Sucrose amendment enhances phytoaccumulation of the herbicide atrazine in Arabidopsis thaliana

Cécile Sulmon a, Gwenola Gouesbet a, Françoise Binet a, Fabrice Martin-Laurent b, Abdelhak El Amrani a, Ivan Couée a,∗

a UMR 6553 ECObio, CNRS, Université de Rennes 1, Campus de Beaulieu, bâtiment 14A, F-35042 Rennes Cedex, France
b UMR Microbiologie et Géochimie des sols, INRA, Université de Bourgogne, 17 rue Sully, BP86510, F-21065 Dijon Cedex, France

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Abstract

Growth in the presence of sucrose was shown to confer to Arabidopsis thaliana (thale cress or mustard weed) seedlings, under conditions of in vitro culture, a high level of tolerance to the herbicide atrazine and to other photosynthesis inhibitors. This tolerance was associated with root-to-shoot transfer and accumulation of atrazine in shoots, which resulted in significant decrease of herbicide levels in the growth medium. In soil microcosms, application of exogenous sucrose was found to confer tolerance and capacity to accumulate atrazine in Arabidopsis thaliana plants grown on atrazine-contaminated soil, and resulted in enhanced decontamination of the soil. Application of sucrose to plants grown on herbicide-polluted soil, which increases plant tolerance and xenobiotic absorption, thus appears to be potentially useful for phytoremediation.

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1. Introduction

The photosystem II inhibitor atrazine, one of the most widely used s-triazine herbicide in maize and sorgho crops (Barceló, 1993), is commonly found as a pollutant in soil and in surface and ground water at concentrations exceeding USA regulation limits (Anon., 1990a,b; Hayes et al., 2002). In some cases, removal of organic pollutants from soil and water can be achieved by remediation methods which generally consist, on one hand, of soil excavation and ex situ treatment by activated carbon adsorption, thermic desorption, oxidation or incineration, or, on the other hand, of in situ treatments using the ability of microorganisms to degrade organic pollutants (Garcinuño et al., 2003).

Remediation by plants (phytoremediation) can be a complement to these traditional methods, especially in sites presenting diffuse pollution (Schröder et al., 2002; Pilon-Smits, 2005). In this case, plants are used to remove, sequestrate and/or detoxify contaminants from soil, water and air (Pilon-Smits, 2005), through phytoextraction/phytoaccumulation, phytostabilization, phytovolatilization, rhizosphere bioremediation or phytotransformation/phytodegradation (Susarla et al., 2002). Phytoremediating plants must be highly tolerant to contaminants (Arthur and Coats, 1998), which limits the number of useful plant species. Naturally tolerant plant species such as legumes (Schnoor, 2002), grasses (Schnoor, 2002), and phreatophytic trees including hybrid poplar (Burken and Schnoor, 1997; Schnoor, 2002) are generally used.

We recently showed that exogenous treatment by soluble sugars, especially sucrose, conferred, under axenic conditions, a very high level of atrazine tolerance to seedlings of the model plant Arabidopsis thaliana, thale cress or mustard
weed, which belongs to the Brassicaceae family (Salmon et al., 2004). Sucrose induced tolerance to other photosystem-targeted herbicides such as simazine, diuron, isoproturon, and to other xenobiotics such as parathion (Salmon, 2004). Sugar-treated plantlets under axenic in vitro conditions maintained photosynthesis and phototrophic growth in the presence of atrazine concentrations that were lethal in the absence of sugar treatment. Thus, sugar treatment conferred efficient tolerance to at least 40 μM (8.6 mg L\(^{-1}\)) atrazine. Comparatively, Arabidopsis plantlets overexpressing the atrazine-biodegrading bacterial enzyme atrazine chlorohydrolase show enhanced tolerance up to 15 μM (3 mg L\(^{-1}\)) atrazine (Wang et al., 2005). Protection was ascribed to signalling interactions between sucrose and atrazine resulting in overexpression of the \(psbA\) gene and enhanced accumulation of the photosystem II D1 protein, which is the protein target of atrazine, and in activation of genes involved in xenobiotic and oxidative stress defence (Salmon et al., 2004, 2006). These effects at the level of gene regulation were in accordance with the signalling effects of soluble sugars on plant growth (Rolland et al., 2002) and on plant responses to abiotic stress (Price et al., 2004; Couée et al., 2006).

