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The Fungicide Phosphonate Disrupts the Phosphate-Starvation Response in *Brassica nigra* Seedlings[1]

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The development of *Brassica nigra* seedlings over 20 d of growth was disrupted by the fungicide phosphonate (Phi) in a manner inversely correlated with nutritional inorganic phosphate (Pi) levels. The growth of Pi-sufficient (1.25 mM Pi) seedlings was suppressed when 10, but not 5, mM Phi was added to the nutrient medium. In contrast, the fresh weights and root:shoot ratios of Pi-limited (0.15 mM) seedlings were significantly reduced at 1.5 mM Phi, and they progressively declined to about 40% of control values as medium Phi concentration was increased to 10 mM. Intracellular Pi levels generally decreased in Phi-treated seedlings, and Phi accumulated in leaves and roots to levels up to 6- and 16-fold that of Pi in Pi-sufficient and Pi-limited plants, respectively. Extractable activities of the Pi-starvation-inducible enzymes phospho*enol*pyruvate phosphatase and inorganic pyrophosphate-dependent phosphofructokinase were unaltered in Pi-sufficient seedlings grown on 5 or 10 mM Phi. However, when Pi-limited seedlings were grown on 1.5 to 10 mM Phi (a) the induction of phospho*enol*pyruvate phosphatase and inorganic pyrophosphate-dependent phosphofructokinase activities by Pi limitation was reduced by 40 to 90%, whereas (b) soluble protein concentrations and the activities of the ATP-dependent phosphofructokinase and pyruvate kinase were unaffected. It is concluded that Phi specifically interrupts processes involved in regulation of the Pi-starvation response in *B. nigra*.

The Phi anion (HPO^{3-}) is an isostere of the Pi anion in which hydrogen replaces one of the oxygens bound to the P atom. In spite of the apparent similarity, most enzymes that catalyze the transfer of Pi groups can distinguish between the two anions. Phi was traditionally regarded as inert with respect to plant and animal metabolism and was once used as a buffer in systems in which Pi was unsuitable (Robertson and Boyer, 1956). Evidence suggesting that Phi is not biologically inert began to accumulate following the discovery that foliar applications or trunk injections of Fosetyl-Al (aluminum tris-O-ethyl Phi) effectively suppress several soil-borne plant diseases caused by pseudofungi belonging to the order Oomycetes, particularly *Phytophthora* sp. (Williams et al., 1977; Fenn and Coffey, 1984; Whiley et al., 1986). It is Phi, released in the plant by hydrolysis of ethyl-Phi, that is responsible for protection of plants against the fungal pathogen (Fenn and Coffey, 1989).

The primary site of Phi's fungicidal action appears to lie within the fungal pathogen and not the host plant (Fenn and Coffey, 1984). This view is corroborated by the observation that 0.1 to 3 mM Phi markedly inhibits the growth of *Phytophthora* mycelia in sterile culture (Smillie et al., 1989). ^{31}P-NMR spectroscopy has revealed that Phi perturbs P metabolism in *Phytophthora* by causing a massive accumulation of poly-P and PPi but not sugar-P or nucleotide-P pools (Griffith et al., 1990; Niere et al., 1990, 1994). Phi's toxicity in *Phytophthora* sp. has therefore been proposed to result from its capacity to increase PPi and hence indirectly inhibit key pyrophosphorylase reactions essential to anabolism (Niere et al., 1994).

It has been generally assumed that the levels of Phi used to control plant pathogenic fungi do not seriously interfere with the growth or metabolism of plants, even though these levels are high relative to many other fungicides such as the acylalanines (Schwinn and Staub, 1987; Smillie et al., 1989). However, a recent study showing inhibition of onion (*Allium cepa*) root growth following treatment with aluminum ethyl-Phi suggests that Phi can interfere with the metabolism of some plants (Sukarno et al., 1993). In this paper, we demonstrate that Phi concentrations comparable to those used to control plant infection by *Phytophthora* drastically disrupt the development of Pi-limited *Brassica nigra* (black mustard) seedlings. Phi treatment of *B. nigra* also decreased the induction of PEPase and PFP by Pi limitation by up to 90%. This suggests that Phi interferes with the Pi-starvation response of *B. nigra*, for which these two enzymes are known to be markers (Duff et al., 1989; Theodorou et al., 1992; Theodorou and Plaxton, 1992, 1994, 1995), thereby exacerbating the effects of Pi deficiency.

