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Effects of phosphite on phosphorus supply and growth of corn *(Zea mays)*

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Abstract

Phosphites are alkali salts of phosphorous acid, e.g. potassium phosphite, which are widely used as fungicides. Recently, phosphite-containing formulations claim to contribute to the phosphorus (P) supply of horticultural crops, too. In this study, therefore, the efficiency of potassium phosphite as a P source for maize plants has been studied in selected plots of a long-term field trial and in a pot experiment.

For the simultaneous determination of phosphate and phosphite, an analytical method was developed that based on ion chromatography including a solid phase extraction step to eliminate co-extractants of the water extracts.

In the field trial, the growth responses of maize to phosphite foliar application led to severe negative growth effects when applied to plants grown under sub-optimal P nutrition. However, this detrimental effect was lower when phosphite was applied to maize under sufficient P nutrition. These tendencies matched the results of the pot experiment. An inhibited growth of the maize plants was observed, after potassium phosphite was applied as the sole P source. The negative effects ranged from a stunted growth of the plants to the complete die off. The detection of phosphite in the harvested maize plants also indicated that an oxidation to phosphate did not occur. Hence, phosphite was not available to the plants as a P source.

Keywords: phosphate, phosphite, maize, plant growth, ion chromatography

Zusammenfassung

Einfluss von Phosphit auf Phosphorversorgung und Wachstum von Mais (*Zea mays*)

Phosphite, Alkalisalze der phosphorigen Säure (H_3PO_3), wurden ursprünglich als Mittel mit fungizider Wirkung eingesetzt. Breite Anwendung finden phosphithaltige Formulierungen seit einiger Zeit im Gartenbau auch als Blattdünger zur Phosphor-(P)-Versorgung. In dieser Studie wurde die Wirksamkeit von Kaliumphosphit als P-Quelle für die Ernährung von Maispflanzen in ausgewählten Parzellen eines Langzeit-Feldexperimentes und in einem entsprechenden Sandkultur-Gefäßversuch untersucht.

Zur parallelen Bestimmung von Phosphat- und Phosphitgehalten in wasserextrahierten Pflanzenproben wurde eine Analysenmethode entwickelt, die basierend auf der Ionenchromatografie eine Reinigungsstufe in Form von Festphasen-Extraktionssäulen (SPE) zur Eliminierung von Matrixeffekten enthält.

Die Ergebnisse der Felduntersuchungen zeigten, dass Mais, der nicht optimal mit P versorgt war, nach Applikation von Kaliumphosphit als Blattdünger mit Blattschädigungen und Wachstumsdepressionen reagierte. Bei ausreichender P-Ernährung war der schädigende Effekt durch die zusätzliche Blattdüngung wesentlich geringer. Im Sandkulturversuch verursachte die Düngung mit Kaliumphosphit als alleinige Quelle für die P-Versorgung der Maispflanzen starke Pflanzenschäden, die von Missbildungen und Wachstumsstillstand bis hin zum vollständigen Absterben der jungen Pflanzen reichten. In der Pflanzensubstanz selbst wurde Phosphit nachgewiesen. Das deutet darauf hin, dass Phosphit zwar von der Pflanze aufgenommen wird, aber im Stoffwechsel nicht vollständig zu Phosphat oxydiert werden kann und somit für die Phosphorernährung der Pflanze nicht zur Verfügung steht.

Schlüsselworte: Phosphat, Phosphit, Mais, Pflanzenwachstum, Ionenchromatografie

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1 Introduction

As early as 1930, a study was carried out to determine the efficiency of various phosphorus (P) containing compounds as fertilizers (MacIntire et al., 1950). Phosphite was determined to be a poor source of nutritional P since plants treated by phosphite grew weakly. Therefore, this compound was eliminated in the market as a potential source of P in plant nutrition. Four decades later, phosphite returned to the agricultural market when it was sold as a fungicide. It was found that phosphite suppressed plant diseases caused by *Phytophthora*. The same fungi caused the potato blight that finally led to the Irish potato famine in 1846. The toxic effect of phosphite to *Phytophthora* has been understood to be acting either by activating defense mechanisms in plants or by directly acting on the fungi itself. One study showed that it affected the fungal pathogens by both modes of action (Jackson et al., 2000).

Phosphite fungicide is first formulated as ethyl phosphonate by reacting phosphite with ethanol to form the ethyl phosphonate anion and an aluminum ion as the counter ion. It is widely marketed under the trademarks *Aliette*® or *Fosetyl-Al* (Bayer CropScience). When applied to plants *Fosetyl-Al* releases phosphite as a product of hydrolysis (Figure 1) which is responsible for the protection of plants against fungal diseases.

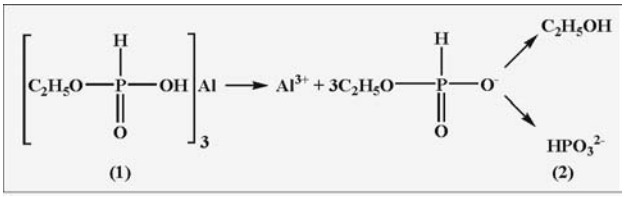

Figure 1:
Decomposition of aluminum ethyl phosphite (1) to phosphite (2) in plant tissues (Saindrenan et al., 1985)

When the patent for the trademark *Fosetyl-Al* was expired, several fungicide manufacturers created phosphite-based fungicides by simple formulation of phosphite with potassium, ammonium, sodium, and aluminum. These salts release phosphite ions in plants, hence, in theory, are as equally effective agent as the pioneer *Fosetyl-Al* in controlling fungal diseases. Thus, *Fosetyl-Al* and other phosphite salts are sold under several brand names: *Fosphite, Prophyt, Phosguard, Nutrol*. They have been continuously used to control *Phythophthora* infections of e.g. avocado, pineapple, and cacao crops. The native trees *Jarra* and *Banksia*, species in Western Australia, are protected against infection by *Phythophthora* through injecting them phosphite fungicides. The oak trees in California, which were known to die of sudden oak death (SOD) caused by a fungal pathogen, were treated by the application of phosphite solution (http:// www.biagro.com/nutri_grow/ng_html/planthealth.html).