In the present work, we investigated how uptake and distribution of atrazine occurred in sucrose-protected Arabidopsis thaliana seedlings and to what extent this uptake could contribute to a decrease of atrazine content in the agar medium surrounding the roots. Application of sugar treatment in a more complex environmental system consisting of soil microcosms further showed that sucrose-induced tolerance and capacity to accumulate atrazine of Arabidopsis plantlets were essentially similar to the processes observed under in vitro conditions. The resulting enhanced decontamination of the soil is discussed in terms of potential use for phytoremediation.

2. Materials and methods

2.1. Plant material under in vitro conditions

In vitro culture in Petri dishes was carried out on 0.8% (w/v) agar in 1× Murashige and Skoog (MS) basal salt mix (Sigma) adjusted to pH 5.7. Sucrose was added during preparation of agar-MS prior to autoclaving. Atrazine (Atrazine Pestanal, Riedel-de-Haën) was sterilized by microfiltration through 0.2 μm cellulose acetate filters and then axenically added to melted agar-MS medium prior to pouring into Petri dishes. Seeds of Arabidopsis thaliana (ecotype Wassilewskija) were surface-sterilized in bayrochlore/ethanol (1:1, v/v), rinsed in absolute ethanol and dried overnight. After sowing, Petri dishes were placed at 4 °C for 48 h in order to break dormancy and homogenize germination, and then transferred to a control growth chamber at 22 °C under a 16-h light period regime at 4500 lux for 15 days of growth.

2.2. Localization and quantification of radiolabelled [ring-U-\(^{14}\)C]atrazine in seedlings under in vitro conditions

Arabidopsis seedlings, previously grown for 15 days on MS-agar medium in the absence or presence of 80 mM sucrose and in the absence or presence of 1 μM atrazine, were transferred either on MS-agar or MS-sucrose-agar media containing 10 μM atrazine including 4.2 KBq of [ring-U-\(^{14}\)C]atrazine (specific activity 382.95 MBq mmol\(^{-1}\)) and then incubated at 22 °C. Seedlings incubated for 16 h in the presence of [ring-U-\(^{14}\)C]atrazine were transferred on MS-agar or MS-sucrose-agar media containing 10 μM unlabelled atrazine during 24 h for a chase experiment of plant-accumulated radiolabelled atrazine. [\(^{14}\)C]Atrazine transfer within plantlets was visualized by autoradiography using a Phosphor Imager. \([^{14}\text{C}]\)Atrazine contained in seedlings was extracted in methanol/water (3:1, v/v), for soil and agar medium; 4:1, v/v, for plant samples) for 2 h under shaking and then kept at 0 °C overnight. Samples were shaken again for 2 h and then centrifuged at 12,000 × g for 5 min. Radioactivity level of the resulting supernatants was determined by liquid scintillation counting.

2.3. Soil microcosm experiments

Soil, which was a silty-clay loam soil (sand 12%, silt 75%, clay 13%), slightly acidic (pH 6.4) with a low content of organic matter, was collected from the first 30 cm layer of a corn agroecosystem at the Institut National de la Recherche Agronomique experimental site of Vezin-le-Coquet (Brittany, France). The soil was air-dried and sieved at 4 mm. Moisture was adjusted to 20% (w/w) with distilled water. PVC cylinders of 9.4 cm internal diameter (Binet and Trehen, 1992) were filled with 1.8 kg of remoistened soil and compacted to a bulk-density of 1.4 g cm\(^{-3}\), thus resulting in soil columns of 10 cm diameter and 15 cm high. Soil moisture was adjusted to its water holding capacity, i.e. 28.1% (w/w). Arabidopsis seeds were sown on the soil surface. Soil microcosms were incubated in a control growth chamber at 12 °C. 75% humidity, under a 16-h light period regime at 8000 lux. When Arabidopsis plantlets reached rosette stage, the number of plants was adjusted to 15 per column and treatments were applied to the soil columns. Chemical treatments, which consisted in 50 ml aqueous solutions of pure atrazine (Atrazine Pestanal, Riedel-de-Haën) and sucrose, were uniformly sprayed on the soil surface. Control soil microcosms were sprayed with an equivalent volume of distilled water.

