents as well as enhanced peroxidase and polyphenol-oxidase activities, while maintaining potato yield at harvest. Similarly, the application of potassium Phi reduced the period between tuber planting and emergence, and increased leaf area and dry matter of potato plants (Tambascio *et al.*, 2014). Moreover, indigenous mycorrhizal colonization increased after Phi application to seed tubers, which has also been reported in other plant species (Howard *et al.*, 2000; Hardy *et al.*, 2001).

Fruit quality may also be enhanced by Phi applications in different horticultural crops. For instance, foliar application of potassium Phi to P-deficient citrus seedlings caused a biochemical response equal to that of calcium phosphate feeding and also restored plant growth (Lovatt, 1990). Similarly, foliar applications of K_3PO_3 to orange trees significantly increased the number of commercially valuable large size fruit, whereas both total soluble solids and the ratio of soluble solids to acid were improved (Lovatt, 1998; 1999). As well, winter pre-bloom foliar applications of Phi to Valencia oranges increased flower number, fruit set and yield, and increased total soluble solids (Albrigo, 1999). Moreover, Phi slightly enhances P_i uptake by citrus mycorrhizas, and stimulates root colonization by the symbiotic fungi (Graham and Drouillard, 1999; Graham, 2011). In citrus and avocado trees, Lovatt and Mikkelsen (2006) reported that a single foliar application of Phi increases floral intensity, yield, fruit size, total soluble solids, and anthocyanin concentrations.

According to Rickard (2000), Phi foliar sprays in citrus trees increased yield. Phosphite also improved soluble solids content, acidity and yield of Navel oranges. Quality of stone fruits was also improved as a result of Phi foliar sprays. Sugar content and soluble solids were significantly higher in Phi-treated solids peaches. Greater firmness in dark red berries, a factor related to premium pricing, was evident in raspberry fruit quality in Phi-treated plants.

In strawberry, Phi irrigation increased the quality of the fruits by activating the synthesis of ascorbic acid and anthocyanins (Moor *et al.*, 2009). Similarly, Estrada-Ortiz *et al.* (2013) reported that Phi applied into the nutrient solution increased anthocyanin content in strawberry too. Anthocyanins act as light attenuators (Ticconi *et al.*, 2001) and also as powerful antioxidants of great importance for plant physiology and human health (Zafra-Stone *et al.*, 2007; Lo Piero, 2015).

Phi is most effective when the rate and the application are properly timed to match the needs of the crop, which depend on the plant genotype (Lovatt and Mikkelsen, 2006), phenological stages and environmental conditions. Moreover, considering that Phi displays hormetic effects, its applications must be strictly supervised to avoid plant damage as a consequence of the toxicity it may cause (Gómez-Merino and Trejo-Téllez, 2015).

Phosphite may also trigger defense mechanisms against a number of abiotic stressors. In Phi-pretreated potato leaves exposed to UV stress, Oyarburo *et al.* (2015) observed that Phi increases chlorophyll content and expression of the psbA gene, which encodes a key photosynthetic protein. Oxidative stress caused by UV-B was also prevented by Phi, which demonstrates that this oxyanion mediates UV-B stress tolerance in potato plants. Therefore, Phi induction is not restricted to plant defense mechanisms against pathogens, but also abiotic stress and primary metabolism have been proved to be altered, while cell wall related proteins also increased in abundance in Phi-treated plants exposed to abiotic stressors (Burra *et al*., 2016).