1.1 Commercialization of phosphite as a fertilizer

The effectiveness of phosphite in controlling plant diseases over-shadowed the potential of phosphite as a fertilizer. However, interest in the nutrient property of phosphite was renewed when Lovatt (1990) discovered that P deficiency in citrus species caused changes in nitrogen metabolism. Through the application of potassium phosphite the biochemical response as well as a normal plant growth were restored. Furthermore, Lovatt showed that fruit set and yield of avocado were improved when potassium phosphite was applied with foliar sprays (Lovatt, 1990a; 1998).

The work of Lovatt led to the first commercialization of phosphite compounds as a fertilizer. The product was patented and sold under the trademark *Nutri-Phite* (Biagro Western Sales Inc), which is potassium phosphite, derived from phosphorous acid, potassium hydroxide, and the organic tripotassium citrate. This product is sold as a P nutrient fertilizer for foliar application and is used in a wide variety of field and horticultural crops. The list of phosphite products that are available in the German market includes the brand names *Kalium Plus* (Lebosol), *Folistar* (Jost), *Frutogard* (SpiessUrania), and *Phosfik* (Kemira GrowHow). All of them are formulated as alkali salts of phosphorous acid and all are still registered under the German fertilizer law. The possibility of registration of phosphite as a P fertilizer could be due to the predefinition in the German fertilizer law that the composition of a P fertilizer should be expressed in terms of P_2O_5 (Kluge and Embert, 2003). This is based on early practices, which report elements as calcined solids that produce oxides, hence, the reporting of P as "% P_2O_5". Therefore, although a fertilizer contains phosphite instead of phosphate this would still be conform to the law if P is declared as P_2O_5 in the fertilizers analyses.

1.2 Conflicting results from the usage of phosphite as a fertilizer

In previous publications, it has been suggested that the phosphite compound is an effective source for the P nutrition of plants. Rickard (2000) compiled the results of greenhouse and field scale experiments obtained from the investigation of crop responses to a commercially sold phosphite fertilizer. The results were shown to demonstrate a consistent trend towards higher yields, and an additional benefit by quality and appearance improvement of the harvested crops. However, in none of these references the P status of the plants has been seriously investigated. In contrast, other recent publications indicate that phosphite is not a superior P source. Hydroponically cultivated tomato and pepper plants treated with phosphite exhibited a significant growth reduction compared to phosphate-fertilized plants (Forster et al., 1998; Varadara-

jan et al., 2002). Potted *Vinca* plants applied with phosphite as sole source of P nutrition grew significantly smaller than those with phosphate fertilizer (Banko and Hong, 2001). Similar deleterious effects of phosphite treatment on the growth and development of plants were observed on *Arabidopsis* plants (Ticonni et al., 2001). Furthermore, phosphite exhibited phytotoxicity when the compound was applied to *Brassica nigra* seedlings and *Brassica napus* cell cultures (Carswell et al., 1996; 1997).

1.3 Phosphite suppresses the responses of plants to P-deficiency

Interestingly, it was found that phosphite is not really utilized in plants but rather acts detrimentally when applied to plants under P deficiency (McDonald et al., 2001). Phosphite tricks P deficient plants into not mimicking typical P deficiency symptoms. When plants are P deficient morphological and molecular responses are activated to increase the P acquisition. The increase in root to shoot ratio, enhancement of root hair formation and hair density and anthocyanin accumulation are hallmarks of morphological responses of plants to P starvation. Molecular responses of plants to P deficiency include the synthesis of enzymes (e.g. acid phosphatase) and transporters (e.g. high affinity plasmalemma phosphate translocator) for scavenging P to be able to adapt to P starvation (Mc Donald et al., 2001). However, phosphite was found out to prevent some of these morphological responses of plants to P deficiency. Ticconi et al. (2001) showed that length and density of root hairs of *Arabidopsis* plants grown under P starvation were significantly reduced when the plants were treated with phosphite, and the accumulation of anthocyans in the leaves was prevented, too. Similarly, the root elongation of tomato plants under P-stress was inhibited resulting in the reduction in root to shoot ratio when the plants were treated with phosphite solution (Varadarajan et al., 2002). In contrast, the root to shoot ratio of P-sufficient plants supplied with phosphite showed minimal changes. This difference indicates that none of the typical P starvation induced symptoms was present in P-deficient plants when fed with phosphite. Moreover, various research studies have shown that the induction of enzymes and transporters characteristic for P deficiency was significantly reduced if not inhibited when phosphite was applied to plants grown under P starvation. Examples are the enzyme acid phosphatase (APase) and the pyrophosphate-dependent phosphofructokinase (PFP), which are activated in the absence of P. Both enzymes were highly suppressed when P-starved *Brassica napus* was treated with phosphite (Carswell et al., 1996; 1997). Phosphite fed to P-starving plants repressed the induction of novel acid phosphatase LEPS2, a phosphate starvation-induced gene (Baldwin et al., 2001). The suppression of multiple P-starvation induced genes (phosphate transporters, phosphatases, novel genes) by phosphite could be verified in tomato and *Arabidopsis* plants cultivated under P-starvation (Liu et al., 1998; Ticconi et al., 2001; Varadarajan et al., 2002). Overall, these findings suggest that the normal perception and response mechanism of plants to P deficiency is being obstructed by a phosphite treatment under sub-optimal P supply.

Maize (*Zea mays*) is a crop with a high growth rate, large leaf areas, and comparative robust leaf surfaces, which has global importance in agricultural production. Therefore, based on the aforementioned results and conclusions, the different effects of P fertilization in form of phosphate, phosphite and both in combination were investigated in selected plots of a long-term field study and in a pot experiment. For this purpose, it was necessary to develop an analytical method for the simultaneous determination of phosphate and phosphite contents in plant materials.