Treatments in preliminary experiments consisted of (i) atrazine treatment from 50 g ha\(^{-1}\) to 2000 g ha\(^{-1}\) (0.035 to 1.4 mg per microcosm), and of (ii) sucrose treatment with solutions ranging from 0.25% to 1.75% (w/v), in the absence or in the presence of 250 g ha\(^{-1}\) or 1000 g ha\(^{-1}\) atrazine. Bioturbation experiments were carried out with earthworms from the same site as the experimental soil. Adult specimens of Aporrectodea caliginosa and Aporrectodea giardi (Bouchez, 1972), which dominated the earthworm community of the agroecosystem (Binet and Le Bayon, 1999), were collected. They were maintained in experimental soil supplied with litter for 10 days at 12 °C for acclimation. Total earthworm biomass per microcosm was 3.38 g (±0.07), with 1.73 g (±0.09) of A. caliginosa and 1.64 g (±0.13) of A. giardi. Atrazine (250 g ha\(^{-1}\) or 1000 g ha\(^{-1}\)), sucrose (1.75% solution) and earthworm treatments resulted in a set of 12 experimental treatments. Five replicates were done per treatment, thus leading to 60 soil microcosms. Earthworms were added at the beginning of the experiment and chemical treatments were applied once 1 week later. Microcosms were further incubated during 30 days. Over the incubation period, the total water supply added to each microcosm was 50 ml.

2.4. Analysis of plant growth and development in soil microcosm experiments

Shoot fresh weight was measured 30 days after atrazine treatment. Pigments were extracted by pounding plantlet leaves in 80% (v/v) acetone, and absorbance of the extracts was measured at 663 nm, 646 nm and 470 nm. Chlorophyll and total carotenoid (xanthophylls and carotenes) levels in these extracts were determined and expressed as μg ml\(^{-1}\) as described by Lichtenthaler and Wellburn (1983). Chlorophyll fluorescence and maximum photosystem II efficiency (\(F_v/F_m\)) were measured with a PAM-210 chlorophyll fluorometer system (Salmon et al., 2004). After dark adaptation for at least 15 min, minimum fluorescence (\(F_o\)) was determined under weak red light. Maximum fluorescence of dark adapted leaf (\(F_m\)) was measured under a subsequent saturating pulse of red light and variable fluorescence (\(F_v = F_m - F_o\)) was determined. Measurements were carried out on 10 plants.

