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# Effects of anthracene on development of an arbuscular mycorrhizal fungus and contribution of the symbiotic association to pollutant dissipation

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**Abstract** The influence of anthracene, a low molecular weight polycyclic aromatic hydrocarbon (PAH), on chicory root colonization by *Glomus intraradices* and the effect of the root colonization on PAH degradation were investigated in vitro. The fungus presented a reduced development of extraradical mycelium and a decrease in sporulation, root colonization, and spore germination when exposed to anthracene. Mycorrhization improved the growth of the roots in the medium supplemented containing 140 mg $l^{-1}$ anthracene, suggesting a positive contribution of *G. intraradices* to the PAH tolerance of roots. Anthracene disappearance from the culture medium was quantified; results suggested that nonmycorrhizal chicory roots growing in vitro were able to contribute to anthracene dissipation, and in addition, that mycorrhization significantly enhanced anthracene dissipation. These monoxenic experiments demonstrated a positive contribution of the symbiotic association to anthracene dissipation in the absence of other microorganisms. In addition to anthracene dissipation, intracellular accumulation of anthracene was detected in lipid bodies of plant cells and fungal hyphae, indicating intracellular storage capacity of the pollutant by the roots and the mycorrhizal fungus.

**Keywords** Mycorrhiza · *Cichorium intybus* · *Glomus intraradices* · Monoxenic culture · Anthracene · PAH

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants of soils and industrial areas. These organic pollutants result essentially from the incomplete combustion of fossil fuels but also from industrial processes and forest fires. Residual contamination of soils by PAHs is of concern because of their toxicity, several being potential carcinogens and mutagens, and because of their persistence due to a low solubility and bioavailability. For all these reasons, the rehabilitation of polluted soils remains difficult. The use of vegetation to improve bioremediation of PAH-polluted soils would be an economic and low maintenance approach. The effect of plant roots on the dissipation of organic pollutants was mainly attributed to an increase in microbial populations, selection of specialized microbial communities in the rhizosphere (Reilley et al. 1996), and improvement of physical and chemical soil conditions.

Enhanced remediation of PAH-contaminated soils by arbuscular mycorrhizal (AM) plants was reported over the past few years (Leyval et al. 2002). The role of AM fungi concerns two aspects: improved establishment and development of plants on polluted soil, and the enhancement of PAH degradation (Leyval et al. 2002). AM development in different plant species is negatively affected when the plants are grown in crude oil-contaminated soils (Cabello 1997) or by addition of industrial soil polluted with PAH (Leyval and Binet 1998). Nevertheless, AM development was shown to enhance plant survival and growth in the presence of PAH (Joner and Leyval 2001). Decreases in phosphorus deficiency (Joner and Leyval 2001), water stress (Augé 2001), and stimulation of oxidative enzyme production (Salzer et al. 1999; Criquet et al. 2000) may contribute to attenuation of the stress due to the organic pollutant. However, mechanisms involved in PAH degradation by mycorrhizal plants are still not clearly understood. An indirect degradation associated with the mycorrhiza-associated microflora was demonstrated (Joner et al. 2001) but no data about the direct implication of AM fungi and/or the colonized roots without other associated microorganisms are available.

In the last three decades, AM were obtained in vitro using root organ cultures (Fortin et al. 2002). These cultures have improved the understanding of the function of AM symbiosis and have shed new light on the molecular

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biology, cytology, genetics, physiology, systematics, and phylogeny of AM fungi (Declerck et al. 2005). Moreover, in vitro culture of mycorrhizal roots was previously used to evaluate the toxicity of different pesticides on root colonization and the symbiotic fungus *Glomus intraradices* (Wan et al. 1998). The present work aim to study in vitro the effect of anthracene on the development of *G. intraradices* and on colonization by the fungus of chicory (*Cichorium intybus* L.) roots transformed by *Agrobacterium rhizogenes*. Monoxenic root cultures were used to evaluate the direct impact of PAH on AM fungal sporulation, hyphal length development, and spore germination, and to measure the PAH dissipation capacity of AM roots free of any other microorganisms.

## Materials and methods

Experiment 1

### *Culture of mycorrhizal chicory roots in the presence of anthracene*

Ri transfer DNA (T-DNA) transformed chicory (*C. intybus* L.) roots (Fontaine et al. 2004), colonized or not by *G. intraradices* (Schenck and Smith) (DAOM 197198), were grown on a modified M medium (Bécard and Fortin 1988) [solidified with 0.05% (w/v) gellan gel (Phytagel, Sigma, St Louis, MO, USA)] supplemented or not with 50 or 250 mg $l^{-1}$ of anthracene for 6 weeks at 27°C in the dark. To prepare PAH-enriched medium, anthracene was dissolved in acetone (12.5 mg anthracene/ml acetone) and individually added to a flask containing 25 ml of M medium to provide final concentrations of 50 or 250 mg $l^{-1}$. After the addition of anthracene, media were sterilized (121°C, 15 min); acetone was removed from the culture medium during autoclaving. Cultures were started from standardized root inoculum of 2-month-old monoxenic cultures of Ri T-DNA transformed chicory roots, colonized or not by *G. intraradices*, and using a 10-mm cork borer. A disk of culture medium containing roots from monoxenic cultures (noncolonized or colonized chicory roots) was placed in the middle of ten replicate petri dishes per treatment. To use homogeneous inoculum, AM colonization was randomly controlled in roots of five inoculum disks.

