Mycorrhiza (2001) 10:155–159 © Springer-Verlag 2001

ORIGINAL PAPER

Erik J. Joner · Corinne Leyval

Influence of arbuscular mycorrhiza on clover and ryegrass grown together in a soil spiked with polycyclic aromatic hydrocarbons

Accepted: 12 June 2000

Abstract The effect of arbuscular mycorrhiza (AM) on white clover and ryegrass grown together in a soil spiked with polycyclic aromatic hydrocarbons (PAH) was assessed in a pot experiment. The soil was spiked with 500 mg kg^{-1} anthracene, 500 mg kg^{-1} chrysene and 50 mg kg^{-1} dibenz(*a*,*h*)anthracene, representing common PAH compounds with three, four and five aromatic rings, respectively. Three treatments and two harvest times (8 and 16 weeks) were imposed on plants grown in spiked soil: no mycorrhizal inoculation, mycorrhizal inoculation (*Glomus mosseae* P2, BEG 69) and mycorrhizal inoculation and surfactant addition (Triton X-100). Pots without PAH were also included as a control of plant growth and mycorrhizal colonization as affected by PAH additions. The competitive ability of clover vis-à-vis ryegrass regarding shoot and root growth was enhanced by AM, but reduced by PAH and the added surfactant. This was reflected by mycorrhizal root colonization which was moderate for clover (20–40% of total root length) and very low for ryegrass (0.5–5% of total root length). Colonization of either plant was similar in spiked soil with and without the added surfactant, but the PAH reduced colonization of clover to half that in non-spiked soil. P uptake was maintained in mycorrhizal clover when PAH were added, but was reduced in non-mycorrhizal clover and in mycorrhizal clover that received surfactant. Similar effects were not observed on ryegrass. These results are discussed in the context of the natural attenuation of organic pollutants in soils.

Keywords Phosphorus uptake · Plant competition · Polluted soil · Polycyclic aromatic hydrocarbons · Toxicity

Paper presented at the COST 8.38 meeting Arbuscular Mycorrhizae and Plant Health under Abiotic Stress, Nancy, France

E.J. Joner (✉) · C. Leyval
Centre de Pédologie Biologique CNRS,
UPR 6831 associée à l'Université H. Poincaré,
17 rue N.D. des Pauvres,
B.P. 5, 54501 Vandoeuvre-les-Nancy Cédex, France
e-mail: joner@cpb.cnrs-nancy.fr
Tel.: +33-3-83510860
Fax: +33-3-83576523

Introduction

Polycyclic aromatic hydrocarbons (PAH) represent a group of persistent and toxic soil pollutants that are of major public concern due to their mutagenic and carcinogenic capacity (Wright 1980). Polluted soils may be poorly vegetated or completely void of vegetation, and plantations may be desirable to reduce wind erosion (Hetrick et al. 1994) and/or to enhance the degradation of organic pollutants through rhizosphere technology. The use of plants in PAH bioremediation schemes is still in its infancy, but positive effects of plants on the degradation of PAH have been observed (Schwab and Banks 1994; Reilley et al. 1996). Two strategies may be employed in bio- and phytoremediation: a costly high-input approach where, e.g., chemicals are added repeatedly to enhance PAH availability and plant growth (additions of, e.g., surfactants and fertilizers), or a low-input approach [natural attenuation, depending only on an intrinsic capacity for biodegradation and physicochemical mechanisms that decrease the pollutant concentration, see e.g. Brigmon et al. (1998)] where sowing may be the only intervention. In either case, grasses have been used extensively due to their fibrous root systems that provide a high root surface area that interacts with soil microorganisms to enhance degradation (Schwab and Banks 1994; Shann and Boyle 1994). Natural attenuation of pollutants should be favoured by mixed swards including N-fixing plants to provide N which is often limiting for both plant growth and microbial degradation of organic pollutants. Arbuscular mycorrhizae (AM) are known to enhance the competitive ability of N-fixing legumes in mixed swards (Hall 1978;

Buwalda 1980), but it is unknown if this may be achieved in polluted soils.