2 Material and methods

2.1 Field trial

The field experiment was carried out at the experimental site of FAL Braunschweig in the frame of an already 20 years lasting long-term experiment. Two different P fertilization treatments were conducted: application of 45 kg ha^{-1} P as triple superphosphate in combination with organic fertilization in form of farmyard manure (4.8 t ha^{-1} DM), and without P fertilization. The P treatment represented P sufficiency with mean P contents of 50.9 and 38.2 mg kg^{-1} P_{CAL} in the upper soil layers (0-15 and 15-30 cm). Without P fertilization, P deficiency occurred with mean available P concentrations of 14.5 and 15.8 mg kg^{-1} P_{CAL}, respectively. Within the crop rotation, the maize (*Zea mays*), variety "Prinz", followed winter wheat/mustard, and was sown at the end of April. Three months after sowing, at growth stage BBCH 51-53, five maize plants of each test plot were treated with P foliar fertilizer (15 mg P per plant dissolved in 9 ml water) using either potassium dihydrogenphosphate (KH_2PO_4) or potassium dihydrogenphosphite (KH_2PO_3). Table 1 shows a summarized scheme of the field trial.

The foliar fertilizer was applied on the three last, fully developed leaves by means of a paintbrush. Afterwards, those leaves were marked with paper tags. Treated plants and control plants were covered with breathable plastic bags for 7 days to avoid washing off by precipitation (Figure 2). The maize plants were harvested two weeks after foliar fertilization.

For the analysis of dry matter yield and P content, each plant was cut into three segments: the bottom segment consisting of the plant parts above the ground, the middle segment consisting of the leaves where foliar fertilization was directly applied and the upper segment consisting of

Table 1:
Design of the field trial (FV4) with different treatments of soil and foliar P fertilization

Soil fertilization	Foliar fertilization
P deficiency: without P fertilization	without (control) with KH_2PO_4 [1)] with KH_2PO_3 [1)]
P sufficiency: 45 kg ha^{-1} mineral P in combination with organic fertilization [2)]	without (control) with KH_2PO_4 [1)] with KH_2PO_3 [1)]

1) 15 mg P as 0.2% w/v KH_2PO_4 or KH_2PO_3 solution, respectively
2) triple superphosphate + farmyard manure (4.8 t ha^{-1} DM)

Figure 2:
Maize plants covered with plastic bags immediately following the foliar fertilization (FV4, Braunschweig 2004)

those leaves, which were not treated with foliar fertilizer, including the flag leaf. In addition, all developed corncobs were analyzed separately. Fresh weights of the plants were recorded directly after cutting and plant dry matter was determined after drying in a kiln at 50 ºC for 48 hours until constancy of weight.

2.2 Pot experiment

The pot experiment was carried out in Mitscherlich pots (volumetric capacity of 8 kg). Cleaned gravel sand was used as substrate; deionized water was utilized for irrigation. Initially, only three different sets of soil P fertilization were created, each consisting of 12 replicates. The first treatment received P fertilization (1,008 mg P per pot) in form of dissolved potassium dihydrogenphosphate (KH_2PO_4), the second treatment the same P amount as dissolved potassium dihydrogenphosphite (KH_2PO_3). Another set of pots was prepared without any P application. All other essential macro and micronutrients were adjusted at a level for optimum supply (Table 2).

28 days after seeding (BBCH 16) the plant density was reduced to four plants per pot and the three soil fertilization treatments were split into three different soil/foliar fertilizing combinations (Table 3). KH_2PO_4 or KH_2PO_3 were applied in a 0.2 % w/v solution.

Table 2:
Composition of the nutrient solutions for the pot experiment (calculated for 1 pot)

Nutrient	Rate [mg]	
N	504	⇨ applied before sowing
	756	⇨ applied at BBCH 12
K	1,288	
Mg	63	
Ca	594	
S	84	
Fe	10	
additional essential micronutrients		

Table 3:
Design of the pot experiment with different treatments of substrate and foliar P fertilization

Substrate treatment	Foliar fertilization
Without P supply	without with KH_2PO_4 [1)] with KH_2PO_3 [1)]
Optimum P supply using KH_2PO_4	without (control) with KH_2PO_4 [1)] with KH_2PO_3 [1)]
Optimum P supply using KH_2PO_3	without with KH_2PO_4 [1)] with KH_2PO_3 [1)]

1) 15 mg P as 0.2 % w/v KH_2PO_4 or KH_2PO_3 solution, respectively

The nutrient solution was applied on the three youngest, fully developed leaves by means of a paintbrush. Afterwards, the foliar-applied leaves were labeled. Four weeks later, those plants that were growing healthy received a second foliar fertilization. All plants that showed extrem leaf damages were harvested at this time. The final harvest of the plants was conducted 90 days after sowing. The plant material was prepared according to the field trial.

2.3 Plant analyses

Several methods have been widely used for the analysis of P in orthophosphate form. However, there are no accepted standard methods presently available for the measurement of P in the phosphite form. A special problem in analyzing phosphite is the dissolution of the phosphite compound into the solution without oxidizing the compound to phosphate. In some published works the phosphite compound was determined with ion chromatography (McDowell et al., 2004; Ouimette and Coffey, 1989; Roos et al., 1999) or with gas chromatography coupled with mass spectrometry (Butts and Rainey, 1971;

Mawhinney, 1983; Smillie and Grant, 1988). A more sophisticated method uses phosphorus nuclear magnetic resonance spectroscopy (Carswell et al., 1996).

In this study the total P content of the plant material was determined employing the molybdenum blue method according to John (1970) after dry combustion of 1 g plant material. Phosphate and phosphite concentrations were simultaneously determined by ion chromatography (IC) adapted to the special requirement of the target analytes.

2.3.1 Sample preparation

0.5 g of finely ground and completely dry plant material was weighed in a plastic vial and 20 ml of deionized water were added. The vial was shaken for 3 hours on a horizontal shaker at 220 rpm and was left to stand for 30 min. After sedimentation, the solution was partly decanted into a 5-ml Eppendorf plastic tube and was centrifuged for 10 minutes at 10,000 g and at 20 ºC. A 2-ml aliquot of the supernatant was filtered through a 0.22 µm Millipore filter (Millipore® Corporation, Bedford, MA, USA) connected to a disposable syringe. The filtrate was then directly passed through a conditioned Bond Elut® C18 cartridge (Varian BV, Middelburg, Netherlands) to eliminate organic co-extractants. The collected outflow was subjected to IC analysis.

