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INFLUENCE OF TWO POLYCYCLIC AROMATIC HYDROCARBONS ON SPORE GERMINATION, AND PHYTOREMEDIATION POTENTIAL OF *Gigaspora margarita-Echynochloa polystachya* SYMBIOSIS IN BENZO[*a*]PYRENE-POLLUTED SUBSTRATE

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Key words: arbuscular mycorrhizal fungi, PAH, rhizosphere

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are ubiquitous microorganisms that occur in contaminated soils. However, little is known about the responses of AMF with organic contaminants such as polycyclic aromatic hydrocarbons (PAH). The first objective of this study was to evaluate the influence of two PAH on spore germination of *Gigaspora margarita* Becker & Hall. Water-agar plates were contaminated with phenanthrene (PHE) and benzo[*a*]pyrene (BaP) at several concentrations: 0, 25 (0.1 mM BaP & 0.15 PHE), 50 (0.2 mM BaP & 0.3 mM PHE), 75 (0.3 mM BaP & 0.45 mM PHE), and 100 μg mL^{-1} (0.4 mM BaP & 0.6 mM PHE), respectively. The second objective consisted on the evaluation of the responses of the symbiosis between *G. margarita* and *Echinochloa polystachya* (H.B.K.) Hitch. to increased concentrations of BaP (0, 25 (0.1 mM), 50 (0.2 mM), 75 (0.3 mM), and 100 mg kg^{-1} (0.4 mM) under plant growth chamber conditions. Spore germination and hyphal length were drastically reduced by PHE. Reduction of spore germination was higher than 90% in presence of PHE. In presence of BaP, spore germination reduction was 42.8% when exposed at 100 μg mL^{-1} (0.4 mM). Spores that germinated in presence of 75 (0.3 mM) and 100 (0.4 mM) μg BaP mL^{-1} had greater hyphal elongation. BaP did not affect shoot dry mass of non-mycorrhizal or mycorrhizal *E. polystachya*. Mycorrhizal plants showed higher dehydrogenase activity in the rhizosphere soil at 0, 0.2 and 0.3 mM BaP, but reduced root polyphenol oxidase activity at 0 and 0.1 and higher at 0.3 mM BaP than non-mycorhizal plants. Dissipation of BaP was higher in non-mycorrhizal plants than mycorrhizal plants. *Echinochloa polystachya* showed an intrinsic capability on dissipating PAH from its rhizosphere.

Palabras clave: hongos micorrízicos arbusculares, HAP, rizósfera

RESUMEN

Los hongos micorrízicos arbusculares (HMA) son microorganismos cosmopolitas que se pueden encontrar en suelos contaminados. No obstante, pocos estudios se han enfocado a la evaluación de las respuestas de HMA ante contaminantes orgánicos