MATERIALS AND METHODS

Chemicals and Plant Material

NADH and EGTA were obtained from Boehringer Mannheim. Tris base was from Schwartz/Mann (Cam-

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Abbreviations: PEPase, PEP phosphatase (EC 3.1.3.60); PFK and PFP, ATP- and PPi-dependent phosphofructokinase, respectively (EC 2.7.1.11 and 2.7.1.90); Phi, phosphonate; PK, pyruvate kinase (EC 2.7.1.40); poly-P, polyphosphate.

bridge, MA), and DTT was from Research Organics (Cleveland, OH). Other biochemicals, coupling enzymes, insoluble PVP, agar (plant cell culture tested), and plant culture boxes were purchased from Sigma. All other reagents were of analytical grade and were obtained from BDH (Toronto, Ontario, Canada). All solutions were prepared using Milli-Q-processed water (Millipore).

Brassica nigra (line L/T) seeds were kindly provided by Dr. Daina Simmonds (Plant Research Centre, Agriculture Canada, Ottawa, Ontario). Seeds were sterilized for 10 min in 6% (v/v) hypochlorite containing 0.02% (v/v) Triton X-100, washed several times with autoclaved distilled water, and then germinated in plant culture boxes (nine per box) on an agar-solidified Murashige-Skoog medium (Murashige and Skoog, 1962) containing 0.7% (w/v) agar, 2% (w/v) Suc, 0.15 or 1.25 mM potassium Pi, and potassium Phi as indicated. The pH of the medium was adjusted to 7.7 prior to autoclaving, which resulted in a final pH of 5.8. The plant culture boxes were maintained in a growth cabinet at 27°C, 75% RH, with a light intensity of 100 μmol m^{-2} s^{-1} and a 12:12-h light:dark regime. Seedlings were harvested 20 d postsowing, and leaves and roots were quickly excised, immersed in liquid N_2, and stored at −80°C.

Enzyme Extraction and Assays

All procedures were carried out at 0 to 4°C. Frozen tissues (0.7–1.3 g) were powdered in liquid N_2 and ground (1:2, w/v) using a mortar and pestle in 50 mM imidazole-HCl (pH 7.5) containing 1.0 mM EGTA, 1.0 mM EDTA, 100 mM KCl, 20% (v/v) glycerol, 0.05% (v/v) Triton-X 100, 1.0 mM DTT, 2.0 mM PMSF, 0.1 mM Fru-6-P, and 1.5% (w/v) insoluble PVP. Homogenates were centrifuged at 14,000*g* for 10 min using an Eppendorf microcentrifuge. Aliquots (10–20 μL) of the resulting supernatants were immediately assayed for PEPase, PFP, PK, and PFK activities by monitoring NADH oxidation at 340 nm and 25°C in a 1-mL final volume. Assay conditions were as described in the following references: for PFP, Theodorou and Plaxton (1992); for PFK, PEPase, and PK, Duff et al. (1989). PFP and PFK assays were initiated by the addition of PPi and ATP, respectively, to the reaction mixtures. All assays were conducted in duplicate, optimized with respect to pH and substrate concentrations, and corrected for any contaminating NADH oxidase activity. Activity in all assays was proportional to time and to the volume of extract added. One unit of enzyme is the amount of enzyme required to catalyze the formation of 1 μmol product min^{-1}.

For analysis of the effect of Phi on in vitro activities of PEPase and PFP, extracts were prepared as described above from roots of 20-d Pi-limited seedlings grown in the absence of Phi. Extracts were desalted as described by Penefsky (1977) on a column of Sephadex G-50 that had been equilibrated in extraction buffer minus PMSF and insoluble PVP. Aliquots of the desalted extracts were assayed as described above for PEPase and PFP activities in the absence and presence of 10 mM Pi or Phi. All results are the means of duplicate determinations performed on two separate desalted extracts and are reproducible to within ±10% SE.

Protein Determination

Protein concentration was determined by the method of Bradford (1976) using bovine γ-globulin as the standard.