2.5. RNA isolation and northern blot analysis in soil microcosm experiments

Total RNA from Arabidopsis plantlets was extracted using the RNAgent Total RNA Isolation System kit (Promega). Equal amounts of RNA (0.2 μg)
were mixed with sample buffer containing ethidium bromide, separated by electrophoresis through 1% (w/v) agarose gel containing 2% (v/v) formaldehyde and 1× MOPS, and were capillary-blotted with 20× SSC onto Zeta-Probe® GT genomic Tested Blotting membranes. RNA was fixed to the membrane by UV crosslinking. A 950 bp polymerase-chain-reaction amplified fragment of the Arabidopsis psbA gene (GenBank accession number X79898) was used to generate a digoxigenin (DIG)-labelled DNA probe by random primed labelling with digoxigenin-11-dUTP using DIG High Prime DNA Labelling® and Detection Starter Kit II (Roche Diagnostics). Hybridized probes were immunodetected with an alkaline phosphatase-conjugated anti-di-goxigenin antibody and visualized with chemiluminescence substrate CSPD following the supplier’s instructions (Roche Diagnostics). Prehybridization (2 h) and hybridization (overnight) were performed in hybridization buffer following the supplier’s instructions (Roche Diagnostics). Prehybridization and hybridization buffer were used for 2 h and then centrifuged at 12,000 g 10 cm) and the bottom (10 cm) of the soil column, dried at 60 °C, and then separately analysed. Before extraction, Arabidopsis plantlets were ground into powder in liquid nitrogen. Atrazine extraction was performed in methanol/water (3:1, v/v, for soil and agar medium; 4:1, v/v, for plant samples) into powder in liquid nitrogen. Atrazine extraction was performed in methanol/water (3:1, v/v, for soil and agar medium; 4:1, v/v, for plant samples) for 2 h under shaking and then centrifuged at 12,000 g 5 min. Supernatants containing atrazine were analysed, after dilution, by ELISA (enzyme linked immunosorbent assay) method. The Atrazine ELISA kit (Abraxis LLC) used in this study is specific of free atrazine, cross-reactivities for deethylatrazine and hydroxyatrazine being, respectively, 1.3% and 1.8%. Atrazine content in the extracts was quantified spectrophotometrically at 450 nm, following the supplier’s instructions.

2.6. Extraction and quantification of atrazine in in vitro and soil microcosms experiments

Atrazine levels were measured in plant and surrounding medium samples. Agar medium was sampled in the vicinity of Arabidopsis root systems. In microcosms, soil samples were collected at the top (0-5 cm), the middle (5-10 cm) and the bottom (10-15 cm) of the soil column, dried at 60 °C, and then separately analysed. Before extraction, Arabidopsis plantlets were ground into powder in liquid nitrogen. Atrazine extraction was performed in methanol/water (3:1, v/v, for soil and agar medium; 4:1, v/v, for plant samples) for 2 h under shaking and then centrifuged at 0 °C overnight. Samples were shaken again for 2 h and then centrifuged at 12,000 × g for 5 min. Supernatants containing atrazine were analysed, after dilution, by ELISA (enzyme linked immunosorbent assay) method. The Atrazine ELISA kit (Abraxis LLC) used in this study is specific of free atrazine, cross-reactivities for deethylatrazine and hydroxyatrazine being, respectively, 1.3% and 1.8%. Atrazine content in the extracts was quantified spectrophotometrically at 450 nm, following the supplier’s instructions.

3. Results

3.1. Effects of sugar-treated atrazine-tolerant Arabidopsis plantlets on atrazine dynamics under in vitro culture conditions

In vitro experiments were realized under axenic conditions on MS-agar media, using atrazine concentrations which are sublethal (0.25 µM) or lethal (1 µM, 10 µM) for Arabidopsis seedlings in the absence of exogenous sucrose (Sulmon et al., 2004). Atrazine-sensitive plantlets (Suc—) accumulated a low level of atrazine, which did not significantly modify atrazine content in the culture medium in comparison to the amount initially applied to the system. In contrast, the presence of exogenous sucrose, which conferred atrazine tolerance to Arabidopsis plantlets and lifted the inhibition of vegetative growth (Sulmon et al., 2004), allowed seedlings to accumulate a high level of atrazine in plant tissues (Fig. 1). Arabidopsis seedlings grown in the presence of 10 µM atrazine may have reached the maximal levels of endogenous atrazine that they can support without tissue injury. Atrazine accumulation in sugar-treated atrazine-tolerant plants was associated with a strong decrease of herbicide level in the surrounding MS-agar medium. A proportion of the initial amount of atrazine was however not recovered, thus suggesting that this amount of atrazine had been transformed, through abiotic or biotic processes, to metabolites or conjugated forms, which were not detected by the ELISA method.