como los hidrocarburos poliaromáticos (HAP). El primer objetivo de este trabajo consideró la evaluación del efecto de dos HAP sobre la germinación de esporas de *Gigaspora margarita* Becker & Hall. Placas con agar-agua fueron contaminadas con fenantreno (PHE) o benzo[*a*]pireno (BaP) en diferentes concentraciones: 0, 25 (0.1 mM BaP y 0.15 PHE), 50 (0.2 mM BaP y 0.3 mM PHE), 75 (0.3 mM BaP y 0.45 mM PHE), y 100 μg mL^{-1} (0.4 mM BaP y 0.6 mM PHE), respectivamente. El segundo objetivo consistió en la evaluación de las respuestas de la simbiosis entre *G. margarita* y *Echinochloa polystachya* (H.B.K.) Hitch. ante la presencia de diferentes concentraciones de BaP (0, 25 (0.1 mM), 50 (0.2 mM), 75 (0.3 mM), y 100 mg kg^{-1} (0.4 mM), bajo condiciones de cámara de crecimiento. La germinación de esporas y longitud hifal fueron significativamente inhibidas por PHE, el cual produjo una disminución del 92%. En el caso de BaP, la germinación de esporas disminuyo en 42.8% ante 100 μg mL^{-1} (0.4 mM). Las esporas que germinaron en presencia de 75 (0.3 mM) y 100 (0.4 mM) μg BaP mL^{-1}, tuvieron mayor elongación hifal. La presencia del BaP no produjo efectos negativos en el peso seco de la parte aérea de *E. polystachya* con la inoculación o no del HMA. Las rizosferas de plantas micorrizadas presentaron mayor actividad deshidrogenasa ante 0, 0.2 y 0.3 mM BaP, pero reducida actividad polifenoloxidasa en la raíz ante 0 y 0.1, y mayor ante 0.3 mM BaP, en comparación con plantas no inoculadas. La disipación del BaP de la rizosfera fue mayor en plantas no inoculadas en comparación con plantas micorrizadas. *Echinochloa polystachya* al parecer tiene una capacidad intrínseca para disipar BaP de su rizosfera.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants that have toxic, mutagenic, and carcinogenic properties (Sutherland *et al*. 1995). To reduce human health risks for these compounds some innovative bioremediation techniques have been developed using phytoremediation (Reynolds and Skipper 2005). However, the extent of the dissipation/degradation of PAH depends on their physical and chemical properties. Benzo[*a*]pyrene is one of the most recalcitrant PAH due to its complex molecular structure, however its biodegradation has been enhanced by free aerobic microorganisms (Juhasz and Naidu 2000) and microorganisms inhabiting the rhizosphere of selected plant species (Newman and Reynolds 2004). High concentrations of PAH in petroleum-contaminated soils limit plant growth and survival by affecting water and nutrient uptake to the roots (De Jong 1980, Merkl *et al*. 2005). Studies have demonstrated that these stressful conditions may be alleviated by the association of plants with certain beneficial rhizosphere microorganisms that contribute to nutrient availability as well as to improve phytoremediation of soil pollutants (Sarand *et al*. 1998, Siciliano and Germida 1998). Microbial associations in the rhizosphere provide higher plant tolerance to inorganic and organic contaminants and contribute to detoxify and degrade soil contaminants (Johansson *et al*. 2004, Barea *et al*. 2005). Arbuscular mycorrhizal fungi (AMF) are ubiquitous microorganisms in most ecosystems and form a mutually beneficial symbiosis with the roots of approximately 80% of all extant plant species (Smith and Read 1997). Benefits of AMF to plants are often related with increased nutrition, water uptake, and enhanced tolerance and survivability to cultural and environmental stresses (Jeffries *et al*. 2003).

Phytoremediation of heavy metals can be enhanced by AMF (Meharg and Cairney 2000, Davies *et al*. 2001). However, some studies have demonstrated that the presence of heavy metals and petroleum hydrocarbons in soils are detrimental to the formation/expression of AMF associations with the roots of higher plants (Davies *et al*. 2001, Cabello 1997, Leyval and Binet 1998). Some species of AMF such as *Gigaspora margarita* Becker & Hall and *Acaulospora delicata* Walker, Pfeiffer & Bloss have been detected in soils chronically contaminated by petroleum in Veracruz, México (Varela *et al*. 2000). Arbuscular mycorrhizal fungal species isolated from petroleum-contaminated soils have been shown to be more efficient in promoting plant growth and nutrient uptake than introduced fungal species (Cabello 1999). In addition, plant survival and growth has been enhanced by the presence of AMF in PAH-contaminated soil (Leyval and Binet 1998). The establishment of AMF in the root system of some plants may represent an important biological process for the dissipation/degradation or alleviation of PAH toxicity (Binet *et al*. 2000, Joner *et al*. 2003). However, the role of AMF on the performance of plants utilized in

phytoremediation of petroleum hydrocarbons has not been well understood.

The objectives of this study were to evaluate the response of *Gigaspora margarita* spores to two PAH compounds (phenanthrene and benzo[*a*]pyrene) and to determine the response of *Gigaspora margarita-Echinochloa polystachya* symbiosis to the presence of benzo[*a*]pyrene in the rhizosphere.

MATERIAL AND METHODS

Spore germination response to phenanthrene and benzo[*a*]pyrene

Spores of *Gigaspora margarita* were collected from the rhizosphere of *Sorghum vulgare* L. trap cultures, and surface sterilized as described by Becard and Fortin (1988). Spores were maintained in microtubes at 4 °C during five days.