2.3.2 Conditioning of Bond Elut® SPE cartridges

The Bond Elut® SPE cartridges (2-ml, 250 mg) were conditioned by passing 2 ml of methanol (HPLC gradient grade, Mallinckrodt Baker BV, Deventer, Netherlands) followed by two times 2 ml deionized water. Subsequently, the samples under study were transferred to the columns.

2.3.3 Ion chromatography system

The analyses were performed using a Metrohm Compact IC 7618 equipped with a suppressor and a conductivity detector (Metrohm IC-Detector 732). The separation was carried out by means of a Metrosep A Supp 5-250 (4.0 x 250 mm) analytical column and a Metrosep A Supp 4/5 guard column. The eluent for the column consisted of 4.0 mM Na_2CO_3 and 3.0 mM NaOH in 10 % acetone and run at 0.7 ml min^{-1}. The suppressor regenerants consisted of 50 mM H_2SO_4 and tridistilled water and run at 3.5 ml min^{-1}. For sample loading, a Metrohm IC Sample Processor 766 was used.

2.3.4 Preparation of standards

A commercially available standard stock solution of orthophosphate (1 g L^{-1}, CertiPUR, Merck KGaA, Darmstadt, Germany) was used as the reference standard for phosphate analysis. A standard stock solution containing a corresponding phosphite concentration (1 g L^{-1}) was prepared by dissolving phosphorous acid in deionized water. A standard containing phosphate and phosphite was prepared from the stock solutions. A five-point calibration was performed at concentration levels of 1, 10, 20, 50, and 100 mg L^{-1}. Phosphate and phosphite contents of the sample material were identified by comparison of the retention time with the reference standards and quantification was performed using an external calibration curve.

2.3.5 Method development for the simultaneous determination of phosphate and phosphite in plant material by ion chromatography

Figure 3 shows the chromatogram of the standard containing 20 mg L^{-1} of phosphate and phosphite. The retention times for phosphate and phosphite were 22 and 15 min, respectively. Total run time was less than 30 minutes.

The analysis of the standard mixture extracted out of a water sample by the Bond Elut® C18 cartridge showed recovery rates for phosphate in the range of 96 to 118 % (mean: 102 %, RSD: 8.7 %, n = 7) while recovery rates for phosphite ranged from 90 to 100 % (mean: 93 %, RSD: 3.8 %, n = 7).

The peak area for phosphate and phosphite showed a high correlation (r = 0.999) within the calibration range under study.

For analytical quality assurance, fortification experiments were conducted. For this purpose, homogeneous maize plant extracts were spiked with a defined amount of phosphate and phosphite: 0.5 ml KH_2PO_4 solution (142 mg phosphate) and 0.5 ml KH_2PO_3 solution (118 mg phosphite). The two target analytes were baseline separated as shown in the chromatogram in Figure 4. Purification via solid phase extraction minimized interfering peaks of organic co-extractants from the plant matrix. Further peaks, e.g. from other inorganic ions, were recorded.

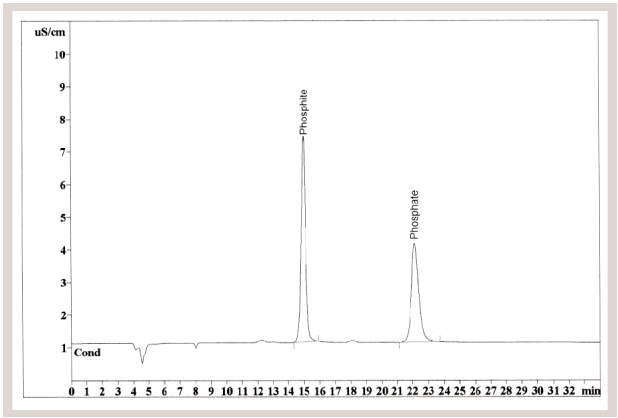

Figure 3:
Chromatogram of a mixted standard containing 20 mg L^{-1} of phosphate and phosphite

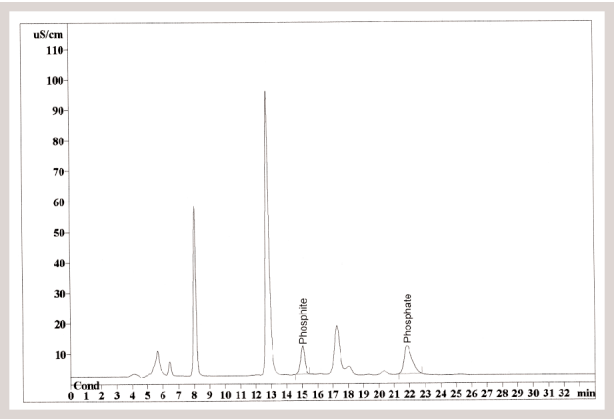

Figure 4:
Chromatogram of phosphate and phosphite in an aqueous maize plant extract of a fortification experiment at a concentration of 208 mg L^{-1} and 180 mg L^{-1}, respectively

Table 4:
Influence of different P fertilization treatments on the dry matter yield of maize plants [g DM per plant] (FV4, Braunschweig 2004)

Soil P fertilization	Foliar P fertilization		
	Without	Phosphate[1)]	Phosphite[2)]
	Plant dry matter [g]		
P deficiency	35 ab	45 b	29 a
P sufficiency	45 a	45 a	42 a

[1)] one-time 15 mg P as 0.2 % w/v KH_2PO_4 solution
[2)] one-time 15 mg P as 0.2 % w/v KH_2PO_3 solution
All values are means of five replicates. Means followed by dissimilar letters in rows are significantly different (Duncan's test, P = 0.05).

However, IC separation performance was sophisticated enough for quantitative analysis of phosphate and phosphite.