Study of atrazine transfer using [14C]atrazine revealed that atrazine was absorbed through roots from the medium and then primarily accumulated in shoots (Fig. 2A,B). However, the rate of accumulation in plants was not constant and decreased with time (Fig. 2B). This could be related to a decrease of atrazine absorption, to biotransformation of atrazine into non-extractable forms, or to an efflux of [14C]atrazine or 14C-containing compounds from plantlets. The highest levels of radioactivity were observed in sucrose-treated seedlings. In contrast, 14C radioactivity accumulation after 16 h of treatment showed a similar level in plantlets previously grown in the absence or in the presence of atrazine (Fig. 2B). After 16 h of incubation in the presence of 10 µM radiolabelled atrazine, the three types of seedlings were submitted to a chase experiment in order to study the displacement of accumulated radiolabelled atrazine by unlabelled atrazine. Autoradiography of these seedlings confirmed that atrazine was transferred from roots to shoots (Fig. 2A). However, liquid scintillation counting showed that seedling shoots after the chase experiment contained less radioactivity than shoots of seedlings that were not submitted to the chase experiment (Fig. 2B). This may suggest the production of non-extractable bound atrazine residues within seedling tissues, or an efflux of [14C]atrazine, or 14C-containing compounds, from seedlings.

3.2. Sucrose-induced atrazine tolerance in soil-grown Arabidopsis plants

Photosynthetic activity of plantlets represents a sensitive marker of atrazine inhibition (Sulmon et al., 2004). Atrazine treatments from 50 g ha⁻¹ to 2000 g ha⁻¹ were applied to experimental soil microcosms. Photosynthetic activity of plantlets began to decrease significantly at 100 g ha⁻¹ atrazine.
The 250 g ha\(^{-1}\) herbicide treatment gave 50% inhibition and was thus defined as sublethal treatment. Treatments of 1000 g ha\(^{-1}\) and 2000 g ha\(^{-1}\) resulted in complete inhibition of photosynthesis activity and plant development. The 1000 g ha\(^{-1}\) treatment was used as lethal atrazine treatment.

Application of 1.75% (w/v) sucrose solution in association with atrazine significantly increased the atrazine tolerance of Arabidopsis plantlets (Fig. 3A). However, the level of atrazine tolerance conferred by sucrose depended on the intensity of herbicide treatment. Whereas sucrose allowed plantlets to recover photosynthesis activity in the presence of sublethal herbicide dose (250 g ha\(^{-1}\)), the lethal herbicide dose (1000 g ha\(^{-1}\)) was shown to maintain partial inhibition on photosynthesis activity in sucrose-treated plantlets (Fig. 3A).

Such a response was also found for plant growth, which remained partially affected by high atrazine concentration in spite of sucrose application (Fig. 3B). This was not observed in our previous in vitro experiments performed on MS-agar medium (Sulmon et al., 2004), and may be related to differences of mineral nutrition and matrix complexity between the MS-agar medium and the soil. Atrazine tolerance in soil-grown plants was however associated with maintenance of high levels of chlorophylls and carotenoids in plantlets (Fig. 4A,B), and with overexpression of the \(psbA\) gene encoding the D1 protein targeted by atrazine (Fig. 4C), which was in accordance with sucrose-induced responses of plantlets to inhibitory concentrations of atrazine under in vitro conditions (Sulmon et al., 2004). The negative effects of sucrose alone on plantlet growth (Fig. 3B) and \(psbA\) expression (Fig. 4C) were in agreement with previous results under in vitro conditions (Sulmon et al., 2004). Moreover, these negative effects are known to be enhanced by low nitrogen conditions (Martin et al., 2002), as is expected to occur in soil. Thus, in soil microcosms, the protective effects should also be ascribed to interactions between sucrose and atrazine, as was shown to be the case under in vitro conditions (Sulmon et al., 2004, 2006).
3.3. Effects of atrazine-tolerant Arabidopsis plants on atrazine dynamics in a contaminated soil