Leyval and Binet (1998) showed that AM colonization of some plant species was negatively affected by increasing additions of an industrial soil polluted with PAH, but not by similar concentrations of a single PAH (anthracene) added to an agricultural soil. Still, AM enhanced survival and plant growth in PAH-contaminated soils. It was, however, not checked whether the AM functioned with respect to P uptake nor to what extent PAH solubility affected toxicity.

The objectives of the present study were thus to investigate the effects of AM on the growth and P uptake of clover and ryegrass grown together in a soil artificially contaminated with PAH. Mycorrhizal effects were assessed at two growth stages on plants exposed to a mixture of three common PAH. A treatment where a surfactant was added was included to evaluate the effect of enhanced bioavailability/toxicity of PAH on mycorrhizal performance.

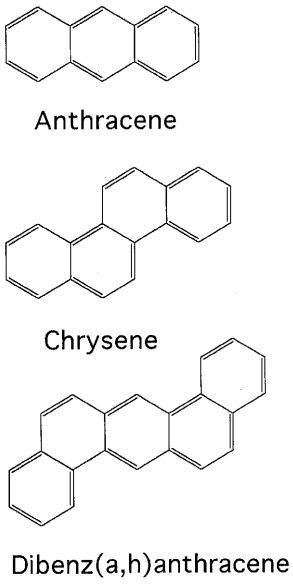

Fig. 1 Structural formulas of the added polycyclic aromatic hydrocarbon compounds

Materials and methods

The experiment had an unbalanced factorial design with three main treatments (no mycorrhizal inoculation, mycorrhizal inoculation and mycorrhizal inoculation plus surfactant addition, all with PAH-spiked soil), two harvest times (8 and 16 weeks) and five replicates per treatment. In addition to this full factorial part, three replicate pots were included to monitor mycorrhizal effects in soil without PAH.

An agricultural topsoil (Table 1) was sieved to <2 mm, partially sterilized (27 kGy gamma rays) and left unamended or spiked with 500 mg kg^{-1} dry soil of anthracene and chrysene, and 50 mg kg^{-1} dry soil of dibenz(*a*,*h*)anthracene (Fig. 1). Soil microorganisms were reintroduced by adding 50 ml kg^{-1} of a soil suspension (50 g of the original soil plus 1 l water blended in a Waring blender for 30 s, sieved to 20 μm and mist-sprayed onto soil during mixing). The spiked soil was then stored for 4 weeks for stabilization and ageing of the PAH amendment. Mycorrhizal pots were inoculated with 500 spores of *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe, P2 (BEG 69; Weissenhorn et al. 1993), from pot cultures with leek. Pots were sown with two pre-germinated seeds of ryegrass (*Lolium perenne*, cv. Barclay) and white clover (*Trifolium repens,* cv. Grasslands huia) and thinned to one plant of each species after 10 days. After seedling emergence, half of the mycorrhizal pots were drenched with a non-ionic surfactant (Triton X-100, 0.15 g kg^{-1}; Sigma) to reach 60% of water holding capacity (WHC). The pots were kept in a growth chamber at 21 °C, 60–70% relative humidity with 300–350 μmol m^{-2} s^{-1} photosynthetically active radiation and watered to 60% of WHC 2–7 times $week^{-1}$.

Shoots and roots were harvested 8 or 16 weeks after seedling emergence. Shoots were dried, weighed, ground, digested (using concentrated HNO_3, pressure vials and a microwave oven) and analysed for P (using an inductively-coupled plasma atomic emission spectrometer, Jobin Yvon JY 32). Roots were dried and weighed after sampling for measurements of root length and AM colonization. Total and colonized root length were determined using a line intersect method (Kormanik and McGraw 1982; Tennant 1975), which distinguished between clover and grass roots on the basis of morphology.

Treatment effects were tested by ANOVA and means were compared by the Newman-Keuls' test for unplanned comparisons.