Furthermore, a narrow peak for phosphite was obtained while the phosphate peak tended to peak broadening. Recoveries for phosphate ranged from 85 to 92 % (mean: 88 %, RSD: 2.8 %, n = 7) while recoveries for phosphite ranged from 73 to 85 % (mean: 79 %, RSD: 5.7 %, n = 7). The experimental lower limit of detection (LLD) for phosphate as well as for phosphite was about 1 µg L^{-1}.

3 Results and discussion

3.1 Growth responses of maize plants to different phosphate and phosphite fertilization

3.1.1 Field trial

An overview of total dry matter yield of field grown single maize plants treated with phosphate and phosphite foliar fertilization is given in Table 4. The results show that the application of phosphate as well as phosphite foliar fertilizer on maize grown with an optimum basic P fertilization (mean plant available P content of the topsoil: 44.5 mg kg^{-1}) had no significant influence on the further growth of the plants. The total dry matter yield of the plants applied with phosphite foliar fertilizer was lower compared to the other treatments, but there were no statistically significant differences.

Under P deficient soil conditions (mean plant available P content of the topsoil: 15.2 mg kg^{-1}) the foliar fertilization with different P compounds entailed measurable differences in the dry matter yield of individual plants. In comparison to the treatment without foliar fertilization, the phosphate application increased the yield by 29 % while the phosphite application caused a yield decrease by 18 %.

In Figure 5, the dry matter yield effect of different foliar fertilization is separately shown for the plant segments bottom, middle and top, and the developed corncobs (spadices).

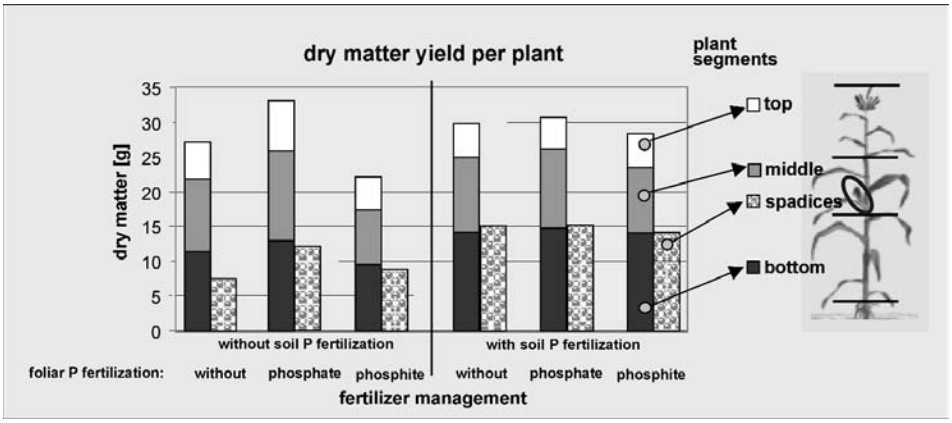

Figure 5:
Influence of P fertilizer management on dry matter yield of separate plant segments of single maize plants (FV4, Braunschweig 2004)

The negative effect of phosphite foliar fertilization was explicitly determined under P deficiency; the dry matter weight of all vegetative plant parts was lower in comparison to the other treatments (Figure 5). Under P sufficient conditions, there were nearly no differences in dry matter yield of the bottom as well as the top plant parts between the three foliar fertilization treatments. In the middle plant parts, where the P foliar fertilizer solutions had been applied, the phosphite induced impairment resulting in lower yields, both of the leaves and the corncobs.

Overall, these data suggest the negative effect of phosphite application to P-deficient plants. The treated leaves featured visible damages in form of broad chlorotic spots and streaks on leaf blades. Leaf margins and leaf tips were necrotic (Figure 6). Such a clearly detrimental effect was not observed when phosphite was applied to the plants grown with optimum P supply via soil. The treated leaves had only slight marginal necroses with measurable, but not significant yield effects (Table 4).

Neither under P sufficient nor under P deficient soil conditions, any foliar injuries were determined after the application of phosphate containing fertilizer solution. Furthermore, an additional growth-stimulating effect by foliar phosphate fertilization could not be ascertained when the P supply via roots was sufficient.

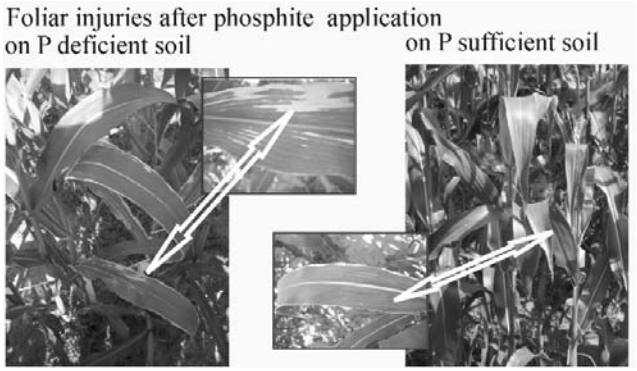

Figure 6:
Comparison of foliar damages after treatment of maize leaves with KH_2PO_3 solution under P deficient (left) and P sufficient (right) soil conditions (FV4, Braunschweig 2004)

3.1.2 Pot experiment

While maize plants adequately supplied with P as phosphate were healthy and normally developed, the plants supplied with P in phosphite form showed an inhibited growth and serious plant damages (Figure 7). In comparison, they were even smaller than the control plants, which did not receive any P fertilization.

Furthermore, the phosphite fertilized plants showed an abnormal habitus. The plants developed lateral tillers with brightened, malformed leaves (Figure 8).

Similar growth reactions of maize plants to phosphite containing fertilizers were observed by Lucas et al. (1979) and by Mitchell et al. (2004). However, it was described that such negative effects of phosphite application did not continuously appear, and its occurrence was year-dependent.

The effect of different foliar fertilization on the dry matter yield of maize plants is shown in Table 5 and Figure 9. When the P nutrition state of the plants was optimal, the additional foliar application of phosphate had a significant albeit small raising effect on the dry matter yield, whereas no effect was obtained by the phosphite foliar fertilization.