HPLC Analysis of Phi and Pi Content of *B. nigra* Leaves and Roots

Tissues frozen in liquid N_2 were lyophilized, and 50-mg aliquots were extracted in 3 mL of 10 mM formic acid using an Ultra-Turrax homogenizer (Janke and Kundel, IKA-Labortechnik, Staufen, Germany) fitted with a microprobe. Homogenates were stored on ice for 30 min and then centrifuged at 6500*g* for 5 min. Resulting pellets were extracted twice by resuspension in 3 mL of 10 mM formic acid, followed by incubation on ice for 5 min and centrifugation as described above. Supernatants were combined to yield 9 mL of formic acid extract/sample. Cations, other than H^+, were removed by eluting the extract through a column of AG50 X 8 cation exchanger (200–400 mesh; Bio-Rad). Extracts were lyophilized to remove the formic acid, reconstituted with 1 mL of water, and subjected to HPLC on an anion-exchange column (4.6 × 75 cm, IC-PAK A HR column; Waters). The eluant consisted of 1.5 mM Na-gluconate, 6 mM boric acid, 1.5 mM Na_2-tetraborate decahydride, 5% (v/v) glycerol, 12% (v/v) acetonitrile, and 2% (v/v) *n*-butanol. Eluted anions were detected using a conductivity detector (LDC; Milton Roy, Rochester, NY), and the HPLC data were acquired and analyzed using an HPLC software package (DAPA Scientific Software, Kalamunda, Australia). Pi and Phi were identified and quantified by comparison with known standards.

^{31}P-NMR Spectroscopy

For ^{31}P-NMR analysis, 200- to 600-mg aliquots of lyophilized tissue were extracted as described above for HPLC analysis, except that 5% (w/v) ice-cold perchloric acid was used in place of formic acid. Perchloric acid was removed by precipitation as potassium perchlorate, and samples were prepared and analyzed as described by Niere et al. (1994).

RESULTS

Effect of Phi on Growth of Pi-Limited and Pi-Sufficient *B. nigra* Seedlings

Seedlings grown on Pi-limited medium in the absence of Phi displayed symptoms typical of Pi deficiency by 20 d (Fig. 1a; Table I). The oldest leaves died prematurely, and yellowing and anthocyanin formation was evident in all but the youngest leaves. Root growth was enhanced relative to shoot growth in Pi-limited seedlings, resulting in an increased root:shoot ratio. These are all classical symptoms associated with Pi deficiency in plants (Christie and Moorby, 1975; Rychter and Mikulska, 1990). Pi-sufficient