Four solutions of phenanthrene (PHE, 96% purity; Sigma-Aldrich® P-2558) using acetone as solvent, were prepared in order to obtain the following concentrations (μg mL^{-1}): 25 (0.15 mM), 50 (0.30 mM), 75 (0.45 mM), and 100 (0.60 mM). Four solutions of benzo[*a*]pyrene (BaP, 97% purity; Sigma-Aldrich® B-1760) were also prepared to obtain the following concentrations (μg mL^{-1}): 25 (0.1 mM), 50 (0.2 mM), 75 (0.3 mM), and 100 (0.4 mM). Each contaminant solution was sprayed on to individual petri dishes containing water-agar (1%). Non-contaminated petri dishes were used as a control treatment. After 48 hours, spores were placed on the water-agar surface. Each plate contained 20 spores, and four plates (n=4) were utilized for each treatment of contamination with either PHE or BaP.

Spores were incubated at 24 °C in the dark for 25 days. The number of germinated spores was evaluated under dissecting microscope. Germinated spores were gently extracted from the water-agar and set them on glass slides to evaluate the hyphal total length in each spore by using a light microscope Reichert Microstar IV, Model 410, and American Optical 10X micrometer.

Data were analyzed as a two-ways factorial experiment through the analysis of variance procedure, and Tukey´s test (a=0.05%) for means separation and multiple regression model were performed (SAS Institute, 2000).

Phytoremediation potential of *Gigaspora margarita-Echinochloa polystachya* symbiosis in a benzo[*a*]pyrene-polluted substrate

River sand was washed and sterilized by three hours of exposition to steam pressure (124.1 kPa, 121 °C). Sand was contaminated with benzo[*a*]pyrene (Sigma-Aldrich®, 97% purity) at doses from 0, 0.1, 0.2, 0.3 to 0.4 mM (0, 25, 50, 75, and 100 mg kg^{-1}, respectively). Contaminant solutions were prepared by the dilution of BaP in 50 mL of acetone. Then, the solution was applied directly to the sand and manually mixed until acetone evaporation. Contaminated sand with its respective concentration of BaP was kept under laboratory conditions during 24 h to allow the solvent evaporation. Glass-dark containers were filled up with 700 g of sand and watered with 100 mL of sterilized (124.1 kPa, 121 °C, 20 minutes) Long Asthon Nutrient Solution (LANS) modified to supply 11 μg mL^{-1} of phosphorus to avoid interference on the symbiosis establishment.

Rooted cuttings of *Echinochloa polystachya* (H.B.K.) Hitchcock (Poaceae) were transplanted to the containers and inoculated with 500 spores of *Gigaspora margarita* (AMF-plant). In addition, non-mycorrhizal (Non-AMF) plants were utilized as a control treatment.

Plants were grown in a growth chamber (28 °C, 75 % relative humidity; 12 h photoperiod; Biotronette 850H, Lab-Line Instruments Inc.), and watered approximately every seven days with a sterilized LANS for the duration of the study. Plants were harvested after 70 days. At this time, shoot dry mass, dehydrogenase (DEH) activity was measured at the rhizosphere (Casida *et al*. 1964), and root polyphenoloxidase (PPO) activity was assessed (Racusen and Foote, 1965). Tolerance of AM symbiosis to BaP was estimated by performing a vital stain technique to determine the fungal alkaline phosphatase activity in roots (Tisserant *et al*. 1993). Fractional infection of *G. margarita* was estimated microscopically as the intensity of colonization of the root cortex (Trouvelot *et al*. 1986).

The extraction of BaP from the rhizosphere sand (100 g) was performed with 200 mL of acetone (Sigma-Aldrich®, 99.5% purity) by the mechanical-shaking extraction procedure (Schwab *et al*. 1999). Extracts were purified by filtering through in a silica gel column, and concentrated to a volume about 3 mL at 60 °C. UV-Absorbance readings were taken at 297 nm (Soroka and Soroka, 2002) with a UV-VIS Hewlett Packard Spectrophotometer (ChemStation 8453). Calibration solutions with BaP were prepared at six concentrations ranging from 0 to 50 μg mL^{-1}. BaP-dissipation was expressed as percentage which was calculated by subtracting the final concentration of BaP (at time 70-days) to the initial recovered concentration of BaP (time zero) of each treatment.