In contrast, the foliar treatment of the P-starving maize plants with the different P solutions resulted in striking growth differences. A significant increase in yield was observed for the phosphate application, the mean dry matter yield was with 25 g DM about 3 times higher than that of the control. Phosphite foliar applied plants were smallest, on average merely 4 g DM were produced (Table 5).

The stunted growth of maize resulting from phosphite as sole P source (Figure 9) indicates that this P compound cannot exploit as a nutrient within the plant metabolism. Under the extremely conditions in a pot experiment, lacking compensatory properties of natural soils, the damaging effect of phosphite for stressed plants became apparent (Figure 10). The additional foliar application of a 0.2 % w/v KH_2PO_3 solution caused irreversible foliar injuries, whose extent was dependent on the basic health

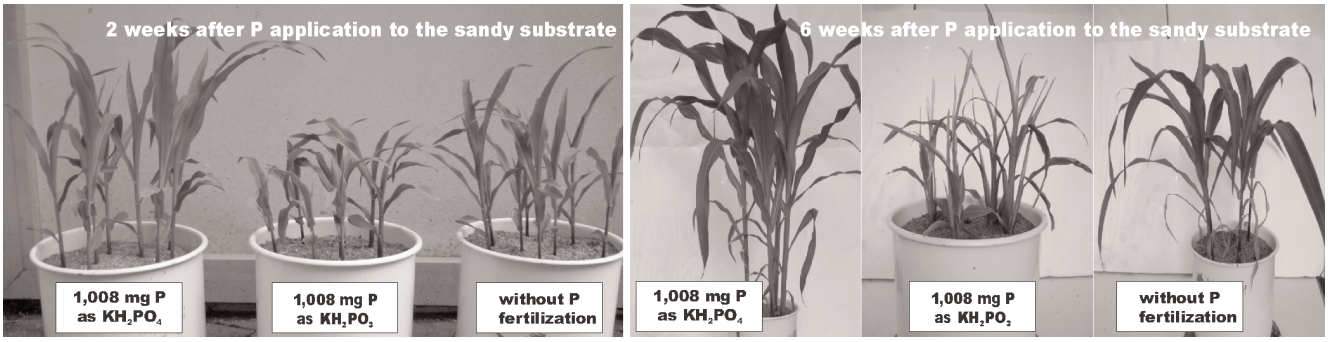

Figure 7:
Growth response of maize plants to different P fertilization: Comparison of the plant development 2 and 8 weeks after the application of the P fertilizer, respectively (pot experiment, Braunschweig 2004)

Table 5:
Effect of different P fertilization treatments on the dry matter yield of maize [g DM] (pot experiment, Braunschweig 2004)

Substrate P fertilization	Foliar P fertilization		
	Without	Phosphate[1)]	Phosphite[2)]
	Plant dry matter [g pot^{-1}]		
Without	9 b	25 c	4 a
Optimum P supply by KH_2PO_4	54 a	59 b	54 a
Optimum P supply by KH_2PO_3	4 b	5 b	1 a

1) 15 mg P as 0.2 % w/v KH_2PO_4 solution
2) 15 mg P as 0.2 % w/v KH_2PO_3 solution
All values are means of 4 replicates. Means followed by dissimilar letters in rows are significantly different (Duncan's test, P = 0.05).

Figure 8:
Single maize plant with lateral, malformed tillers - 6 weeks after substrate fertilization with KH_2PO_3-solution (pot experiment, Braunschweig 2004)

status of the plants. In part, the maize plants died off because of the extreme nutritional disorder.

In accordance with the results of the field trial, these data show the injurious effect of phosphite when applied

Figure 9:
Effect of foliar fertilization with phosphate and phosphite containing solutions at different P nutrition states on the growth of maize plants (pot experiment, Braunschweig 2004)

to P-deficient plants, whereas the P-sufficient plants continued to grow normally after a short stagnation period, immediately following the phosphite application. The growth increasing effect of phosphate foliar fertilizing verified in the pot experiment shows that the application time is vitally important: the foliage uptake of phosphate by maize plants seems to be more effective at an early developmental stage (BBCH 16).

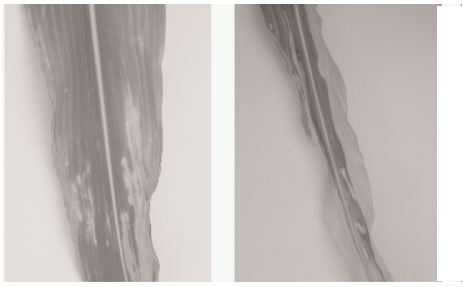

Figure 10:
Leaf necroses on P sufficient (left) and P deficient (right) maize plants after phosphite foliar fertilization (pot experiment, Braunschweig 2004)

3.2 Influence of different P fertilization management on the total P, phosphate and phosphite content of maize plants

3.2.1 Field trial

Two weeks after foliar treatment, measurable differences in the total P content were detected in the tissues of the maize plant segments (Figure 11).

In general, the mean P content, calculated for the whole plants, was slightly higher under P sufficient soil conditions. Increased P concentrations were determined in the foliar fertilized maize plants, at which this effect primarily arose from the elevated P contents of bottom segments, and respectively, the middle segments, where the foliar P was applied (Figure 11).

The highest P concentrations were determined when phosphite was used for the foliar fertilization. Ratjen (2005) describes similar results for experiments with zucchinis (*Cucurbita pepo* L.) and concludes that phosphite was better absorbed by leaves than phosphate. The accumulation of P within the leaf tissue together with absent positive growth effects indicates that phosphite did not completely participate in the plant metabolism (Carswell et al., 1996). Similar results are reported by Lovatt (1990a) from experiments with avocados (*Persea americana*). The author explains the higher P contents by a higher phosphite uptake rate in connection with a missing dilution effect caused by the negative effect on growth.

The results presented in Table 6 and 7 reveal that the distribution of phosphate and phosphite among different parts of the plants was similar.