### *G. intraradices development in the presence of anthracene*

Spore formation and extraradical mycelium development were followed during the 6-week incubation period by observation under a low power stereo microscope at 10 to 40× magnification. Number of spores was determined using the method described by Declerck et al. (2001) and total hyphal length was assessed with the gridline method (Giovanetti and Mosse 1980) in five replicated dishes.

### *Determination of AM colonization and anthracene accumulation*

After spore enumeration and hyphal length determination ($n$=5 replicates), the roots were collected from the medium by solubilizing the solidified media for 1 h under agitation in 5 vol of sodium citrate buffer (10 mM, pH 6) (Doner and Bécard 1991). Roots and hyphae were filtered through a predried cellulose filter (porosity 8 μm) by vacuum filtration. Direct observations of root and fungal samples collected from cultures grown in M medium supplemented with anthracene were undertaken with an epifluorescent microscope on some fragments of roots as described by Verdin et al. (2005) [Nikon, Eclipse E600; excitation 345 nm, emission 485 nm (diamidino phenyl indole, DAPI) and 520 nm (fluorescein isothiocyanate, FITC)] and recorded on Kodak Elite Chrome 400 film.

The roots collected from each replicate ($n$=5) were subdivided in two subsamples to determine root colonization and to visualize lipid bodies. The first root subsamples from each replicate were cleared in KOH and stained with Chlorazol black E (Brundrett et al. 1994) to determine root colonization by the method of McGonigle et al. (1990). Sudan red 7B was used according to Brundett et al. (1991) to visualize lipid bodies in plant cells and intraradical fungal hyphae. The staining procedure was performed with the second root subsamples cleared in KOH and stained with trypan blue in lactophenol as described by Phillips and Hayman (1970). Fluorescence of anthracene in lipid bodies of stained roots was visualized with an epifluorescent microscope [excitation 345 and 520 nm (FITC)]. The slides were dipped in sudan red 7B solution for 1 h at room temperature and rinsed in water. Lipid bodies appeared stained in red in light microscopy.

### *Intracellular enzyme activities: laccase and peroxidase*

*Preparation of crude cell-freeextracts* Lyophilized roots (five replicates) were ground in a mortar with sand and homogenized in a phosphate buffer (100 mM, pH 7.8) containing EDTA (1 mM) and dithiothreitol (1 mM). The homogenate was centrifuged (10,000×$g$, 15 min, 4°C) and supernatants were used as crude enzymatic extracts.

Enzyme activities in root extracts were followed by spectrophotometric assays. The specific enzymatic activities were expressed as nkat $mg^{-1}$ protein. Intracellular proteins were measured with the method of Lowry et al. (1951) using BioRad DC Protein Assay Kit (500-0116) and bovine serum albumin as standard.

*Laccase activities (EC 1.10.3.2.)* Two different protocols were used to determine laccase activities. Firstly, activities were determined by oxidation of 16 μM syringaldazine in phosphate buffer 0.1 M, pH 6 (Criquet et al. 2000), and secondly by oxidation of 50 μM 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) in 0.1 M sodium acetate buffer (pH 5) (Childs and Bardsley 1975) as described by Verdin et al. (2004). Oxidation rates of the

different substrates were monitored at 525 and 420 nm, respectively.

*Peroxidase activities (EC 1.11.1.7)* Peroxidase was assayed colorimetrically using ABTS as the electron donor at 20°C using the method of Mitchell et al. (1994) based on the method of Shindler et al. (1976). The assay mixture consisted of 100 µl of enzyme extract in reaction buffer (500 mM ABTS, 250 µM $H_2O_2$, and 25 mM acetate buffer, pH 4.4). The formation of the radical cation was monitored at 412 nm ($\varepsilon$=32.4×103 M cm).

### *Anthracene extraction*

To evaluate the quantity of anthracene remaining in the culture medium after autoclaving, M medium supplemented with 50 or 250 mg $l^{-1}$ of anthracene was autoclaved, anthracene was extracted and quantified as described previously. Anthracene losses due to autoclaving were estimated at approximately 40% anthracene. Thus, final concentrations in the supplemented culture medium (50 and 250 mg $l^{-1}$) were 30±3.58 and 140±6.13 mg $l^{-1}$, respectively, when roots were introduced.