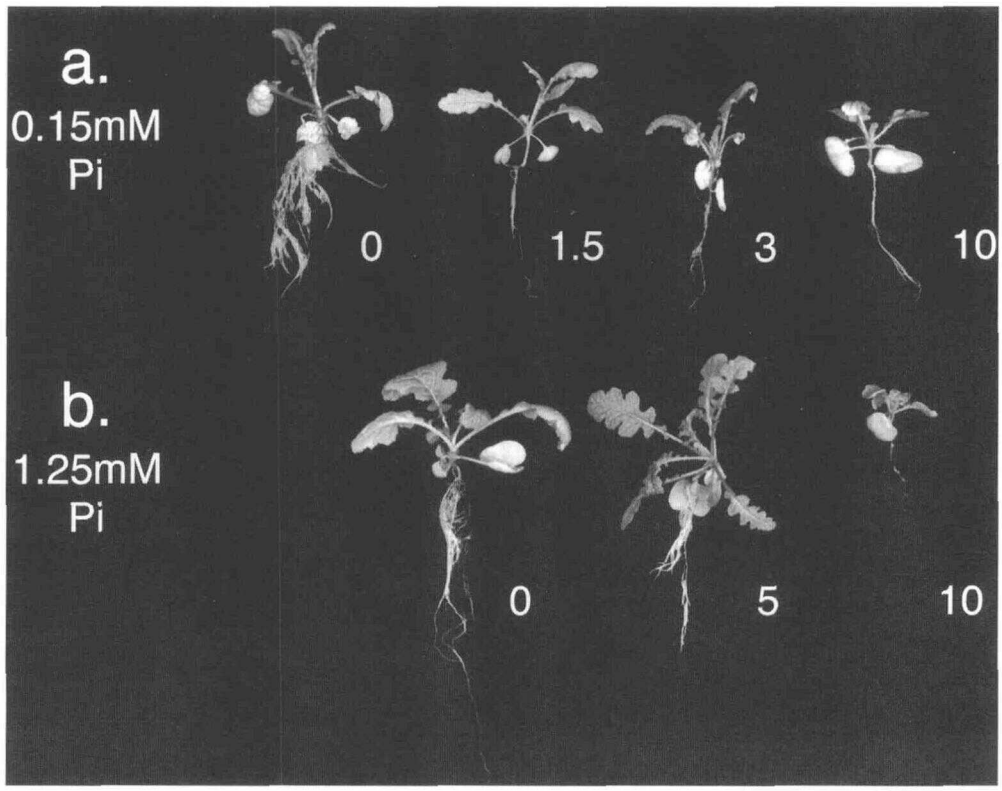

Figure 1. Pi-limited and Pi-sufficient 20-d *B. nigra* seedlings grown in the absence and presence of Phi. Growth medium contained 0.15 mM Pi (a) or 1.25 mM Pi (b) with 0, 1.5, 3, 5, or 10 mM Phi as indicated.

seedlings showed none of these symptoms (Fig. 1b; Table I).

The addition of 1.5, 3, or 10 mM Phi to the nutrient medium dramatically altered the growth and morphology of 20-d Pi-limited *B. nigra* seedlings (Fig. 1a; Table I). Total fresh weights and the root:shoot ratios of Pi-limited plants progressively decreased to about 40% of control values as the medium concentration of Phi was increased to 10 mM (Fig. 1a; Table I). By contrast, the presence of 5 mM Phi had no effect on the growth or morphology of Pi-sufficient seedlings (Fig. 1b; Table I). However, when Pi-sufficient seedlings were grown on 10 mM Phi, total fresh weights were reduced by about 50%, although root:shoot ratios were only slightly decreased (Fig. 1b; Table I).

Table I. *Root and shoot fresh weights and root:shoot ratios of 20-d B. nigra seedlings grown in Pi-sufficient (1.25 mM) or Pi-limited (0.15 mM) medium containing various concentrations of Phi*

All values represent the mean weights ± SE of 15 seedlings. Values within each column determined to be significantly different ($P < 0.05$) by the Student's *t* test are denoted by different superscript letters.

Treatment	Root Fresh Wt	Shoot Fresh Wt	Root:Shoot Ratio
	mg		
1.25 mM Pi	$79^{a} \pm 9$	$810^{a} \pm 40$	$0.098^{a} \pm 0.012$
+5 mM Phi	$81^{a} \pm 5$	$810^{a} \pm 29$	$0.100^{a} \pm 0.007$
+10 mM Phi	$34^{b} \pm 1$	$402^{b} \pm 12$	$0.084^{b} \pm 0.004$
0.15 mM Pi	$52^{c} \pm 7$	$319^{c} \pm 27$	$0.163^{c} \pm 0.023$
+1.5 mM Phi	$24^{d} \pm 5$	$244^{d} \pm 20$	$0.098^{a} \pm 0.020$
+3 mM Phi	$12^{e} \pm 1$	$202^{d} \pm 19$	$0.059^{d} \pm 0.007$
+10 mM Phi	$9^{f} \pm 1$	$138^{e} \pm 22$	$0.065^{d} \pm 0.010$

Analysis of Pi and Phi Content of *B. nigra* Seedlings

The levels of formic acid-extractable Pi and Phi in the Pi-sufficient versus Pi-limited *B. nigra* seedlings are compared in Table II. Phi was readily absorbed and accumulated to levels more than 6- and 16-fold that of Pi in Pi-sufficient and Pi-limited plants, respectively (Table II). Maximal concentrations of Phi were about 2.8 and 3.7 μmol g^{-1} fresh weight in the leaves and roots, respectively, of Pi-limited seedlings grown on 10 mM Phi. Phi concentrations in Pi-sufficient seedlings were generally lower than those in the corresponding tissues of the Pi-limited seedlings.

Growth of Pi-sufficient seedlings in the presence of 5 or 10 mM Phi caused an approximate 35% decline in root Pi concentrations (Table II). Moreover, a 70% decrease in Pi levels of leaves of Pi-sufficient plants was observed at 10 mM Phi. In contrast, root Pi levels were largely unaffected when Pi-limited seedlings were grown on 3 or 10 mM Phi, whereas those of the leaves were decreased by about 40% relative to the control without Phi (Table II).

^{31}P-NMR Spectroscopy

The ^{31}P-NMR spectra of perchloric acid extracts of leaves and roots revealed that Pi and Phi were the only P-containing compounds present in sufficient abundance to

Table II. *Distribution of Phi and Pi in 20-d B. nigra seedlings grown in Pi-sufficient (1.25 mM) or Pi-deficient (0.15 mM) medium in the presence of various concentrations of Phi*

Except where indicated all values represent the means of two analyses from separate experiments. Each analysis consisted of five determinations on a bulked sample; individual means are shown in parentheses and are reproducible to within ±5% SE.

