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Short communication

Consequences of phosphate application on glyphosate uptake by roots: Impacts for environmental management practices

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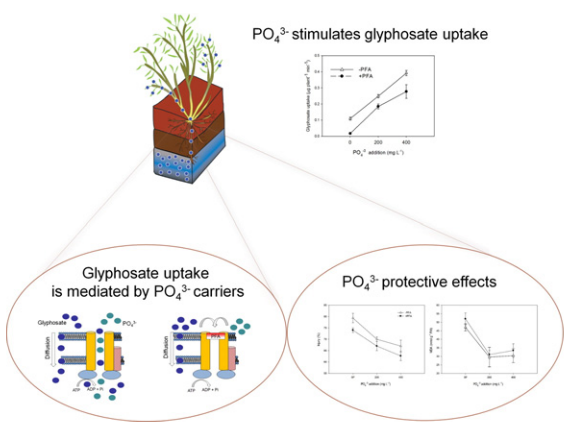
HIGHLIGHTS

- We studied the mechanisms of glyphosate uptake by willow roots.
- Glyphosate uptake is mediated by PO_4^{3-} carriers.
- PO_4^{3-} increased root cell membrane stability and glyphosate uptake by roots.
- PO_4^{3-} has a clear role in glyphosate toxicity and remediation.

GRAPHICAL ABSTRACT



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ABSTRACT

Phosphate (PO_4^{3-}) fertilization is a common practice in agricultural fields also targets for glyphosate application. Due to their chemical similarities, PO_4^{3-} and glyphosate compete for soil adsorbing sites, with PO_4^{3-} fertilization increasing glyphosate bioavailability in the soil solution. After PO_4^{3-} fertilization, its concentration will be elevated in the soil solution and both PO_4^{3-} and glyphosate will be readily available for runoff into aquatic ecosystems. In this context, man-made riparian buffer strips (RBS) at the interface of agricultural lands and waterways can be used as a green technology to mitigate water contamination. The plants used in RBS form a barrier to agricultural wastes that can limit runoff, and the ability of these plants to take up these compounds through their roots plays an important role in RBS efficacy. However, the implications of PO_4^{3-} for glyphosate uptake by roots are not yet clearly demonstrated. Here, we addressed this problem by hydroponically cultivating willow plants in nutrient solutions amended with glyphosate and different concentrations of PO_4^{3-}, assuring full availability of both chemicals to the roots. Using a phosphate carrier inhibitor (phosphonophormic acid—PFA), we found that part of the glyphosate uptake is mediated by PO_4^{3-} transporters. We observed, however, that PO_4^{3-} increased glyphosate uptake by roots, an effect that was related to increased root cell membrane stability. Our results indicate that PO_4^{3-} has an important role in

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glyphosate physiological effects. Under agricultural conditions, PO_4^{3-} fertilization can amplify glyphosate efficiency by increasing its uptake by the roots of undesired plants. On the other hand, since simultaneous phosphate and glyphosate runoffs are common, non-target species found near agricultural fields can be affected.

1. Main

Since the introduction of glyphosate-resistant (GR) plants, glyphosate has become the most widely used herbicide globally (Coupe et al., 2012). Once applied in the field, glyphosate invariably penetrates into the soil, where it can be adsorbed onto soil particles, and phosphate (PO_4^{3-}) content appears to be one of the most important factors driving this glyphosate binding (Borggaard, 2011). As PO_4^{3-} and the methylphosphonic group of glyphosate compete for the same adsorbing sites (Bott et al., 2011; Clua et al., 2012), the capacity of soils to adsorb PO_4^{3-} determines glyphosate availability in soil solutions, and increases in soil PO_4^{3-} concentrations may result in increased glyphosate availability (Bott et al., 2011). Phosphorous is an essential element for plants, participating in crucial physiological events, and PO_4^{3-} fertilization is a common agricultural practice (Lopes, 2004). In agricultural fields where glyphosate has been applied, PO_4^{3-} fertilization may influence soil herbicide bioavailability (Laitinen et al., 2007). Furthermore, these two compounds may be readily available for runoff, and be leached in adjacent waterways.

The detrimental effects of agricultural PO_4^{3-} inputs to aquatic systems have been widely discussed (Correll, 1998; Chien et al., 2011), and attention has more recently been focused on the presence of glyphosate and its detrimental effects on aquatic ecosystems (Annett et al., 2014). In this context, implantation of man-made riparian buffer strips (RBS) at the boundary of the agricultural lands may limit runoff and decrease water contamination. The ability of these plants to perform efficient root uptake of these agricultural wastes plays an important role in RBS efficacy (Krutz et al., 2005; Lin et al., 2011). It was advance that glyphosate may compete with PO_4^{3-} for membrane carriers (Denis and Delrot, 1993; Morin et al., 1997), as it occurs with soil absorption sites. Therefore, the following questions arise: 1) Does glyphosate compete with PO_4^{3-} for root uptake? 2) Under situations of high availability of both compounds, how does PO_4^{3-} influence glyphosate uptake by plants?