A factorial (2^5) experiment with 10 treatments was set in a completely randomized design. There was one plant per container which was a single replication

(n=5). Statistical analysis was performed with the statistical analysis system (SAS Institute, 2000). Data were analyzed by variance analysis (ANOVA), and Tukey´s test (α =0.05) for mean comparison and multiple regression model were performed (SAS Institute, 2000).

RESULTS

Spore germination response to phenanthrene and benzo[*a*]pyrene

Spore germination was drastically reduced at all concentrations of PHE (0.15 to 0.6 mM). Concentrations ≥ 25 µg PHE mL^{-1} were detrimental to the germination of the *G. margarita* spores (**Fig. 1a**). Spore germination reduction by PHE was >92 % in comparison with the spore germination observed at non-contaminated medium (**Fig. 1a**). Hyphal growth and elongation were also significantly ($P \leq 0.01$) reduced by PHE (**Fig. 1b**).

Benzo[*a*]pyrene reduced spore germination percentage (**Fig. 2a**) at all concentrations but not to the same extent as PHE. For instance, spore germination showed a reduction > 40 % in the presence of 100 (0.4 mM) µg BaP mL^{-1} in comparison with the germination percentage obtained from non-contaminated spores (**Fig. 2a**). In contrast to the PHE effects, germinated spores showed non-significant enhanced hyphal elongation in response to this contaminant. The average longest hyphal lengths (69.2 and 102.0 mm) were observed at the two highest concentrations of BaP, 75 (0.3 mM) and 100 (0.4 mM) µg mL^{-1}, respectively (**Fig. 2b**).

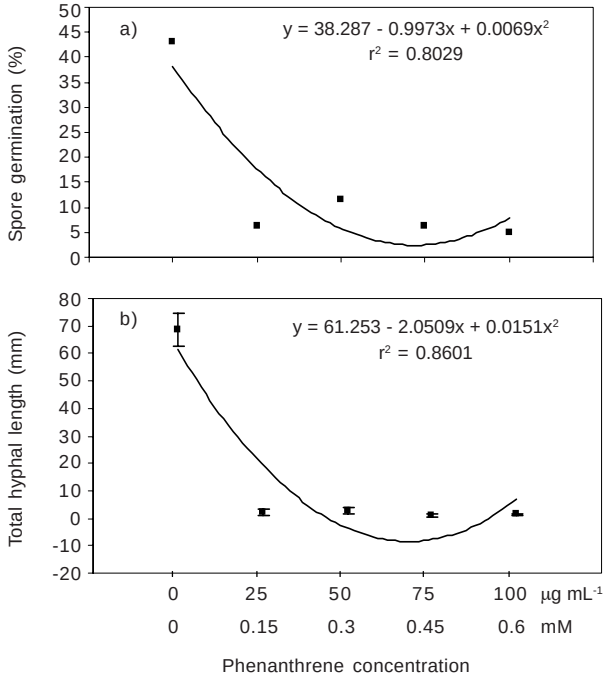

Fig. 1. Effect of phenanthrene concentrations on spore germination (a), and total hyphal length (b) of *Gigaspora margarita*, after 25 days of incubation. n=4. Means ± Standard Error