After 6 weeks of incubation, roots (five replicates) were separated from the culture media as previously described by solubilizing the solidified media in 5 vol of sodium citrate buffer, and were recovered by filtration. Roots were rinsed with 10 ml of dichloromethane to remove weakly bound anthracene. This volume (containing adsorbed anthracene) was added to the solubilized medium and the total fraction (aqueous and organic) was extracted three times with an equal volume of dichloromethane. Anthracene extracted from this total fraction corresponded to residual anthracene in the culture medium. The washed roots were lyophilized, dry weight was determined, and anthracene contained in the roots was extracted with a soxhlet apparatus (100 ml dichloromethane during 16 h at 70°C, 1 recycling per hour).

To quantify anthracene dissipation from media inoculated by colonized or noncolonized roots, comparisons were made to a root-free control. Petri dishes containing M medium initially supplemented with 50 or 250 mg $l^{-1}$ of anthracene were prepared without roots, and concentrations of anthracene lost from these root-free controls were measured after 6 weeks of incubation as described previously.

Anthracene dissipation was estimated as anthracene recovered from root-free control (minus residual anthracene in the culture medium + anthracene accumulated in roots).

*High-pressure liquid chromatography (HPLC) analysis* Amounts of anthracene in both dichloromethane fractions (medium or roots) were determined and compared to the root-free control using pyrene as an internal standard. Anthracene quantification was performed by reverse-phase HPLC (Waters, Milford, CA, USA) analysis with a methanol–water gradient elution and a 5-µm Waters Symmetry-reversed-phase C18 column (length 150 mm, width 2.1 mm, and column temperature 30°C). Anthracene was detected and identified with a variable wavelength detector (Waters 996 UV photodiode array detector, Waters, Milford, CA, USA) operating at 254 nm. The following gradient was used with a flow rate of 0.2 ml $min^{-1}$ throughout: 0 min, 20% $H_2O$ and 80% methanol; 10 min, 100% methanol (hold for 30 min); and 50 min, 20% $H_2O$ and 80% methanol. The relative elution time for anthracene was 1.82 min.

## Experiment 2

### *Measure of spore germination*

Spores of *G. intraradices* were isolated from a 4-month-old monoxenic culture after solubilization of the M medium with a citrate buffer according to Doner and Bécard (1991). The spores were subsequently isolated with a pipette under a binocular microscope and placed individually in petri dishes (55-mm in diam.) containing 10 ml of M medium supplemented with 50 or 250 mg $l^{-1}$ of anthracene. Petri dishes were incubated at 27°C in the dark and spore germination was monitored at regular intervals under a binocular microscope (10 and 40× magnification). Germination was considered to have occurred if one germ tube was clearly visible. The germination of 40 spores was observed for each treatment. These germination tests were done in triplicate. Percentages of spore germination were converted to arcsine values before the analysis of variance.

## Chemicals

Anthracene was purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Dichloromethane and methanol were purchased from SDS (Peypin, France) and Mallinkrodt Baker France (Noisy-le-Sec, France), respectively. All chemicals were at least 99% pure.

## Statistical data analysis

Analysis of variance (ANOVA) was carried out with the statistical program STATGRAPHICS release 4.0 (Manugistic, Rockville, MD, USA). The method used to discriminate between the means was the Student–Newman–Keuls multiple comparison procedure ($P$<0.05). ANOVA was conducted on the original data except for the germination tests (see above) and normality of the data was checked with the Hartley test before the use of the multiple comparison procedure.

# Results

## Effect of anthracene on root growth

Figure 1 shows the effect of a low (30 mg $l^{-1}$) and a high (140 mg $l^{-1}$) anthracene concentration on the dry weight after 6 weeks of growth of chicory roots colonized by

*G. intraradices*. The presence of anthracene in the culture medium induced a biomass reduction greater than 70% when compared with roots grown without the pollutant. Anthracene had a more toxic effect on the development of noncolonized chicory roots at 140 mg $l^{-1}$. Response of colonized roots did not seem to be linked to anthracene concentrations.

## Effect of anthracene on fungal development and mycorrhization

During the first days after inoculation, transformed chicory roots grown on the medium without anthracene developed many lateral roots. After 3 to 4 weeks, roots completely covered the petri dishes when grown in absence of the pollutant. Extraradical hyphal extension was observed at the same time. During the first 3 weeks of growth, mycorrhiza development in roots remained particularly low (about 10%); the first new generation of spores was formed after 3 weeks. Microscopic observations of stained chicory roots showed the presence of arbuscules, vesicles, and intraradical hyphae in roots after 6 weeks on both anthracene-supplemented and anthracene-nonsupplemented media. Chicory root colonization by *G. intraradices* was significantly reduced in the presence of anthracene at 30 and 140 mg $l^{-1}$ (Fig. 2) and the frequency of vesicles was lower in roots grown on polluted than nonpolluted media. No difference was observed in colonization or vesicles frequency in chicory roots grown on 30 and 140 mg $l^{-1}$ of anthracene.