Treatment	Roots		Leaves	
	Phi	Pi	Phi	Pi
		μmol g⁻¹ fresh wt		
1.25 mM Pi	–[a]	0.35 (0.31, 0.38)	–	1.54 (1.21, 1.87)
+5 mM Phi	0.71 (0.61, 0.81)	0.25 (0.21, 0.29)	0.28 (0.23, 0.32)	1.17 (1.13, 1.21)
+10 mM Phi	1.56 (1.53, 1.58)	0.24 (0.22, 0.26)	2.06 (1.83, 2.26)	0.46 (0.43, 0.48)
0.15 mM Pi	–	0.26 (0.23, 0.28)	–	0.50 (0.39, 0.61)
+1.5 mM Phi	3.17 (3.03, 3.31)	0.27 (0.21, 0.32)	2.15 (2.14, 2.15)	0.30 (0.29, 0.31)
+3 mM Phi	2.64 (1.90, 3.38)	0.26 (0.18, 0.33)	2.81 (2.53, 3.08)	0.29 (0.21, 0.36)
+10 mM Phi	3.7[b]	0.23[b]	2.79 (2.12, 3.45)	0.36 (0.35, 0.37)

[a] –, Nil. [b] Single analysis.

be detected by this technique (data not shown). There was no measurable accumulation of PPi or poly-P in any tissue and no evidence for the conversion of Phi to other compounds.

Effect of Phi on Protein Content and Activities of PEPase, PFP, PK, and PFK

Pi limitation slightly decreased the amount of protein extracted from roots but had a more marked effect on leaves, in which a 25% reduction was observed (Table III). Phi treatment had little or no effect on the soluble protein concentration of leaves or roots of Pi-sufficient or Pi-limited seedlings (Table III).

Extractable activities of PEPase, PFP, PK, and PFK from leaves and roots of 20-d-old *B. nigra* seedlings are shown in Figure 2. The presence of 5 or 10 mM Phi had no significant effect on the activity of any of the four enzymes from either tissue of Pi-sufficient seedlings, relative to the non-Phi-treated controls (data not shown). Consistent with previous studies (Duff et al., 1989; Theodorou et al., 1992; Theodorou and Plaxton, 1992, 1994, 1995), there was a significant (approximately 2- to 3-fold) induction, relative to Pi-sufficient seedlings, in the activities of PEPase and PFP, but not PK and PFK, in leaves and roots of the Pi-limited seedlings (Fig. 2). However, when Pi-limited plants were grown on 1.5, 3, or 10 mM Phi, the degree of induction of leaf or root PEPase and PFP by Pi limitation was reduced by 40 to 90% (Fig. 2). The deleterious effect of Phi on the induction of these enzymes by Pi stress tended to be more marked in roots than in leaves but was highly significant ($P < 0.01$, Student's t test) in all cases. In contrast, Phi treatment of Pi-limited seedlings had no significant effect ($P > 0.05$, Student's t test) on the extractable activities of either PK or PFK in leaves or roots (Fig. 2).

The effect of 10 mM Pi versus 10 mM Phi on the in vitro activities of PEPase and PFP was measured using desalted extracts prepared from roots of 20-d-old Pi-limited seedlings grown in the absence of Phi. PEPase was inhibited 80 and 20% by 10 mM Pi and 10 mM Phi, respectively, whereas PFP was inhibited 60% by 10 mM Pi. In contrast, 10 mM Phi had no effect on in vitro PFP activity. Although 10 mM Phi was capable of inhibiting the activity of PEPase in vitro, this inhibition was much less than that caused by 10 mM Pi. If we assume that 1 g fresh weight is equivalent to 1 mL cellular volume, the range of Phi concentrations attained in the various Phi-treated *B. nigra* tissues can be estimated from the data of Table II to be about 0.2 to 4 mM. Therefore, given that the cellular contents were diluted at least 100-fold in the assay medium, direct inhibition by Phi cannot account for the large reduction in the extractable activities of PFP and PEPase from tissues of the Phi-treated, Pi-limited *B. nigra* seedlings (Fig. 2).