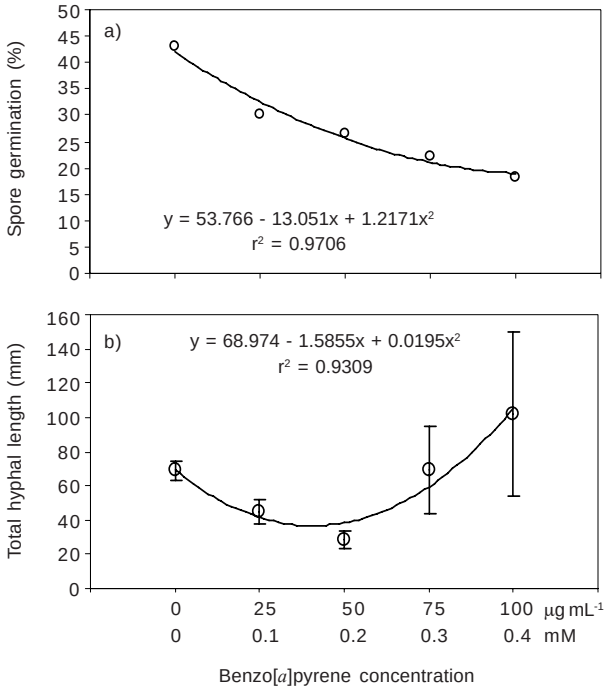

Fig. 2. Effect of benzo[*a*]pyrene concentrations on spore germination (a), and total hyphal length (b) of *Gigaspora margarita*, after 25 days of incubation. n=4. Means ± Standard Error

Phytoremediation potential of *Gigaspora margarita-Echinochloa polystachya* symbiosis in a benzo[*a*]pyrene-polluted substrate

Echinochloa polystachya roots were colonized by *G. margarita* when growing at BaP-contaminated substrate. Shoot dry mass was not significantly enhanced by the mycorrhiza nor reduced by BaP-contamination (**Fig. 3**).

Dehydrogenase activity was significantly ($P \leq 0.05$) higher in the rhizosphere of AMF-plants at 0, 0.2, and 0.3 mM BaP when compared to non-AMF plants (**Fig. 4a**). BaP-contamination did not significantly affect DEH activity of AMF-plants, which showed similar DEH activity than AMF-plants at non-contaminated substrate. No significant differences on rhizosphere

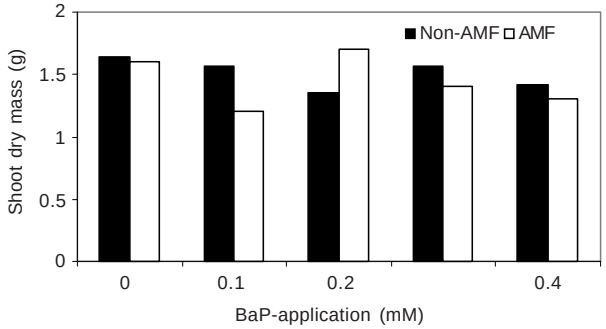

Fig. 3. Effect of benzo[*a*]pyrene (BaP) application on shoot dry matter of *Echinochloa polystachya* without (Non-AMF) or with the inoculation of *Gigaspora margarita* (AMF), after 70 days. n=5. No significant differences were observed among treatments at 5% level

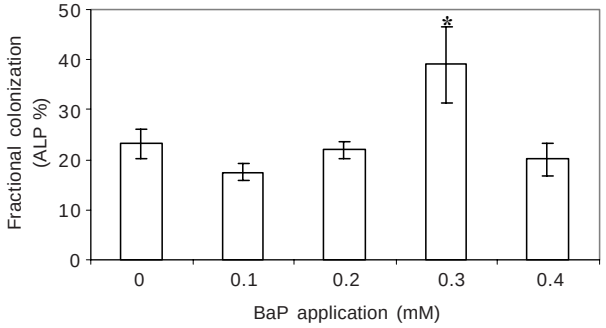

Fig. 5. Fractional infection of *Gigaspora margarita* in roots of *Echinochloa polystachya* exposed at several doses of benzo[*a*]pyrene (BaP) revealed as alkaline phosphatase vital stain, after 70 days. n=5. Means ± Standard Error. * Indicate significant differences among treatments at 5% level

soil DEH activity between AMF- and non-AMF plants were observed at 0.1 and 0.4 mM BaP (**Fig. 4a**). Root PPO activity was significantly (*P≤0.05*) diminished in AMF-plants in the presence of 0 and 0.1 mM BaP in comparison to non-AMF plants. However, AMF-plants show significant greater PPO (*P≤0.05*) than non-AMF plant exposed to 0.3 mM BaP (**Fig. 4b**). No significant differences on root PPO activity between AMF- and non-AMF plants were observed at 0.2 and 0.4 mM BaP (**Fig. 4b**).