After 6 weeks of incubation, intense sporulation and a high density of extraradical hyphae were observed with many branched, absorbing structures being formed as generally observed in monoxenic cultures (Bago and Cano 2005). Spore production and development of extraradical hyphae by *G. intraradices* grown on a medium without anthracene were much higher than in medium supplemented with anthracene where slower development of chicory roots was accompanied by very low spore formation and extraradical hyphae development (Table 1). The mycelium of *G. intraradices* grown with the pollutant also produced branched, absorbing structures and did not appear stunted.

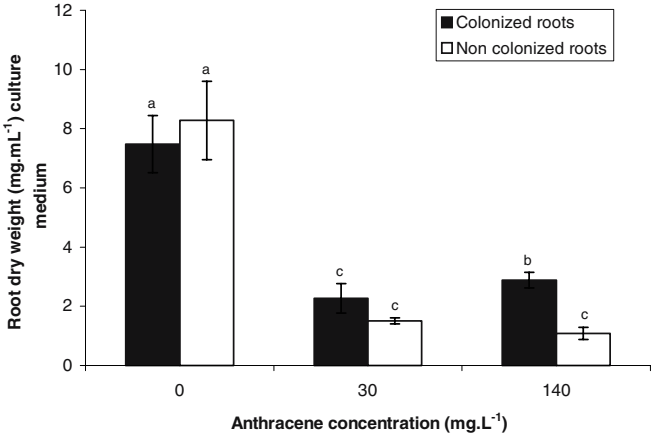


**Fig. 1** Root dry weight of transformed chicory roots (*C. intybus*) colonized or not by *G. intraradices* after 6 weeks of growth in the absence (control culture) or presence of anthracene at different concentrations (30 and 140 mg $l^{-1}$). The means were obtained from five replicates. In all plots, data are presented as mean±SD. *Bars* sharing the same *letters* are not significantly different according to the Student–Newman–Keuls test ($P<0.05$)

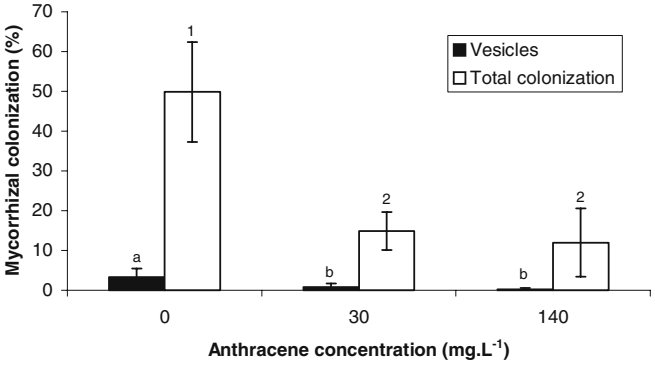


**Fig. 2** Estimation of mycorrhizal colonization of transformed chicory roots (*C. intybus* L.) by *G. intraradices* after 6 weeks of growth in the presence of anthracene at two concentrations (30 and 140 mg $l^{-1}$). The means were obtained from five replicates. In all plots, data are presented as mean±SD. *Bars* sharing the same *letters* or *numbers* are not significantly different according to the Student–Newman–Keuls test ($P<0.05$)

## Effect of anthracene on spore germination

In control cultures without anthracene, spores of *G. intraradices* first germinated after 1 day and germinated up to 80% within 2 to 3 days (Fig. 3). Thereafter, the number of germinated spores gradually increased and reached 100% after 20 days of incubation. In the presence of anthracene, spore germination followed a similar pattern but an inhibitory effect on germination was observed (Fig. 3). At 140 mg $l^{-1}$, anthracene significantly reduced the number of germinating spores after 30 days (75%) compared to the control.

## Residual anthracene in growth medium of mycorrhizal chicory roots

Mycorrhizal or nonmycorrhizal chicory roots were grown during 6 weeks in the presence of high (140 mg $l^{-1}$) and low (30 mg $l^{-1}$) anthracene concentrations and the residual amount of anthracene in the medium was extracted and estimated (Table 2). Abiotic anthracene losses occurred during incubation time. Residual anthracene concentrations were quantified by comparison with the root-free control and were estimated at 110±5.76 and 22±2.09 mg $l^{-1}$. Residual, accumulated, and dissipated anthracene was therefore estimated with respect to 110 and 22 μg/petri dish. Table 2 shows that the quantities of residual anthracene in the medium were similar after growth of mycorrhizal and nonmycorrhizal roots in the presence of low and high anthracene concentrations, and corresponded