Since Arabidopsis plantlets showed tolerance to atrazine in the presence of sucrose, their potential to remove the herbicide from the soil was evaluated. The 250 g ha\(^{-1}\) and 1000 g ha\(^{-1}\) atrazine treatments resulted in the application of, respectively, 175 mg and 700 mg herbicide on soil columns. After 30 days of incubation, control soil columns, which did not receive sucrose, were found to exhibit lower atrazine contents than expected (Fig. 5A), thus suggesting that atrazine was partly transformed, by abiotic or biotic processes, into undetected derivative forms. Whereas the 28% loss of atrazine in the 1000 g ha\(^{-1}\) treatment, which resulted in total seedling death, may originate from soil-related processes as described in other studies (Binet et al., 2006), the 50% level of non-retrieved atrazine observed in the 250 g ha\(^{-1}\) treatment (Fig. 5A) may be partly ascribed to absorption of herbicide by plants (Fig. 5B), which were present under these conditions of sublethal treatment. Addition of sucrose to soil decreased atrazine content in soil for both herbicide treatments and resulted in a concentration-dependent atrazine accumulation in plant tissues per g fresh weight (data not shown), and in the global plant biomass (Fig. 5B). Sucrose-treated plants in the 1000 g ha\(^{-1}\) atrazine treatment showed atrazine concentrations of up to 3000 ng per g fresh weight (FW), which was 5 times higher than the endogenous atrazine concentration leading to death of non-treated plants. However, whereas the presence of sucrose led to a decrease of approximately 50 µg and 160 µg atrazine, for, respectively, the 250 g ha\(^{-1}\) and 1000 g ha\(^{-1}\) herbicide treatments (Fig. 5A), the accumulation of atrazine in plant biomass did not exceed 300 ng (Fig. 5B).
This limited amount of atrazine in plants could be ascribed to the small number of plants and small plant biomass in each microcosm.

This disappearance of atrazine could have been ascribed to stimulation, by sucrose and/or root exudates, of endogenous atrazine-degrading bacteria in soil. However, since a similar disappearance of atrazine was reported in axenic in vitro experiment (Fig. 1), it was also likely that sucrose-treated plants may transform atrazine into metabolites or conjugated forms after absorption.

Atrazine distribution showed a decreasing gradient from the top (0-5 cm) to the bottom (10-15 cm) of soil columns (Fig. 5C,D), in agreement with other studies (Kersanté, 2003). Atrazine loss associated with sucrose application observed in 250 g ha\(^{-1}\) atrazine-treated microcosms (Fig. 5A) resulted from a decrease of herbicide content at all the levels of the soil column (Fig. 5C). In contrast, atrazine loss from microcosms that were treated with 1000 g ha\(^{-1}\) atrazine was entirely localized in the upper level of the soil column (Fig. 5D). Such differences may reflect the fact that Arabidopsis roots in 1000 g ha\(^{-1}\) atrazine-treated microcosms did not reach the middle and bottom of the soil column. Moreover, plantlets treated with 1000 g ha\(^{-1}\) atrazine in the presence of sucrose exhibited lower shoot development than plantlets grown in the presence of sucrose and sublethal atrazine dose (Fig. 3B), which may be linked to a decrease of root development.

The presence of earthworms affected soil remediation. Thus, earthworm-containing microcosms treated with 250 g ha\(^{-1}\) atrazine, which presented photosynthesizing plants (data not shown), displayed the same total atrazine content, whether they were submitted to sucrose application or not (data not shown). In these microcosms, there was a significant decrease of atrazine content in plants per g fresh weight (Fig. 6A) and in the total biomass (data not shown) in comparison to herbicide levels measured in plantlets grown in the absence of earthworms. Earthworms thus seemed to affect, directly or indirectly, atrazine absorption by roots. Moreover, earthworm activity in soil was found, in the absence of plants, to homogenize atrazine distribution in the soil column and to decrease atrazine levels in the upper part of the column (Fig. 6B).