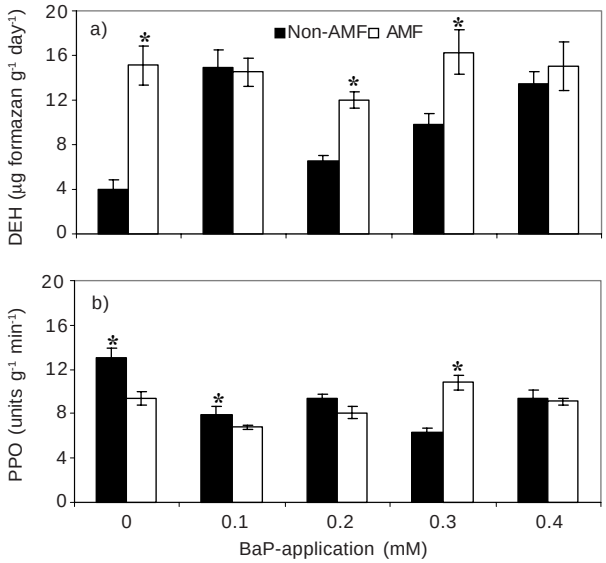

Fig. 4. Rhizosphere dehydrogenase (DEH) (a) activity and root polyphenoloxidase (PPO) activity (b) of *Echinochloa polystachya* with benzo[*a*]pyrene (BaP) contamination (Non-AMF) or with the inoculation of *Gigaspora margarita* (AMF), after 70 days. n=5. Means ± Standard Error. * Indicate significant differences between Non-AMF and AMF plants at 5% level

Arbuscular mycorrhizal symbiosis was not affected by BaP-contamination (**Fig. 5**). However, the application of BaP at 0.3 mM produced significant (*P≤0.05*) increases in AMF-colonization when compared to the colonization in AMF-plants at 0 mM BaP. Colonization was greater than 35%, as indicated by the fungal metabolic activity measured by alkaline phosphatase vital stain. No mycorrhizal colonization was observed in non-AMF plants.

Benzo[*a*]pyrene dissipation from the rhizosphere was significantly higher in non-AMF plants at 0.1, 0.2, and 0.3 mM BaP than AMF-plants (**Fig. 6**). No significant differences were observed in the dissipation of BaP between Non-AMF and AMF-plants exposed at 0.4 mM BaP (**Fig. 6**). In general, the extent of BaP-dissipation from the rhizosphere of either non-AMF or AMF-plants decreased as BaP-contamination increased (**Fig. 6**).

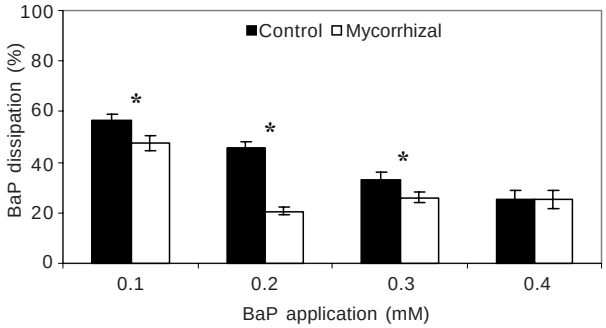

Fig. 6. Benzo[*a*]pyrene (BaP) dissipation from the rhizosphere of *Echinochloa polystachya* with or without the inoculation of *Gigaspora margarita*, after 70 days. n=5. Means ± Standard error. * Indicate significant differences between Non-AMF and AMF plants at 5% level

DISCUSSION

Spore germination response to phenanthrene and benzo[*a*]pyrene

This is one of the first reports detailing the response of *Gigaspora margarita* spore germination to the presence of phenanthrene and benzo[*a*]pyrene. Spore germination was significantly reduced by all tested concentrations of PHE and BaP. However, although no significant differences were observed among treatments, hyphal length was stimulated by the presence of BaP at 75 (0.3 mM) and 100 (0.4 mM) $\mu g\ mL^{-1}$.