Table III. *Soluble protein concentration of roots and leaves of 20-d B. nigra seedlings grown in Pi-sufficient (1.25 mM) or Pi-limited (0.15 mM) medium in the presence of various concentrations of Phi*

All values represent the means ± SE of triplicate determinations.

Treatment	Protein Concentration	
	Roots	Leaves
	mg g⁻¹ fresh wt	
1.25 mM Pi	5.28 ± 0.04	11.23 ± 0.09
+5.0 mM Phi	5.45 ± 0.10	10.89 ± 0.11
+10.0 mM Phi	5.07 ± 0.06	11.17 ± 0.08
0.15 mM Pi	4.62 ± 0.08	8.21 ± 0.05
+1.5 mM Phi	4.70 ± 0.04	8.11 ± 0.06
+3.0 mM Phi	4.58 ± 0.07	8.12 ± 0.04
+10.0 mM Phi	3.96 ± 0.04	8.05 ± 0.03

DISCUSSION

The magnitude of Phi's deleterious effect on the growth of *B. nigra* seedlings was inversely correlated with the concentration of Pi in the nutrient medium. Significant growth inhibition occurred with as little as 1.5 mM Phi in Pi-limited seedlings, whereas reduced growth of the Pi-sufficient seedlings was observed at 10 mM but not 5 mM Phi (Fig. 1; Table I). The negative impact of Phi on the development of Pi-limited *B. nigra* was most pronounced in roots (Fig. 1a), in which 10 mM Phi caused an 80% reduction in fresh weight relative to that of Pi-limited controls (Table I). Similarly, reduced root growth has been observed

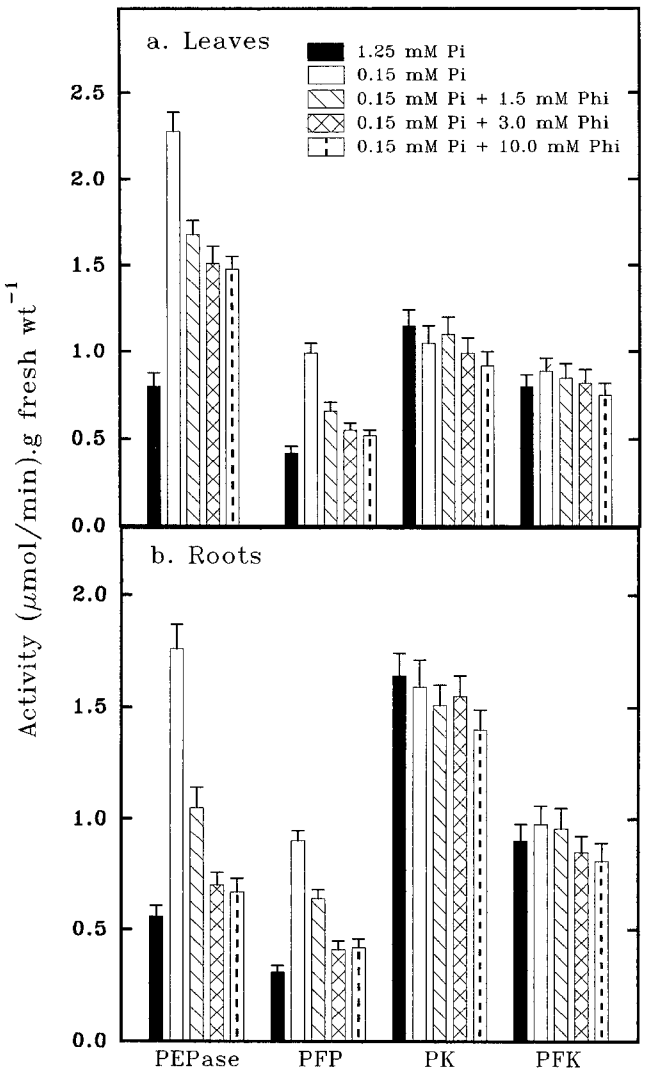

Figure 2. Activities of PEPase, PFP, PK, and PFK from leaves (a) and roots (b) of 20-d *B. nigra* seedlings grown in the presence of 1.25 or 0.15 mM Pi and Phi concentrations as shown. Data are the means (+SE) of four independent determinations.

in onion plants treated with aluminum ethyl-Phi (Sukarno et al., 1993).