Novel approaches for Phi use: development of dual fertilization and weed control systems

Though in nature plants are not capable of using Phi as a phosphorus source, genetic engineering is making it possible to use it as an alternative fertilizer and herbicide. López-Arredondo and Herrera Estrella (2012) developed a dual fertilization and weed control system by generating transgenic *Arabidospsis* and tobacco plants harboring a phosphite oxidoreductase (ptxD) bacterial gene, which are able to use Phi as a sole phosphorus source. Under greenhouse conditions, these transgenic plants require 30-50% less P_i input when fertilized with Phi to achieve similar productivity to that obtained by the same plants using Pi fertilizer and, when in competition with weeds, accumulate 2-10 times greater biomass than when fertilized with P_i. Similarly, Manna *et al.* (2016) engineered rice plants with a codon-optimized ptxD gene, demonstrating that ectopic expression of this gene led to improved root growth, physiology and overall phenotype in addition to normal yield in transgenic plants in the presence of Phi. Furthermore, Phi functioned as a translocative, non-selective, pre- and post-emergent herbicide. According to López-Arredondo and Herrera Estrella (2012) and Manna *et al.* (2016), Phi use as a dual fertilizer and herbicide might be of paramount importance for agricultural sustainability and food security, since this approach may mitigate the excessive use of phosphorus fertilizers and diminish eutrophication and the development of herbicide resistance. However, the novel use of Phi as fertilizers would imply a much larger input than its current use as a biostimulant or plant defense inductor against pathogens. Even if applied at 50% of the total P, its high mobility in the soil may cause accumulation in aquifers with generalized pollution, with negative impacts in different ecosystems. Furthermore, this novel approach would require the use of genetically modified (GM) plants that cannot be cultivated in many countries, including the European Union (EU). In general, public opinion currently appears to be biased against foods derived from GM organisms. As a consequence, in many countries GM crops are facing release restrictions, which have to be taken into consideration when designing programs aimed at using GM strategies. Moreover, the effects of Phi on human health are unknown, and the EU has lowered the Maximum Residual Level (MRL) of Phi in agricultural products. Despite the fact that under experimental conditions this technology has proved effective in reducing not only phosphorus fertilizer use but also the growth of the tested weeds, field trials with a variety of soil and environmental conditions are required to validate its commercial implementation. So far, no commercial transgenic plant harboring a recombinant ptxD protein is available in the market.

In terms of genetic engineering, López-Arredondo and Herrera-Estrella (2013) reported a novel selectable system for the selection of transgenic plants under in vitro and greenhouse conditions based on phosphite metabolism. Subsequently, Kanda *et al.* (2014) reported the application of phosphite dehydrogenase gene as a novel dominant selection marker for yeasts. Recently, Nahampun *et al.* (2016) developed a system using Phi as an effective selectable marker for Agrobacterium-mediated plant transformation. Therefore, novel technologies for Phi application are under development, and new avenues for the usage of Phi are foreseen, which could be of great significance for the development of future horticulture.

Phosphite in the market

A considerable number of commercial Phi-containing products are currently offered in the market. All these products are formulated as alkali salts (i.e. potassium-, ammonium-, sodium-, calcium-, magnesium-, aluminum-Phi, among others) of phosphorous acid. Though Phi does not contribute to P-nutrition in plants under natural conditions, most of these products have been registered as fertilizers. However, experimental evidence indicates that Phi functions as a pesticide against a number of bacteria, fungi, oomycetes and nematodes, as well as a potential biostimulator in crop production, rather than as a proper P source. However, agrochemical companies still commercialize Phi as a fertilizer, rather than as a pesticide. This is especially remunerative for those companies, as they avoid spending significant time and money on registering an agricultural pesticide (Gómez-Merino and Trejo-Téllez, 2015). Nevertheless, in 2013, the European Union (EU) changed the designation of Phi-containing compounds as both fertilizers and pesticides to only pesticides. This evolution is currently affecting international exports of foods to the EU that have been treated with Phi and certainly will influence the future use of Phi in horticulture worldwide. Importantly, on January 1, 2016, the EU maximum residue limit (MRL) for fosetyl-Al for several fruits and vegetables reverted back to the detection level set at 2 mg kg^{-1}, from 75 mg kg^{-1} set before 2015 (EU, 2016; USDA, 2016). Imports of berries and other commodities that use fosetyl or other phosphonate crop inputs will likely be threatened by the return to the default MRL. Therefore, crop producers that use phosphite-containing products and ship their horticultural products to the EU should review the EU's MRLs for fosetyl-Al to assess whether they are in compliance (Gómez-Merino and Trejo-Téllez, 2015).