Beside the middle plant segments consisting of those leaves, which the fertilizer solution was directly applied to, the corncobs were characterized by relative high contents of phosphate and phosphite, respectively, without as well as with foliar fertilization. The high concentration of both P compounds detected in the corncobs can be explained by the internal nutrient cycle within the generative phase of plants: the developing spadices with grains are storage organs and thus strong nutrient sinks.

The detection of phosphite in all plant parts after foliar treatment confirms the phloem and xylem mobility of this compound. Evidently, the translocation of P in phosphate form from the foliar fertilized plant parts into the growing younger leaves was easier than that of phosphite (Table 6 and 7). Depending on the basic P nutrition of the maize plants, the phosphate content of the top segments was higher or at least at the same level than that of the middle part. By contrast, in comparison to the phosphite content of the middle part a significant lower phosphite concentration was measured in the top parts of both P deficient and sufficient maize.

The calculation of the total phosphate and phosphite uptake by the single plant segments is shown in Table 8. There were nearly no differences in phosphate incorporation of the vegetative plant organs between the maize plants grown on plots with and without mineral P fertilization via soil and without additional foliar fertilization. Under P deficiency, some plants (e.g. maize or red clover) are able to produce root exudates in form of organic acid anions (e.g. citrates) that decrease the soil pH value and increase the P solubility by ligands exchange in the rhizosphere (Beißner, 1997; Keller, 2000). Both the bottom and the top plant segments incorporated lower amounts of phosphite. The lower phosphate incorporation into the spadices primarily resulted from the development of a fewer number of corncobs due to the limited P supply. The phosphate foliar fertilization caused positive yield effects, which increased also the phosphate uptake. Apparently, the P fertilized maize plants could exploit P offered by foliar application more intensively than the P deficient plants. The lower phosphate amount in the plant tops under P sufficiency could be explained by the advanced

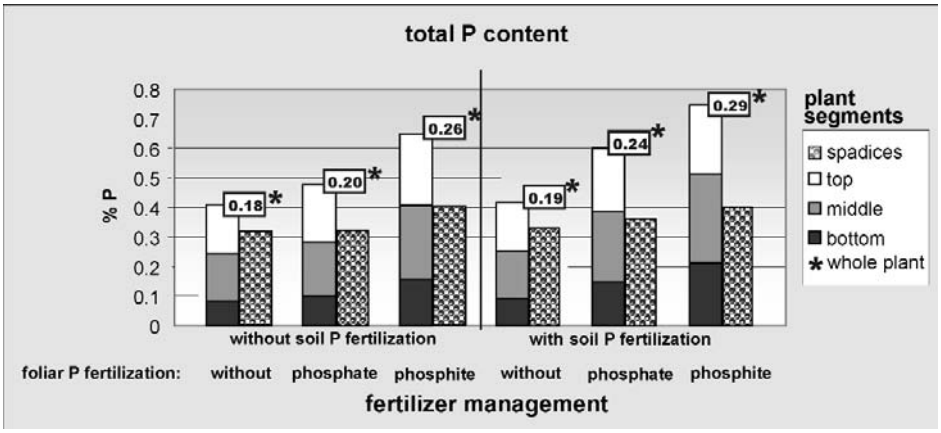

Figure 11:
Influence of the P fertilization management on the mean P content of maize plants and its allocation in separate plant segments (bottom, middle, top, and spadices) at BBCH 61-63 (FV4, Braunschweig 2004)

Table 6:
Phosphate and phosphite contents of single maize plant segments, affected by different foliar P fertilization under P deficient soil conditions (FV4, Braunschweig 2004)

P deficient maize plants

	Plant segment	Foliar P fertilization		
		Without	Phosphate[1)]	Phosphite[2)]
Phosphate content [mg kg^{-1}]	spadices	6.38 c/A	6.76 c/B	5.71 c/B
	top	2.61 b/A	3.30 b/B	2.62 b/A
	middle	2.38 b/A	2.48 ab/A	2.50 b/A
	bottom	1.27 a/A	1.71 a/B	1.61 a/B
Phosphite content [mg kg^{-1}]	spadices	<LLD	<LLD	1.75 c
	top	<LLD	<LLD	0.91 a
	middle	<LLD	<LLD	1.31 b
	bottom	<LLD	<LLD	0.91 a

[1)] foliar application of 15 mg P as 0.2% w/v KH_2PO_4 solution
[2)] foliar application of 15 mg P as 0.2% w/v KH_2PO_3 solution
All values are means of 5 replicates. Means followed by dissimilar small letters (in columns) and capitals (in rows), respectively, are significantly different (Duncan's test, P = 0.05).

Table 7:
Phosphate and phosphite contents of single maize plant segments, affected by different foliar P fertilization under P sufficient soil conditions (FV4, Braunschweig 2004)

P sufficient maize plants

	Plant segment	Foliar P fertilization		
		Without	Phosphate[1)]	Phosphite[2)]
Phosphate content [mg kg^{-1}]	spadices	5.65 b/A	7.40 d/B	5.73 b/A
	top	1.87 a/b	3.39 b/B	3.05 a/B
	middle	2.13 a/A	3.89 c/B	2.72 a/A
	bottom	1.38 a/A	2.38 a/B	2.08 a/B
Phosphite content [mg kg^{-1}]	spadices	<LLD	<LLD	1.37 b
	top	<LLD	<LLD	0.63 a
	middle	<LLD	<LLD	1.28 b
	bottom	<LLD	<LLD	1.01 ab

[1)] foliar application of 15 mg P as 0.2% w/v KH_2PO_4 solution
[2)] foliar application of 15 mg P as 0.2% w/v KH_2PO_3 solution
All values are means of 5 replicates. Means followed by dissimilar small letters (in columns) and capitals (in rows), respectively, are significantly different (Duncan's test, P = 0.05).

development stage of these plants where additional P is preferably stored in the corncobs.

In general, the results show that maize plants foliar fertilized with KH_2PO_4 contained higher amounts of phosphate than all other treatments. It is assumed that the plants are able to absorb phosphate by their leaves and to directly use it for assimilation processes. Maize, growing on P deficient conditions, first will invest the additional foliar fertilizer phosphate in increased growth of vegetative organs, whereas at optimal P nutrition more and bigger corncobs will be developed (see Figure 5).