4. Discussion

The presence of atrazine-tolerant plantlets in contaminated media significantly decreased herbicide contents in different surrounding substrates, whether MS-agar or natural soil (Figs. 1 and 5A,C,D). However, atrazine decrease in sucrose-treated soil could have been related to sucrose stimulation of endogenous atrazine-degrading bacteria, which were initially present in the experimental soil (Binet et al., 2006). Bacterial consortia can metabolize atrazine, even though they have never been exposed to this herbicide (Bouquard et al., 1997; Yassir et al., 1998). However, atrazine disappearance in soil microcosm was in agreement with atrazine disappearance from the medium in axenic in vitro experiments (Fig. 1). Moreover, decrease of herbicide content in sucrose-treated soil was associated with significant accumulation of free atrazine in Arabidopsis plantlets, whether in terms of ng
per g FW or of ng in the total plant biomass (Fig. 5). Finally, whereas atrazine decrease in soil microcosms containing atrazine-tolerant plants was more important in the presence of sucrose (Fig. 5), soil microcosms without plants and treated with 1000 g ha\(^{-1}\) atrazine presented the same decrease of atrazine, whether they were treated with sucrose or not (data not shown).

However, a large part of the atrazine amount removed from the medium, particularly in soil experiment, was not recovered using atrazine-specific detection protocols (Figs. 1 and 5), thus suggesting that atrazine was transformed within plantlets and accumulated principally as metabolites or related compounds. This was in agreement with the work of Gray et al. (1996), who found that atrazine level accumulated in plants of an atrazine-resistant biotype of *Abutilon theophrasti* did not exceed 25% of the amount of herbicide initially absorbed. Metabolization of atrazine has been reported in studies of atrazine-tolerant crop, tree or weed species (De Prado et al., 1995, 2000; Gray et al., 1996; Burken and Schnoor, 1997; Raveton et al., 1997; Plaisance and Gronwald, 1999; Cherifi et al., 2001).

In plants, atrazine can thus undergo different metabolic pathways: (i) 2-hydroxylation (Shimabukuro, 1967; Raveton et al., 1997), which leads to hydroxyatrazine, (ii) N-dealkylation (Shimabukuro, 1967), which results in removal of one or both alky side chains, and (iii) conjugation with the tripeptide glutathione (Lamoureux et al., 1970; Shimabukuro et al., 1970), or with glucose (Hatzios, 2000). In particular, atrazine conjugation to glutathione, which results in non-reactive atrazine-related compounds and is associated with vacuolar sequestration of conjugates (Martinoia et al., 1993; Coleman et al., 1997), constitutes the major atrazine detoxification pathway in some atrazine-tolerant plants (Shimabukuro et al., 1971; De Prado et al., 1995, 2000; Gray et al., 1996; Plaisance and Gronwald, 1999; Cherifi et al., 2001). Since expression of several GST genes in *Arabidopsis* seedlings is induced by herbicides, among which atrazine and metolachlor (Wagner and Mauch, 2000; Wagner et al., 2002; Sulmon et al., 2006), which are known to be detoxified by conjugation to glutathione (O’Connell et al., 1988; Jablonkai and Hatzios, 1991; Scarpioni et al., 1991; Gullner et al., 2001), it was likely that the high amount of atrazine that was removed from MS-agar medium and microcosm soil (Figs. 1 and 5) could be accumulated in *Arabidopsis* plantlet shoots as atrazine-glutathione conjugates. Alternatively, the sucrose treatment may enhance glucose conjugation of atrazine by providing glucose for glucosyltransferase-catalysed reactions (Sulmon et al., 2004). However, chase experiments of radiolabelled compounds with unlabelled atrazine (Fig. 2B) resulted in a decrease of radiolabelled compounds in methanolic extracts from shoots. Although Lao et al. (2003) have reported that *Arabidopsis* seedlings were able to export 3,4-dichloroaniline from root to culture media after absorption and N-glucosylation, absence of radioactivity signal in seedling roots on autoradiography (Fig. 2A) indicated that such an efflux process may not be involved. Mechanisms of herbicide efflux from seedling shoots have, to our knowledge, never been reported. The decrease of detectable radioactivity in methanolic plant extracts after 24 h of chase with unlabelled atrazine could result from biochemical transformation of \([^{14}\text{C}]\)atrazine into bound non-extractable \(^{14}\text{C}\)-residues, as previously described in an atrazine-tolerant biotype of *Abutilon theophrasti* (Gray et al., 1996; Shimabukuro, 1967). Binding of atrazine degradation products to lignin in the apoplast, which generally originates from the glutathione conjugation pathway (Lamoureux et al., 1991), is indeed considered to be a detoxification mechanism (Burken and Schnoor, 1997) and could thus contribute to limit toxic effects of high amounts of atrazine in cells. Since biotransformation of atrazine has never been reported, to our knowledge, in *Arabidopsis*, sucrose would seem to induce metabolic or conjugation pathways that are not usually expressed in the plant.