Table 1 Characteristics of the spiked soil

Sand (%)	70
Silt (%)	19
Clay (%)	11
Organic matter (%)	2.54
pH ($CaCl_2$)	6.6
Olsen-P (mg P kg^{-1})	26

Results

Shoot dry weight was 100% and 30% higher for mycorrhizal clover at 8 and 16 weeks, as compared to non-mycorrhizal clover, respectively, whereas shoot dry weight of ryegrass was not affected at either harvest (Table 2). Mycorrhizal inoculation resulted in approximately 20% of the clover roots being colonized at both harvest times, and 0.5% and 5% of ryegrass roots at 8 and 16 weeks, respectively (Table 3). Total root biomass was not affected by the mycorrhiza, but the root length density of clover was higher in mycorrhizal treatments at both harvests (Table 3). As a consequence, the proportion of clover was enhanced for both shoot biomass and root length density in the mycorrhizal treatments. P uptake was enhanced by mycorrhiza and reduced by PAH for clover, but not for ryegrass (Table 4). The P concentration in mycorrhizal plants was similar in soil with and without PAH.

The addition of a surfactant enhanced the concentration of soluble PAH in the soil solution by a factor of approximately 100 (results not shown) and reduced shoot dry weight, P uptake (first harvest only) and root length for clover, but did not affect any of these parameters for ryegrass. Root colonization (percent colonized

Table 2 Dry weight of shoots and roots of non-mycorrhizal (*NM*) and mycorrhizal (*Myc.*) plants grown in soil with or without polycyclic aromatic hydrocarbons (*PAH*) and a surfactant (*Surf.*). Values within a column followed by the same letter are not significantly different ($P<0.05$) according to Newman-Keuls' test ($n=5$). *Harvest 1* Harvest at 8 weeks, *harvest 2* harvest at 16 weeks

	Treatment	Shoot dry weight		Proportion of clover (%)	Total shoot dry weight (g)	Total root dry weight (g)	Root/shoot ratio
		Clover (g)	Ryegrass (g)				
Harvest 1	NM	0.182 a	1.199 a	13	1.381	0.210 a	0.15
	Myc.	0.355 b	0.955 a	27	1.310	0.205 a	0.16
	Myc. + surf.	0.222 a	0.990 a	18	1.212	0.201 a	0.17
	Myc. no PAH	0.604 c	1.028 a	37	1.632	0.227 a	0.14
Harvest 2	NM	1.169 d	6.085 b	16	7.254	2.139 b	0.29
	Myc.	1.785 e	5.621 b	24	7.406	2.221 b	0.30
	Myc. + surf.	1.650 e	5.232 b	24	6.882	2.317 b	0.34

Table 3 Root length density and AM colonization of clover and ryegrass. Values within a column followed by the same letter are not significantly different ($P<0.05$) according to Newman-Keuls' test ($n=5$). Abbreviations as in Table 2

	Treatment	Root length density (cm cm^{-3})		Proportion	AM colonization (%)	
		Clover	Ryegrass	Clover (%)	Clover	Ryegrass
Harvest 1	NM	2.2 a	7.3 b	23	0	0
	Myc.	3.2 c	6.5 b	33	21 a	0.4 a
	Myc. + surf.	2.3 ab	6.1 b	27	17 a	0.6 a
	Myc. no PAH	3.1 bc	3.9 a	44	42 b	0.1 a
Harvest 2	NM	2.8 abc	27.4 c	9	0	0
	Myc.	6.5 d	24.0 c	21	17 a	4 b
	Myc. + surf.	5.7 d	26.7 c	18	24 a	6 b

Table 4 P in shoots of clover and ryegrass. Values within a column followed by the same letter are not significantly different ($P<0.05$) according to Newman-Keuls' test ($n=5$). Abbreviations as in Table 2

	Treatment	P concentration (mg g^{-1})		P content (mg plant^{-1})	
		Clover	Ryegrass	Clover	Ryegrass
Harvest 1	NM	2.9 b	4.1 b	0.5 a	4.9 a
	Myc.	3.2 bc	4.2 b	1.1 b	4.1 a
	Myc. + surf.	2.9 b	3.8 b	0.6 a	3.8 a
	Myc. no PAH	3.5 c	4.3 b	2.1 c	4.4 a
Harvest 2	NM	1.8 a	3.1 a	2.1 c	18.5 b
	Myc.	2.0 a	3.1 a	3.6 d	17.2 b
	Myc. + surf.	1.9 a	3.1 a	3.1 d	16.0 b

root length) was not affected by surfactant addition for either plant species.