Because of the widespread use of Phi in agriculture, several environmental and human health concerns have arisen. For instance, microbial species that are currently sensitive to Phi may become immune to it. Indeed, Guest and Grant (1991) reported a naturally occurring Fosetyl-Al resistant isolate of *Phytophthora cinnamoni*, and at least two Phi-resistant *Phytophthora* strains have been generated by chemical mutagenesis (Fenn and Coffey, 1984). Furthermore, the regular use of Phi treatments of crop plants may exert a strong selective pressure for microorganisms that are able to utilize Phi as a P source, and in turn, a significant selective pressure against organisms unable to utilize Phi as a source of P may take place. As a result, these changes could have adverse effects in the ecosystem as a whole. Despite results on the effect of Phi on symbiotic microbes (i.e. mycorrhizal fungi and nitrogen-fixing bacteria) are controversial (Despatie *et al*., 1989; Sukarno *et al*., 1993), further investigations into this area would be relevant. It is well documented that Phi disrupts plant metabolism, especially under suboptimal P-availability (Carswell *et al*., 1996; Carswell *et al*., 1997; Forster *et al*., 1998; Varadarajan *et al*., 2000). Hence, its use must be performed under strict control. Furthermore, one has to take into consideration regulatory issues aimed at warranting not only the efficacy of the product, but also its harmlessness to human or animal health when present at the levels likely to be encountered in the environment or food products. Importantly, the effectiveness of Phi as a fungicide relies on its stability within the plant for a long time and its high mobility in the same way as P_i does, often ending up in fruit tissue. Consequently, there is an obvious need to document Phi levels in food products derived from Phi-treated crop plants, and to ensure that long-term consumption of these products poses no threat to the public that consume them (McDonald *et al*., 2001).

Conclusions and final considerations

Novel genetic engineering platforms for the effective development of Phi-mediated dual fertilization and weed control systems have emerged in recent years. These platforms have allowed the use of Phi as a potential fertilizer alternative to P_i. However, in nature, plants are not able to metabolize Phi as a sole P-source, and its use can cause deleterious effects to plant cells if its administration is not properly managed (i.e. applied in the presence of sufficient P_i, at adequate levels). Since Phi displays hormetic effects (i.e. promoting positive responses at low dosages, but negative effects when breaking the physiological threshold leading to damage or cell death), its application must be under control and supervision to ensure better responses in non-biotech crops. In this review, we have provided evidence (fig. 3) that Phi can be used as a pesticide and a biostimulator in horticulture. Apart from having proved to be effective in the control of different plant pathogens, Phi improves plant performance by activating a number of molecular, biochemical and physiological mechanisms leading to induction of plant defense responses and improvement of crop production and productivity parameters.

As a biostimulator in horticulture, when nutritional P_i levels are optimal, the application of Phi may increase fruit quality (i.e. by activating the synthesis of antioxidant metabolites) and may also bring to bear increased flower and fruit set as well as better fruit quality and improved responses to environmental stimuli and stress agents. Apart from considering the P_i status of the plant, Phi efficiency is more evident when its rate and application are properly timed to fulfill the requirements of crop plants in order to stimulate physiological processes (Lovatt, 2013), which in

Fig. 3 - Experiments testing different concentrations of Phi on Brassica juncea and B. campestris under greenhouse conditions. Under our experimental conditions, Phi, at low concentrations and with sufficient Pi, enhanced antioxidant compounds concentration.
Fig. 3 - Esperimenti con diverse concentrazioni di fosfito su piante di Brassica juncea *e* B. campestris *alleviate in serra. Il fosfito a basse concentrazioni e con sufficiente dotaizone di fosfato ha aumentato la concentrazione di composti antiossidanti.*

turn depend on plant genotypes, environmental conditions, agronomic management, source and dosage of Phi to be used (Gómez-Merino and Trejo-Téllez, 2015).