**Table 1** Effect of different concentrations (30 and 140 mg $l^{-1}$) of anthracene on the extraradical hyphae development and the spore production of *G. intraradices*

| Treatments | Control | Anthracene (30 mg $l^{-1}$) | Anthracene (140 mg $l^{-1}$) |
|---|---|---|---|
| Extraradical hyphal length (mm) | 9,570±6,214[a] | 1,012±687[b] | 308±66[b] |
| Spore number | 570±217[a] | 56±13[b] | 5±7[b] |

Data are presented as means±SD. The means were obtained from five replicates. The data were subjected to ANOVA. Values followed by the same letters are not significantly different according to the Student–Newman–Keuls test ($P<0.05$)

approximately to 50% of the initial quantity of pollutant added to the medium.

### Accumulation of anthracene in lipid bodies of root cells and AM fungus

Anthracene quantities accumulating in colonized chicory roots after 6 weeks of growth were 157.5±10 and 742.5±65 μg/petri dish for cultures grown in presence of low and high anthracene concentrations, respectively (Table 2). The quantity of accumulated anthracene was significantly lower in colonized than in noncolonized roots.

It is possible to detect the presence of anthracene in cells because this compound exhibits autofluorescence due to the presence of aromatic cycles in their structure (Verdin et al. 2005). Fluorescence of tissues only occurred in the presence of anthracene where it was observed in chicory roots (Fig. 4a, 1 and 2), hyphae (Fig. 4b, 1 and 2), and spores of the AM fungus (Fig. 4c, 1 and 2). Using sudan red 7B (Brundett et al. 1991), we demonstrated that anthracene accumulated in lipid bodies of AM fungal hyphae and plant cortical cells (Fig. 5).

### Anthracene dissipation in mycorrhizal chicory root cultures

Table 2 shows that the quantity of anthracene dissipation was significantly higher in mycorrhizal roots than in nonmycorrhizal roots with 133±15 and 612.5±47.5 μg/petri dish of dissipated anthracene observed in colonized roots grown in the presence of 30 and 140 mg $l^{-1}$ of anthracene, respectively, and only 55.75±10 and 272±37.5 μg/petri in noncolonized roots, respectively.

### Laccase and peroxidase activities in mycorrhizal roots grown in the presence of anthracene

Laccase activities were not detected in mycorrhizal or nonmycorrhizal chicory roots and in the presence of anthracene, peroxidase specific activities were reduced in the noncolonized roots (Fig. 6). Percentage inhibition was about 90% in presence of both (30 and 140 mg $l^{-1}$) anthracene concentrations. This effect of anthracene on peroxidase specific activities was not observed in colonized roots.

## Discussion

Our results show for the first time a direct impact of anthracene on AM fungi and an increased tolerance of mycorrhizal roots to high anthracene concentration, compared to nonmycorrhizal roots. In the presence of anthracene, development of extraradical hyphae was inhibited and spore production was particularly low when compared to control cultures. Chicory root colonization by *G. intraradices* was significantly affected by the introduc-

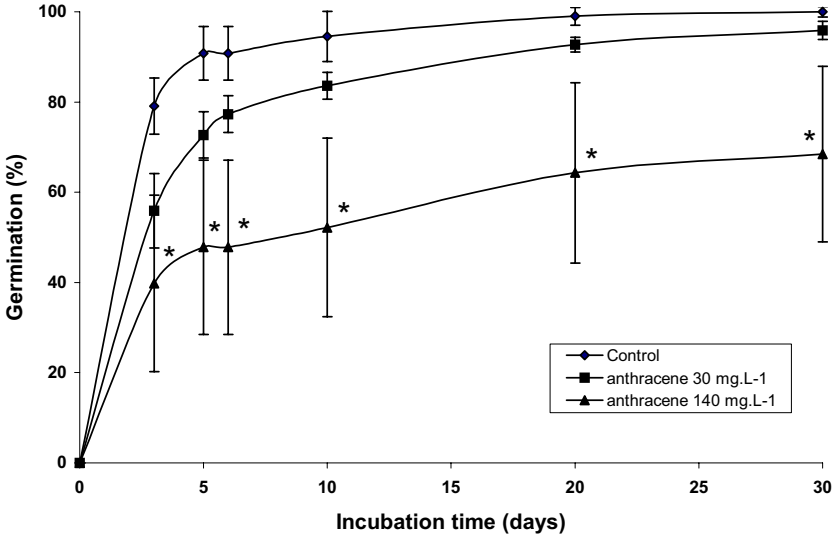


**Fig. 3** Time-course of *G. intraradices* spore germination in the presence of anthracene at different concentrations. Forty spores were observed for each treatment. The means were obtained from three replicates. Data of germination percentage were converted to arcsine values before the analysis of variance for comparing the means according Student–Newman–Keuls test. *Points* with an *asterisk* were significantly different from control ($P<0.05$)