Although not available information was found in the literature about the effects of petroleum hydrocarbons on spore germination, some studies have demonstrated that factors such as temperature, pH, soil moisture, root exudates, and presence of inorganic and organic chemicals may affect the spore germination, hyphal growth and branching from several AMF species (Tommerup and Briggs 1981, Tommerup 1984, Clark 1997, Chiocchio *et al*. 2000, Akiyama *et al*. 2005). It was observed that crystals of PHE had larger size when compared to crystals produced by BaP when applied to the water-agar (data not presented). The toxic effect of PHE to the spores may potentially be explained by the particle size of this contaminant allowing higher spore surface area covered by the crystals. Such exposure could have created unfavorable conditions related with dehydration of the surrounding zones of the spores due to the hydrophobic properties of PHE and other PAH (Cerniglia 1992). Thus, limiting spore hydration and consequently inhibiting its germination under this stressful condition. In this respect, arbuscular mycorrhizal spores have four developmental phases during germination, which appear to have different hydration requirements that may affect spore germination and hyphal growth/branching (Tommerup 1984). Based on the achieved spore germination from non-contaminated medium, we suggest that the presence of PAH on the medium may prevent the hydration of the spores, thus inhibiting or delaying germination. However, further studies are suggested to clarify the effect of PAH on both germination and viability of AMF spores.

Phytoremediation potential of *Gigaspora margarita-Echinochloa polystachya* symbiosis in a benzo[*a*]pyrene-polluted substrate

Petroleum hydrocarbons, including PAH, may retard plant growth by affecting water and nutrient uptake (De Jong 1980, Merkl *et al*. 2005), which may alter plant physiology and therefore reduce plant tolerance and survival to organic contaminants. Under these conditions, AMF may enhance plant water and nutrient uptake and subsequent growth and adaptation in contaminated substrates (Smith and Read, 1997). However, under our experimental conditions, BaP-contamination and AMF-inoculation did not significantly reduce or enhance plant growth.

Soil rhizosphere enzymatic activities have been proposed as indicators of contamination and detoxification of organic pollutant in soils (Gianfreda *et al*. 2005). In general, BaP-contamination and *Gigaspora margarita* inoculation resulted in modifications on the rhizosphere DEH and root PPO activities. Inoculation of *G. margarita* resulted in a 275 % increase in DEH activity when compared with non-AMF control plants (0 mM BaP). At the contaminated rhizosphere, the benefit of AMF inoculation on DEH was evident at 0.2 and 0.3 mM BaP. Inoculated plants had an 84 % and 66 % increase in DEH, respectively, when compared with their respective non-AMF plants. In addition, AMF-plants had a 68% increase in PPO activity at 0.3 mM BaP when compared to respective non-AMF plants. These results may be an indication of potential benefits of *G. margarita* on plants growing at BaP-contaminated substrate. It has been suggested that soil enzymatic activities including DEH and PPO, are affected by both concentration and type of PAH in the soil, and soil properties including organic matter content and pH (Ma *et al*. 2003, Baran *et al*. 2004). The DEH activity has been demonstrated to have more consistent response to organic contaminants, suggesting that it would be a sensitive indicator to soil contamination (Trasar-Cepeda *et al*. 2000). Dehydrogenase activity is recommended to assess overall microbial activities (Günther *et al*. 1996), and it may be an indicator of the activity of AMF in the rhizosphere contaminated with PAH.

This is one of the first reports detailing the mycorrhizal condition of *Echinochloa polystachya*. This plant has been experimentally utilized in phytoremediation of petroleum- and BAP-contaminated soil (Rivera-Cruz 2001). Benzo[*a*]pyrene did not adversely affect root colonization of *Gigaspora margarita* and its efficiency on phosphate transfer (Tisserant *et al*. 1993) from the contaminated soil to the roots, as indicated by the alkaline phosphatase activity of this fungus. However, it has been shown that AMF colonization is negatively affected by the presence of petroleum hydrocarbons as well as by mixtures or single PAH in soils (Cabello 1997, Leyval and Binet 1998, Gaspar *et al*. 2002, Liu *et al*. 2004).