Pots without added PAH, but inoculated with AM, supported larger clover plants (shoots) with twice the amount of root colonization relative to the corresponding treatment with PAH. There was no difference in shoot P concentration. The only observed effect on ryegrass was that root length at the first harvest was enhanced due to PAH addition. Only inefficient (small and pale) nodules were observed on clover roots, but these were not quantified.

Discussion

The present experiment demonstrated that AM may colonize and enhance the growth of white clover when grown in competition with ryegrass in a soil artificially contaminated by PAH. The decisive role of AM on plant biodiversity and inter-species competition is well documented for both natural (Grime et al. 1987; Gange et al. 1993) and disturbed ecosystems (Reddell and Milnes 1992; Smith et al. 1998), including mixed clover/ryegrass swards (Crush 1974; Crush 1995). This is the first indication that the competitive ability of clover may be enhanced in a similar manner in soil containing phytotoxic concentrations of PAH.

A toxic effect of PAH on clover was evident at the level of growth and AM colonization examined, and the negative effect on growth was aggravated by the addition of a surfactant that increased PAH solubility. Colonization and P concentration of clover were unaffected by enhanced PAH solubility, indicating that clover plants may be more sensitive to PAH than the AM fungus. In our previous studies (Leyval and Binet 1998) white clover and ryegrass were grown separately in a soil with a higher PAH content (4 g kg^{-1}) and comparable P content (20 mg $NaHCO_3$-extractable P kg^{-1}). In that case both plant species benefited to the same extent from forming AM with the same fungus as used here; the colonization of ryegrass was higher than in the present experiment (6% after 40 days) and the corresponding mycorrhizal growth enhancement reached a maximum of 50%. This benefit from forming AM was likely due to the amelioration of other types of stress than P deficiency, as indicated by the absence of any influence of AM or PAH on growth and P uptake of ryegrass in the present experiment.

A possible advantage conferred by AM in PAH-contaminated soils is that AM plants may subsist better under water stress (e.g. Sanchez-Diaz and Honrubia 1994). PAH-contaminated soils are more or less hydrophobic, and thus plant growth may be limited by water uptake and access to mineral nutrients which are dissolved in inaccessible soil water (Wilson and Jones 1993). In addition, indirect effects of AM acting through modified root architecture (Hooker and Atkinson 1996), improved membrane integrity due to improved P status (Graham et al. 1981) or enhanced production of oxidative enzymes (Salzer et al. 1999) may improve the performance of AM plants in the presence of organic pollutants.

In the context of natural attenuation of organic pollutants in soils, the use of N-fixing and/or mycorrhizal symbionts could potentially provide nutrients and other growth factors that in their absence would require repeated fertilization or result in poor plant establishment. A high proportion of mycotrophic and/or N-fixing plants in a mixed sward potentially offers other benefits related to bioremediation: N-fixing plants may increase soil N levels, a factor that often limits microbial degradation of organic pollutants (Morgan and Watkinson 1989). Members of the *Leguminosae* have been shown to have extracellular root peroxidases with particularly high activity (Gramss and Rudeschko 1998), a group of enzymes that are capable of oxidizing PAH (Cavalieri et al. 1983) and that show increasing activity upon AM colonization (Salzer et al. 1999).

AM inoculation enhanced the growth of clover relative to ryegrass, as well as the degradation of the added PAH above non-mycorrhizal controls in the present experiment (PAH data will be presented elsewhere). Though the process of PAH degradation and the associated mechanisms have not been identified, we claim that AM, through their impact on plant performance and on soil microbial community biology (see e.g. Andrade et al. 1997), improve conditions for PAH degradation, and thus constitute a biological factor that should be considered for the bioremediation of organically polluted soils.

Acknowledgements The soil used in the reported experiment was kindly supplied by Dr S.P. McGrath and co-workers. The experiment was part of a project funded by the European Commission (contract no. ENV4-CT97–0602).