**Table 2** Residual anthracene in the culture medium, anthracene accumulated in chicory roots, and anthracene dissipated (μg) in culture of transformed chicory roots (*C. intybus*) colonized or not by *G. intraradices* after 6 weeks of growth in the presence of anthracene at two concentrations (30 and 140 mg $l^{-1}$)

| Initial anthracene concentration in medium (mg $l^{-1}$) | | 50 | | 250 | |
|---|---|---|---|---|---|
| Anthracene concentration in medium (mg $l^{-1}$) | After autoclaving | 30±3.58 | | 140±6.13 | |
| | After incubation | 22.03±2.09 | | 110.9±0.51 | |
| Anthracene quantity/petri dish (μg) | After incubation | 550.75±52.25 | | 2,752.5±12.75 | |
| | | Quantity (μg) | Anthracene quantity/root dry weight (μg $μg^{-1}$) | Quantity (μg) | Anthracene quantity/root dry weight (μg $μg^{-1}$) |
| Noncolonized root cultures | Residual anthracene in the culture medium | 297.5±12.75 | 0.32 | 1,545±107.5 | 2.29 |
| | Anthracene accumulated in roots | 197.5±10 | 0.21 | 935±65 | 1.39 |
| | Anthracene dissipation | 55.75±10 | 0.06 | 272.5±37.5 | 0.40 |
| Colonized root cultures | Residual anthracene in the culture medium | 260±23.75 | 0.18 | 1,382.5±77.5 | 0.77 |
| | Anthracene accumulated in roots | 157.5*±10 | 0.11 | 742.5*±65 | 0.42 |
| | Anthracene dissipation | 133*±15 | 0.09 | 612.5*±47.5 | 0.34 |

Data of anthracene quantities in table represent means of 5 values
*Significant difference between means for colonized and noncolonized roots according to the Student–Newman–Keuls test ($P<0.05$)

tion of anthracene into the growth medium, suggesting that hydrocarbon contamination inhibits the colonization of the roots. These results, obtained in monoxenic conditions, are in agreement with those reported in in situ experiments by Cabello (1997) who found significantly reduced AM propagule density in crude oil-contaminated soil. Leyval and Binet (1998) also showed that mycorrhizal colonization of some plant species was negatively affected in industrial soil polluted with PAH. Although it seems evident that anthracene has a direct effect on extraradical fungal development, the effect could also be indirect because the colonized root growth was also dramatically reduced.

In vitro chicory root growth was also affected by anthracene and root dry weight was reduced by 87% in the presence of the pollutant at a concentration of 140 mg $l^{-1}$. This is consistent with the data of Wieczorek and Wieczorek (2006) who reported a decrease of lettuce and radish biomass, especially root biomass after foliar and soil application of anthracene. Our results demonstrated a positive contribution of *G. intraradices* to anthracene tolerance of roots with a better development of roots growing in the presence of 140 mg $l^{-1}$ anthracene when they were colonized by the AM fungus. This effect was also observed when using benzo[a]pyrene, a high molecular weight PAH when using another plant species (Ri T-DNA transformed carrot root) (data not shown).

PAH were described as toxic to plant on physiology. Photosynthesis is inhibited (Huang et al. 1996) but this toxicity is limited by the low solubility of these compounds (Gray et al. 1994). Another suggested effect is the reduced ability of plants to take water and nutrients from contaminated soil (Reilley et al. 1996). AM fungi are known to improve water and nutrients uptake by plants (Augé 2001; Linderman 1997) and may therefore be important for water uptake in PAH-polluted soils. In this respect, mycorrhizal fungi producing dense and long extraradical hyphae should be particularly efficient in protecting the host plant against PAH toxicity.

Transformed chicory roots grown in vitro were able to contribute to anthracene dissipation in the culture medium without the involvement of microorganisms. To be sure that the estimated anthracene dissipation was not entirely due to abiotic losses, root-free controls were used and losses after incubation were estimated to be about 24%. Adsorption of anthracene onto plastic dishes and glass materials probably contributed to these losses. Photooxidation, another process that could explain these abiotic losses recorded during incubation, does not seem likely because the chicory root cultures were incubated in the dark. Our results are in accordance with those obtained in situ by Reilley et al. (1996) who showed an increase in the dissipation of anthracene and pyrene by fescue and alfalfa. Anderson et al. (1993) have listed plant species that can facilitate the dissipation of hazardous organic compounds. The degree of xenobiotic dissipation in the rhizosphere appears to be greater for monocotyledones and grass plants, and the difference was attributed to different root wall constituents or root exudates (Shann and Boyle 1994).