The efficiency of BaP dissipation from the rhizo-

sphere of *E. polystachya* was dependent on the concentration of this PAH in the substrate as well as on the mycorrhizal condition. In the first case, *E. polystachya* contributed on 56 % and 45 % of BaP-dissipation at 0.1 mM and 0.2 mM BaP concentration in the substrate, respectively. This efficiency was decreased when plants were exposed to 0.3 and 0.4 mM whose BaP-dissipation extent was 33% and 25 %, respectively. In contrast, mycorrhizal condition of *E. polystachya* had lower BaP dissipation at 0.1 mM (47 %). At 0.2, 0.3 and 0.4 mM BaP, *Gigaspora margarita* inoculation significantly reduced the BaP-dissipation in the rhizosphere presenting in average 23.9 % of efficiency. These results are contrary to those reported for the inoculation of alfalfa with *Glomus caledomiun* in BaP-contaminated substrate (Liu *et al*. 2005). Although it has been demonstrated that AMF species from contaminated areas are more effective on stimulating plant growth and phytoremediation or petroleum hydrocarbons than introduced AMF species (Cabello, 1999), *Gigaspora margarita* (isolated from a chronically contaminated soil in Mexico, Quiñones *et al*. 2004) did not enhance either plant growth or BaP-dissipation from the rhizosphere of *E. polystachya*. It seems that differences on ecological adaptations of AMF and their host plant combination may result in weak or strong indirect benefits on phytoremediation of petroleum-contaminated soils as suggested by Cabello (2001) and Joner and Leyval (2003b).

Echinochloa polystachya seems to have an inherent root mechanism for the BaP removal, but it has not been clarified. Phytoremediation performance of PAH in soils is dependent on the synthesis and activity of oxidoreductases at the contaminated rhizosphere, which can be selectively enhanced by the presence of AMF (Criquet *et al*. 2000). Nakajima *et al*. (1996) suggested that plant cells may accumulate glycosil conjugates presumably derived from pyrene uptake by leaves or roots. However, Ryan *et al*. (1988) suggested that the extent of absorption of lipophilic compounds by roots is a complex but not significant process. However, more recently, Binet *et al*. (2000) demonstrated that some PAH can be either adsorbed to the root surface or slightly accumulated in both roots and shoots of *Lolium perenne* L. cv. Barclay. In summary, the fate of PAH-transformation products in plant tissue is still unclear and we suggested that plant species and ecotypes may be determinant factors on phytoremediation of PAH in soils.

In our experimental conditions, the inoculation of *Gigaspora margarita* did not induce more dissipation of BaP in the rhizosphere even though mycorrhizal plants showed more stable DEH and root PPO activities. There was no apparent correlation between enzymatic activities and BaP-dissipation in the rhizosphere of *E. polystachya*. Binet *et al*. (2000) reported that *Glomus mosseae* did not affect either plant growth or dissipation/degradation of selected PAH in the rhizosphere of *Lolium perenne*. Nevertheless, the ability of AMF-plants to dissipate/degrade PAH in the rhizosphere may be dependent on the mycorrhizal dependency of the host (Leyval and Binet, 1998). For instance, AMF-inoculation to alfalfa has been demonstrated to enhance the degradation of BaP (Liu *et al*. 2004), but it did not stimulate more PAH dissipation/degradation in the *Lolium perenne* rhizosphere (Binet *et al*. 2000).

Although AMF have not been demonstrated to posses a specific physiological mechanism to contribute directly on PAH-degradation, it is clear that *Gigaspora margarita* may influence plant tolerance under contaminated substrates with PAH. It has been proposed that AMF might reduce the root adsorption and thus, the toxicity of PAH (Binet *et al*. 2000) by unknown physiological fungal mechanisms. Due to the limited dissipation of BaP in the mycorrhizosphere of *E. polystachya*, it is suggested that AMF may contribute on the stabilization/sequestration of PAH in the soil. We concur with Gaspar *et al*. (2002) in suggesting that one probable mechanism for PAH stabilization is the accumulation of PHE in spores of AMF; furthermore we suggest that the external mycelium may also play a significant role in sequestration and accumulation of PAH. In addition, AM-fungal biomass may indirectly create favorable microhabitat for microbial activity (Rilling and Steinberg 2002), which may also stimulate hydrocarbon degradation. Furthermore, the induction of higher synthesis of oxidative enzymes responsible for the degradation or transformation of PAH by plants may represent another possible indirect benefit of AMF in contaminated rhizosphere (Joner and Leyval 2003a). However, further studies are required in order to clarify the physiological role of these symbiotic fungi on phytoremediation of organic contaminants, and to identify the physiological mechanisms of either AMF or AMF-plants, that contribute to improve their tolerance and adaptation under petroleum hydrocarbon-contaminated conditions.

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