Whereas sucrose-treated seedlings grown on MS-agar medium exhibited complete tolerance to high atrazine concentrations, sucrose-treated seedlings in microcosms showed partial tolerance to 1000 g ha\(^{-1}\) herbicide treatment, thus leading to limited shoot and root development (Fig. 3B), and to a decrease

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**Fig. 6.** Effects of earthworm bioturbation on soil remediation by sugar-treated atrazine-tolerant *Arabidopsis* plantlets and on atrazine distribution in the soil column. (A) Experimental soil microcosms containing photosynthesizing *Arabidopsis* plantlets were subjected to 250 g ha\(^{-1}\) atrazine, in the absence or in the presence of 1.75% sucrose and in the absence or in the presence of earthworms. (B) Experimental soil microcosms containing no *Arabidopsis* plantlet shoots were subjected to 250 g ha\(^{-1}\) atrazine in the absence or in the presence of earthworms. Atrazine concentration in *Arabidopsis* plantlets (A) and distribution of atrazine in the three levels of the soil column (B) are given. Values are the mean (±S.E.M.) of four measurements on seedlings (A) and on the three levels of soil column (B), in each of the five replicates.
of soil remediation, particularly in the middle and bottom of the soil column (Fig. 5D), which were not reached by plant roots. Since mineral nutrition, and particularly nitrogen nutrition, strongly influences effects of carbon nutrition on plant development (Martin et al., 2002), decrease of sucrose efficiency could be ascribed to bioavailability of nitrogen. Moreover, homogenization effects of earthworms decreased atrazine content in the upper part of the column (Fig. 6), where plant action seemed to be the most efficient in the absence of earthworms (Fig. 5). This was associated with a decrease of atrazine absorption by plants (Fig. 6). Thus, processes affecting the concentration of bioavailable herbicide in the medium could limit absorption by roots. If sucrose undergoes a similar transfer as that of atrazine within the soil column, earthworm bioturbation activities may also reduce sucrose availability, which would contribute to decrease atrazine tolerance of Arabidopsis plantlets.

5. Conclusions

Sucrose treatment can enhance plant tolerance to xenobiotics, xenobiotic accumulation in plants, and decontamination of pollutants surrounding plants. Sucrose treatment could thus be used to overcome the trade-off between xenobiotic tolerance/detoxification and biomass accumulation by conferring tolerance to species known to exhibit high biomass production, which would thus lead to the improvement of phytoremediation techniques. Conversely, for a given plant species, sucrose treatment could be useful to extend the range of pollutants that can be tolerated and decontaminated. However, abiotic and biotic mechanisms influencing atrazine and sucrose fate in soil microcosms could hamper phytoremediation efficiency. Thus, although further work is needed to optimize the effects of sucrose treatment, application of sucrose on xenobiotic-polluted soil to increase plant tolerance and xenobiotic absorption appears to be potentially useful for phytoremediation.

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