To address these questions, plants were grown in a hydroponic system to assure high availabilities of both glyphosate and PO_4^{3-} to the root systems of a fast growing willow cultivar (*Salix miyabeana* cultivar SX64). Willow species have been successfully used in various phytoremediation programs, from soil decontamination to organic waste disposal (Mirck et al., 2005). In Quebec, willow plants have been used to establish riparian buffer strips to control the runoff of agricultural wastes (Hénault-Ethier et al., 2014) and, in that context, it becomes important to evaluate the ability of willows to take up glyphosate, as well as the implications of different agricultural practices on glyphosate-uptake efficacy by this species. First, we evaluated the involvement of PO_4^{3-} transporters in glyphosate uptake by willow roots. Using a PO_4^{3-} transporter inhibitor (phosphonophormic acid - PFA) we were able to demonstrate the involvement of PO_4^{3-} transporters in glyphosate uptake by roots as glyphosate concentrations in plant tissues (roots and leaves) and glyphosate uptake decreased in the presence of PFA (Fig. 1). The involvement of phosphate carriers in glyphosate uptake has been observed in protoplasts (Bott et al., 2011; Clua et al., 2012), improving our understanding of the glyphosate's membrane transport, and the differential distribution of this herbicide in different plant tissues (Denis and Delrot, 1993). However, to our knowledge, the importance of PO_4^{3-} carriers in primary glyphosate uptake by roots has not been described before.

Interestingly, we observed glyphosate in the tissues of plants exposed to both PFA treatments and PO_4^{3-} additions (Fig. 1). According to Denis and Delrot (1993), PO_4^{3-} carriers are mostly involved in glyphosate uptake at low herbicide concentrations in protoplasts. This energy-dependent uptake was superimposed, however, on a linear diffusional process under increased xenobiotic concentrations (Denis and Delrot, 1993). Similarly, we demonstrated here that high PO_4^{3-} concentrations in nutrient solutions increase glyphosate diffusion into roots (Fig. 1). These results lead us to our second question regarding the involvement of PO_4^{3-} in glyphosate uptake by roots.

The addition of PO_4^{3-} significantly increased glyphosate uptake by roots and its concentration in plant tissues (roots and leaves) (Fig. 1). Recent studies have shown that as PO_4^{3-} fertilization increases glyphosate remobilization from soil matrices, which then becomes more available for root uptake (Bott et al., 2011; Beltrano et al., 2013). In these studies, however, the adsorption of PO_4^{3-} to soil matrices reduced PO_4^{3-} availability in the soil solution, but neither the potential competition between PO_4^{3-} and glyphosate for carrier sites, nor PO_4^{3-} effects on glyphosate uptake under situations of high availability of both chemicals were investigated. In the present study, we observed that despite their competition for the same membrane carriers, PO_4^{3-} fertilization clearly increased plant glyphosate's uptake. To better understand this stimulatory effect of PO_4^{3-}, we examined root cell injury by measuring cell membrane stability (CMS) (Fig. 2), and found that PO_4^{3-} fertilization decreased root cell injuries induced by glyphosate (Fig. 2). Additionally, a negative correlation was observed between root cell injury and root PO_4^{3-} concentrations ($r = -0.824$ and $r = -0.950$ for PFA treated and untreated plants respectively).

Increased phosphorous nutrition is known to protect plants from environmental stress (i.e., trace-elements contamination) through phosphorous stimulation of antioxidant systems, thus preventing oxidative tissue damage (Gomes et al., 2013, 2014b). Like trace-elements (Gomes et al., 2013, 2014b), glyphosate is known to induce oxidative stress in plants (as reviewed by Gomes et al., 2014a). Here, we demonstrated that glyphosate induced lipid peroxidation (malondialdehyde — MDA contents, from 29 to 52%) in willow roots (Fig. 2). PO_4^{3-}-protective effects in glyphosate-treated roots were observed, as lipid peroxidation decreased with increasing PO_4^{3-} concentration (Fig. 2). Lipid peroxidation in biological membranes is the most obvious symptom of oxidative stress in plants, and has been directly linked to cell membrane constitution and stability (Yajima et al., 2009). By protecting cell membranes against oxidative bursts, PO_4^{3-} may favor root absorption, and explain the greater glyphosate concentrations observed in PO_4^{3-} fertilized plants.

Raising PO_4^{3-} fertilization levels in glyphosate treated soils leads to increased glyphosate availability for root uptake and increases the deleterious effects of glyphosate in plants (Bott et al., 2011). However, it is important to note that, in addition to releasing glyphosate into the soil solution, PO_4^{3-} adsorption onto soil binding sites can create situations of low PO_4^{3-} availability for plants impairing their growth. In this context, PO_4^{3-} fertilization should be considered in studies of glyphosate toxicity since we observed that despite PO_4^{3-} competition with glyphosate for root absorbing sites (PO_4^{3-} transporters), PO_4^{3-} fertilization assured high phosphorous contents (which normally range from 0.1 to 1% (Sanchez, 2006)) and also increased glyphosate uptake by willow roots.

These findings have crucial impacts on environmental management strategies at different levels: 1) Glyphosate efficiency — if we assume that PO_4^{3-} increases glyphosate uptake by roots in weed species (as demonstrated here for willow), the co-application of PO_4^{3-} and glyphosate should lead to higher assimilation of glyphosate by these undesirable plants, thus contributing to herbicide efficiency; 2) glyphosate toxicity — it is known, on the other hand, that both glyphosate and phosphate readily runoff agricultural fields to surrounding areas, resulting in glyphosate exposure of non-target plants. In these cases, PO_4^{3-} fertilization and/or the resuspension of PO_4^{3-} present in soils and sediments