Anthracene dissipation was higher in the presence of mycorrhizal chicory roots than in the presence of nonmycorrhizal roots. This result is in agreement with

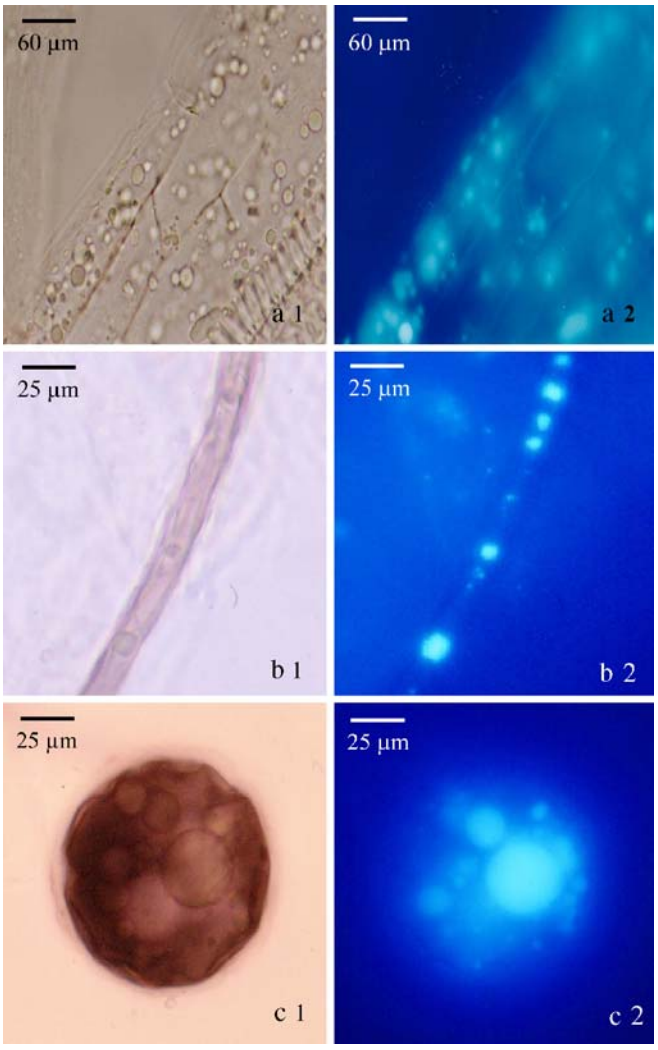


**Fig. 4** Accumulation of anthracene into *G. intraradices* colonized chicory roots grown on anthracene-supplemented medium (×100 objective lens). **a** Chicory root cells, **b** fungal hyphae, and **c** fungal spore. *1* Bright field and *2* fluorescence using standard DAPI filter set. No fluorescence was detected in lipid bodies of the control in comparison to cultures grown in the presence of PAH

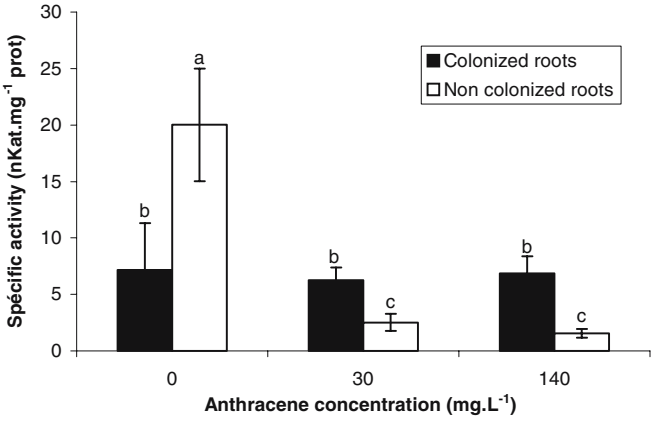


**Fig. 6** Intracellular peroxidase specific activities of transformed chicory roots (*C. intybus* L.) after 6 weeks of growth in the absence (control culture) or presence of anthracene at different concentrations. The means were obtained from five replicates. In all plots, data are presented as mean±SD. *Bars* sharing the same *letters* are not significantly different according to the Student–Newman–Keuls test ($P<0.05$)

the studies of Binet et al. (2001) who showed that the biodegradation of anthracene was greater in soil from a mycorrhizal rhizosphere than in a nonmycorrhizal soil. Mechanisms involved in pollutant degradation by mycorrhizal plants are not clear. AM fungi are supposed to play roles in the establishment of plant cover in polluted soils and in the modification of PAH degradation rates or pathways (Leyval et al. 2002). Extraradical mycelium extending from the root system may influence the maintenance and activity of microbial communities in the rhizosphere, including PAH-degrading microorganisms. Indirect degradation associated to the mycorrhiza-associated microflora was demonstrated (Joner et al. 2001). The present experiments in monoxenic conditions demonstrated a positive contribution to anthracene dissipation of the AM association in the absence of any other microbial communities. However, it is not possible to conclude whether the AM fungus directly dissipates anthracene or indirectly contributes to dissipation by improving root growth. Increased dissipation (2.25 to 2.5 times) of mycorrhizal roots could be due to the higher biomass and, in fact, when the ratios of anthracene dissipation/root dry weight were compared, they were similar when mycorrhizal and nonmycorrhizal roots were grown in the highest anthracene concentration.