That Phi is phloem mobile in higher plants has been established by the work of Ouimette and Coffey (1989, 1990), who detected the anion in (a) roots of avocado following foliar application of Phi and (b) in root exudates of tomato and avocado following incubation of the roots in a solution containing 25 mM Phi. Likewise, the results presented in Table II demonstrate that Phi was readily assimilated and translocated by Pi-sufficient and Pi-limited *B. nigra* seedlings. Although leaf and root Pi pools generally decreased in the Phi-treated seedlings (Table II), Phi did not cause any detectable accumulation of PPi. Poly-P, which rarely occurs in higher plants (Harold, 1966; Jeffrey, 1968), was also undetectable in the Phi-treated *B. nigra* seedlings. In these respects, the effects of Phi on *B. nigra* are very different from those that it elicits in *Phytophthora* sp., in which approximately 1 mM Phi causes massive accumulation of PPi and poly-Ps and a significant increase in Pi (Griffith et al., 1990; Niere et al., 1990, 1994).

A plausible explanation for the growth inhibition of Pi-sufficient *B. nigra* seedlings by 10 mM Phi was the 3- to 4-fold reduction of leaf Pi elicited by this treatment (Table II). Phi has been shown to interfere with the assimilation of Pi in various *Phytophthora* sp. by competing with Pi for plasmalemma transporters (Barchietto et al., 1989; Griffith et al., 1989; Grant et al., 1992). Owing to the central role played by Pi in the regulation of the reductive pentose phosphate pathway and photosynthate partitioning, photosynthesis is particularly vulnerable to decreases in intracellular Pi levels (Sivak and Walker, 1986). The mechanism by which Phi causes the observed alterations in Pi distribution between roots and leaves is unknown.

The most striking effect of Phi on *B. nigra* was its attenuation of the Pi-starvation response. When the medium Pi was reduced from 1.25 to 0.15 mM, cellular Pi levels were markedly reduced (Table II), and PEPase and PFP activities were induced by about 2- to 3-fold (Fig. 2). These results are consistent with our previous studies of suspension cells and seedlings of *B. nigra*, which have demonstrated a marked Pi-starvation-dependent (a) reduction in internal Pi and adenylate but not PPi levels and (b) induction of PEPase and PFP, enzymes that produce Pi as a byproduct while bypassing the adenylate-requiring PK and PFK, respectively (Duff et al., 1989; Theodorou et al., 1992; Theodorou and Plaxton, 1992, 1994, 1995). Although the addition of Phi to the nutrient medium further depleted leaf Pi concentrations of the Pi-limited seedlings by up to 40% (Table II), this treatment also reduced the induction of PEPase and PFP activities by Pi stress by 40 to 90% (Fig. 2). In contrast, Phi treatment of Pi-limited seedlings had negligible effects on soluble protein levels and the extractable activities of PK and PFK (Table III; Fig. 2). This indicates that the inability of Phi-treated *B. nigra* seedlings to fully induce PEPase and PFP during Pi stress does not arise from nonspecific toxicity of the Phi anion. This, along with Phi's suppression of other typical Pi-deficiency symptoms such as increased root:shoot ratios (Fig. 1; Table I) and anthocyanin production, suggests that Phi specifically interferes with processes involved in regulation of the Pi-starvation response in *B. nigra*.

Our results show that Phi can have a profound detrimental influence on the development of *B. nigra* seedlings at concentrations comparable to those required to control infection of plants by pathogenic *Phytophthora* or to restrict *Phytophthora* growth in culture (Smillie et al., 1989). However, it is unlikely that the effects of Phi on higher plants would be of significance in the control of diseases caused by *Phytophthora* sp. unless the plant was under Pi stress when Phi was applied. P metabolism in *Phytophthora* is sufficiently different from that in higher plants to allow Phi to selectively inhibit the fungal pathogen without inflicting severe damage on its host. However, under conditions of Pi deficiency, the presence of Phi clearly disrupts those adaptations of *B. nigra* that may assist in increasing Pi assimi-

lation and/or Pi conservation. The result is that Pi-limited *B. nigra* seedlings that have assimilated significant amounts of Phi behave as though they are Pi sufficient, when in fact their Pi content is very low. It therefore appears that a possible site of Phi action in these seedlings may be at the level of the signal transduction pathway that activates the genes coding for glycolytic bypass enzymes such as PFP and PEPase and for other features of the Pi-starvation response. If so, the Phi anion may represent a useful tool with which to investigate the signal transduction pathway by which higher plants perceive and respond to Pi stress at the molecular level.

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