With 276 plant genome sequencing projects either completed or currently under way (Gómez-Merino *et al.*, 2015; www.ncbi.nlm.nih.gov/genome/browse/), better understanding of the molecular mechanisms of P use efficiency can be achieved. It will be worthwhile to know how and to what extent Phi alters molecular processes that trigger defense responses and improved fruit yield and quality in either wild or genetically- engineered crop plants. In biotech crops engineered to use Phi as a nutrient source, it has the potential to act as an economical and effective agricultural input that would enable us to grow crops in soils with low P_i availability, address the problems of P depletion and herbicide resistance, and mitigate excessive P use to a considerable extent. However, there are several concerns regarding the widespread use of Phi in horticulture, including the development of Phi-resistance in pathogens, the effect of Phi on soil microflora, and the possible threat to public health. Therefore, there is an obvious need to study and document all these phenomena.

In summary, Phi can stimulate positive effects in crop plants if it is properly combined with P_i. In conventional agronomic systems (i.e. non-biotech crops), Phi serves as a pesticide and biostimulator that enhances yield, quality and plant performance under abiotic stressors. Thanks to its particular mobility throughout the whole plant, Phi can be applied in different ways: fertigation, foliar spray, trunk spray, trunk injection, trunk paint, in-furrow or as a soil drench. Therefore, determining the right method of application, as well as the most appropriate source, rate, and phenological stage for different horticultural species and cultivars, remains an unmet challenge.

To ensure efficient Phi use, horticultural producers must work closely with professional consultants, taking into consideration not only technical issues related to its application, but also international regulations governing the export of horticultural crops with residual levels of Phi, especially to the EU.

Abstract

Phosphite (Phi; H_2PO_3- or HPO_3^{-2}) or its conjugate phosphorous acid (H_3PO_3), a reduced form of inorganic phosphate (P_i; H_2PO_4- or HPO_4^{2}-), has increasingly been used as a pesticide against various species of plant pathogens of importance in horticulture. Indeed, Phi may control and/or induce resistance against pathogenic bacteria such as *Erwinia amylovora* and *E. caratovora*, as well as the oomycete genera *Peronospora, Plasmopara, Phytophthora* and *Pythium*, the fungi genera *Alternaria, Rhizoctonia* and *Macrophomina*, and the nematode species *Meloidogyne javanica, Pratylenchus brachyurus, Heterodera avenae* and *Meloidogyne marylandi*, among others. In recent years, Phi has emerged as a potential biostimulator improving yield and quality of a number of crop species, and inducing better performance of plants exposed to abiotic stress factors. In conventional agricultural systems, Phi has not been proved to have a direct effect on plant nutrition, and should not be considered as a proper fertilizer. Nonetheless, novel genetic engineering approaches are currently allowing its use in alternative P fertilization and weed control, albeit its commercial application is still at issue. Though this innovative technology could address the imminent danger of phosphate reserve depletion and multiple herbicide tolerance in an increasing number of weeds, environmental and human health concerns need to be critically approached. Its role as inductor of beneficial metabolic responses in plants is more evident in conditions of P_i-sufficiency. Additionally, Phi applications are more efficient when its rate and utilization are properly timed to meet the requirements of crop plants in order to stimulate physiological processes, which in turn depend on plant genotypes, environmental conditions, agronomic management, source and dosage of Phi to be used. This paper outlines recent research advances on the impact of Phi as a pesticide, biostimulant, and a dual fertilizer and herbicide in horticulture, and discusses potentialities and challenges of its use, especially those related to its impact in the environment and human health.

Keywords: Phosphorus, phosphorous acid, pesticide, biostimulant, alternative fertilizer, herbicide

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