The mechanisms that could be involved in protecting plants from oxidative stress are the oxidation of the pollutant by activated oxygen species and enhancement of oxidoreductases (Leyval et al. 2002). The improvement of anthracene dissipation by mycorrhizal roots may involve enzymatic biotransformation of the PAH by the symbiotic association. In our experiments, a correlation between

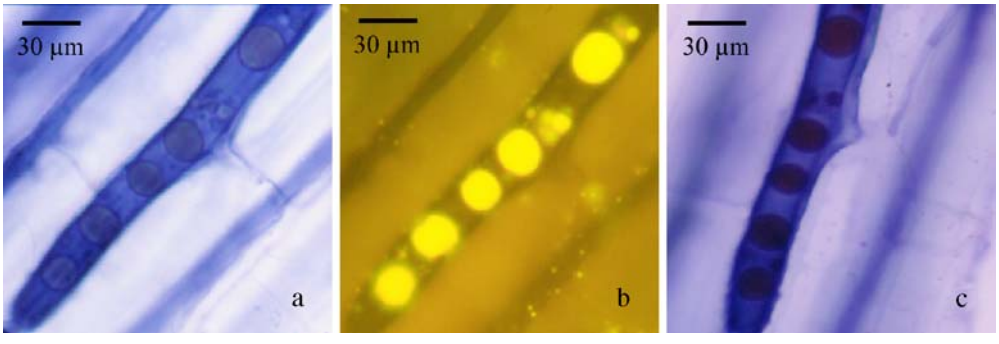


**Fig. 5** Light microscope observation of the intracellular elements in *G. intraradices* hyphae grown on anthracene medium (×100 objective lens). **a** bright field, **b** fluorescence using standard FITC filter set, and **c** bright field after Sudan Red 7B staining. Due to the staining directly on the slide, the preparation has slightly moved

intracellular activity of peroxidases and anthracene dissipation by mycorrhizal roots was established. Peroxidases (EC. 1.11.1.7, donor: $H_2O_2$ oxidoreductase) are ubiquitous. These oxidative enzymes are known to be able to oxidize various xenobiotics in fungi (Bumpus 1989; Haemmerli et al. 1986). They oxidize a variety of phenolic compounds and were implicated in many different physiological processes, such as incorporation of phenolics into cell walls, lignification, wound healing, and pathogen defense (Gaspar et al. 1991). The positive effect of mycorrhization may not be restricted to plant protection but also contribute to PAH dissipation. Many fungi are known as potent mediators of PAH dissipation due to the action of their oxidoreductases (Gramss et al. 1999; Rama et al. 1998; Rama-Mercier et al. 1998), but their growth in soil may be slow and limit their action (Eggen and Sveum 1999). The use of symbiotic fungi growing independently of carbon availability in soil may thus be advantageous, and merit their inclusion in phytoremediation schemes.

Results from the present work indicate that the dissipation of anthracene from the culture medium of mycorrhizal and nonmycorrhizal chicory roots had another origin: accumulation in lipid bodies of plant cells and hyphae. Although it was suggested that lipophilic organic pollutants, including PAH, are strongly associated with the soil organic fraction and not expected to be susceptible to plant uptake (Binet et al. 2000; Wild and Jones 1992; Simonich and Hites 1995), plant uptake and accumulation of phenanthrene and pyrene from soils was recently demonstrated (Gao and Zhu 2004). Moreover, Gao and Zhu (2004) obtained results, which indicate a positive correlation between lipid contents and PAH concentrations in roots. Our study shows for the first time the presence of anthracene in lipid bodies of plant root and AM fungal mycelium, providing direct evidence of the uptake of this hydrophobic pollutant. It was demonstrated in previous studies that PAH accumulate in lipid bodies of *Fusarium solani*, a mitosporic fungus able to efficiently degrade PAH (Verdin et al. 2005). Lipid bodies interact with the endoplasmic reticulum where membrane-bound cytochrome P450 enzymes, known to be involved in PAH metabolism, are localized (Müllner and Daum 2004), and there is increasing evidence that lipid bodies may play important roles in various aspects of lipid trafficking (Murphy 2001). Bidirectional movement of lipid bodies along coenocytic AM fungal hyphae was observed where storage lipid bodies circulate from extraradical hyphae to intraradical fungal structures and vice versa (Bago et al. 2002). Consequently, AM fungal lipid bodies may participate in the intracellular translocation of anthracene in hyphae.

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