References

Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ (1997) Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant Soil 192:71–79

Brigmon RL, Bell NC, Freedman DL, Berry CJ (1998) Natural attenuation of trichloroethylene in rhizosphere soils at the Savannah river site. J Soil Contam 7:433–453

Buwalda J (1980) Growth of a clover-ryegrass association with vesicular-arbuscular mycorrhizas. N Z J Agric Res 23:379–383

Cavalieri EL, Rogan EG, Roth RW, Saugier RK, Hakam A (1983) The relationship between ionization potential and horseradish peroxidase/hydrogen peroxide-catalyzed binding of aromatic hydrocarbons to DNA. Chem Biol Interact 47:87–109

Crush JR (1974) Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Growth and nodulation of some herbage legumes. New Phytol 73:743–749

Crush JR (1995) Effect of VA mycorrhizas on phosphorus uptake and growth of white clover (*Trifolium repens* L) growing in association with ryegrass (*Lolium perenne* L). N Z J Agric Res 38:303–307

Gange AC, Brown VK, Sinclair GS (1993) Vesicular arbuscular mycorrhizal fungi – a determinant of plant community structure in early succession. Funct Ecol 7:616–622

Graham JH, Leonard RT, Menge JA (1981) Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. Plant Physiol 68:549–552

Gramss G, Rudeschko O (1998) Activities of oxidoreductase enzymes in tissue extracts and sterile root exudates of three crop plants, and some properties of the peroxidase component. New Phytol 138:401–409

Grime JP, Mackey JML, Hillier SH, Read DJ (1987) Floristic diversity in a model system using experimental microcosms. Nature 328:420–442

Hall I (1978) Effects of endomycorrhizas on the competitive ability of white clover. N Z J Agric Res 21:509–515

Hetrick BAD, Wilson GWT, Figge DAH (1994) The influence of mycorrhizal symbiosis and fertilizer amendments on establishment of vegetation in heavy metal mine spoil. Environ Pollut 86:171–179

Hooker JE, Atkinson D (1996) Arbuscular mycorrhizal fungi-induced alteration to tree-root architecture and longevity. Z Pflanzenernähr Bodenkd 159:229–234

Kormanik PP, McGraw AC (1982) Quantification of vesicular-arbuscular mycorrhiza in plant roots. In: Schenck NC (ed) Methods and principles in mycorrhizal research. American Phytopathological Society, St Paul, Minn., pp 37–45

Leyval C, Binet P (1998) Effect of polyaromatic hydrocarbons in soil on arbuscular mycorrhizal plants. J Environ Qual 27:402–407

Morgan P, Watkinson RJ (1989) Hydrocarbon degradation in soils and methods for soil biotreatment. CRC Crit Rev Biotechnol 8:305–333

Reddell P, Milnes AR (1992) Mycorrhizas and other specialised nutrient-acquisition strategies – their occurrence in woodland plants from Kakadu and their role in rehabilitation of waste rock dumps at a local uranium mine. Aust J Bot 40:223–242

Reilley KA, Banks MK, Schwab AP (1996) Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. J Environ Qual 25:212–219

Salzer P, Corbiere H, Boller T (1999) Hydrogen peroxide accumulation in *Medicago truncatula* roots colonized by the arbuscular mycorrhiza-forming fungus *Glomus intraradices*. Planta 208:319–325

Sanchez-Diaz M, Honrubia M (1994) Water relations and alleviation of drought stress in mycorrhizal plants. In: Gianinazzi S, Schüepp H (eds) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhäuser, Basel, pp 167–178

Schwab AP, Banks MK (1994) Biologically mediated dissipation of polyaromatic hydrocarbons in the root zone. In: Anderson TA, Coats JR (eds) Bioremediation through rhizosphere technology. American Chemical Society, Washington D.C., pp 132–141

Shann JR, Boyle JJ (1994) Influence of plant species on in situ rhizosphere degradation. In: Anderson TA, Coats JR (eds) Bioremediation through rhizosphere technology. American Chemical Society, Washington D.C., pp 70–81

Smith MR, Charvat I, Jacobson RL (1998) Arbuscular mycorrhizae promote establishment of prairie species in a tallgrass prairie restoration. Can J Bot 76:1947–1954

Tennant D (1975) A test of a modified line intersect method of estimating root length. J Ecol 63:995–1001

Weissenhorn I, Leyval C, Berthelin J (1993) Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy-metal polluted soils. Plant Soil 157:247–256

Wilson SC, Jones KC (1993) Bioremediation of soils contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. Environ Pollut 88:229–249

Wright AS (1980) The role of metabolism in chemical mutagenesis and chemical carcinogenesis. Mutat Res 75:215–241