Nevertheless, in comparison to untreated control plants, after foliar application of KH_2PO_3 higher phosphate contents in the spadices and the top part could be also observed. This increase in the phosphate content may be due to a partial oxidation of incorporated phosphite to phosphate in the plant tissue.

3.2.2 Pot experiment

Under extreme P deficiency - without soil and foliar fertilization - the P content of the maize plants in the pot experiment was only about 0.1 % (Table 9). This is far below the critical P content of the youngest mature leaf of 0.26 % as given by Reuter and Robinson (1997). Adequate P levels were obtained by two times foliar fertiliza-

Table 8:
Total phosphate and phosphite incorporation in single maize plant segments, affected by different foliar P fertilization under P deficient and sufficient soil conditions (FV4, Braunschweig 2004)

Foliar P fertilization	Plant segment	Phosphate uptake [µg plant^{-1}]		Phosphite uptake [µg plant^{-1}]	
		P deficiency	P sufficiency	P deficiency	P sufficiency
Without	spadices	48	84	<LLD	<LLD
	top	14	10	<LLD	<LLD
	middle	25	24	<LLD	<LLD
	bottom	14	20	<LLD	<LLD
With phosphate[1)]	spadices	82	109	<LLD	<LLD
	top	24	14	<LLD	<LLD
	middle	30	46	<LLD	<LLD
	bottom	22	34	<LLD	<LLD
With phosphite[2)]	spadices	41	81	12	19
	top	12	15	4	3
	middle	20	26	10	12
	bottom	15	29	9	14

[1)] foliar application of 15 mg P as 0.2 % w/v KH_2PO_4 solution
[2)] foliar application of 15 mg P as 0.2 % w/v KH_2PO_3 solution
All values are means of 5 replicates.

Table 9:
Influence of the P fertilization treatment on the total P content of maize plants (pot experiment, Braunschweig 2004)

Substrate P fertilization	Foliar P fertilization		
	Without	Phosphate[1)]	Phosphite[2)]
	Total P content [%]		
Without	0.11	0.59	0.84
Optimum P supply by KH_2PO_4	0.37	0.72	1.03
Optimum P supply by KH_2PO_3	1.18	1.09	2.47

[1)] foliar application of 15 mg P as 0.2 % w/v KH_2PO_4 solution
[2)] foliar application of 15 mg P as 0.2 % w/v KH_2PO_3 solution
All values are means of 4 replicates.

Table 10:
Phosphite content of maize plants in depending on different P fertilization treatments (pot experiment, Braunschweig 2004)

Substrate P fertilization	Foliar P fertilization		
	Without	Phosphite[1)]	
	Phosphite content [mg kg^{-1}]		
Without	<LLD		7
Optimum P supply by KH_2PO_4	<LLD	top	11
		middle	15
		bottom	9
Optimum P supply by KH_2PO_3	16		24

[1)] foliar application of 15 mg P as 0.2 % w/v KH_2PO_3 solution
All values are means of 4 replicates.

tion with 15 mg P in form of KH_2PO_4 solution at intervals of 4 weeks. The maize plants grew up without visible symtons. However, they were smaller than plants that received the P fertilizer via roots and leaves in combination. In those plants, P concentrations between 0.37 % (soil fertilization only) and 0.72 % (with additional foliar fertilization) were determined. The extremely high P contents in the plant material of the phosphite-fertilized treatments were rated as being toxic. The damaging growth effect of this P compound applying as fertilizer to young maize plants was reflected in diminished biomass development coupled with substantial visible detrimental effects. Particularly, the combined application of phosphite soil and foliar fertilization had negative consequences. These results are in accordance with those of Barrett et al. (2004) who reported on phosphite toxicity depending on inner plant concentration. Furthermore, species-specific differences in spray retention and chemical uptake are decisive for application effects (Chung and Kwon, 1992; de Ruiter et al., 1990; Schreiber and Schonherr, 1992). Barret (2001) specified morphogenetic properties (leaf shape, phyllotaxy, the existence of leaf hairs, and the distribution and position of stomata) as determining parameters.

Because of the very poor development of the maize plants in the phosphite treatment a separation into plant

segments was not possible. The whole plant analysis of these plants yielded a phosphite concentration of 16 mg kg^{-1} dry matter (Table 10).

Whether there was no other P source, the plants took up phosphite from the sandy substrate via roots. However, they were not able to use this P compound effectively for assimilation processes. Thus, the plant development was visibly inhibited (see Figure 7). Further fertilization with phosphite via foliar application increased the phosphite content in the plant to 24 mg kg^{-1} (Table 10). This very high phosphite content may be due to the low biomass development, which exceeded not more than 5 g per pot (see Table 5). Whole plant analysis of maize grown in pots without P soil fertilization showed a phosphite content of 7 mg kg^{-1} dry matter following phosphite foliar treatment.

The segmental plant analysis of the maize plants optimum fertilized by phosphate showed that the phosphite concentrations ranged from 9 to 15 mg kg^{-1} in all plant parts following the foliar application by phosphite. The data support phloem and xylem mobility of phosphite.

4 Conclusions

The detection of different phosphite concentrations in all aboveground segments of phosphite fertilized maize plants indicates that this P compound is well absorbed by plant roots. After phosphite foliar application, this compound was also detectable in all parts of maize plants, which proves its phloem and xylem mobility. The phosphite accumulation was notably high in developing corncobs. Phosphite is obviously stable within the plant metabolism process as only small amounts appeared to be oxidized to phosphate. The reduced growth observed in phosphite treated plants was especially evident under conditions of P deficiency. This could result from a suppression of the natural mechanisms of plants to respond to P deficiency. These results should be considered as an aspect of the German fertilizer law: in the future, the P content of marketable mineral fertilizers is to disclose specifically in terms of soluble phosphate or phosphite instead of generalized "P_2O_5", as hitherto.

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