BIOLOGIA PLANTARUM 47 (4): 637-639, 2003/4

BRIEF COMMUNICATION

Effects of di-*n*-butyl phthalate on mycorrhizal and non-mycorrhizal cowpea plants

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Abstract

Cowpea (*Vigna sinensis* L.) plants were inoculated with arbuscular mycorrhizal fungus (*Acaulospora laevis*) to investigate the effects of different concentrations of di-*n*-butyl phthalate (DBP; 0, 10, and 100 mg kg^{-1}) added to soil on their growth. Mycorrhizal plants were less affected by high concentration of DBP (100 mg kg^{-1}) than non-mycorrhizal ones. Also the uptake and transformation of DBP by mycorrhizal plants differed from that of non-mycorrhizal plants.

Additional key words: *Acaulospora laevis*, root and shoot biomass, *Vigna sinensis*.

Di-*n*-butyl phthalate (DBP) is one of the commercial phthalate esters (PAEs), which are important and popular additives in many industrial products. However, PAEs are also environmental pollutants due to their hepatotoxicity (Seth 1982), mutagenicity (Kozumbo *et al.* 1982) and carcinogenicity (Kluwe 1982). Numerous studies have been reported on the biodegradation of PAEs in natural water (Taylor *et al.* 1981, Johnson and Heitkamp 1984, Walker *et al.* 1984), wastewater (Nozawa and Maruyama 1988, Wang *et al.* 1996) and soil (Inman *et al.* 1984, Wang *et al.* 1997). However, only few reports deal with the fate of PAEs in the soil-plant systems (Shea *et al.* 1982, Schmitzer *et al.* 1988, Yin *et al.* 2003), and to our knowledge no studies have been conducted in the soil-mycorrhizal plant systems.

Therefore, we chose DBP which is the most frequently identified PAEs in diverse environmental samples, as representatives of PAEs and designed this experiment to study the responses of mycorrhizal and non-mycorrhizal plants to the different concentrations of DBP added to soil.

Soil was collected from a farmland in Qinlin town (Nanjing, China), with pH 6.95, total N 1.6 g kg^{-1}, total P 1.47 g kg^{-1}, total K 18.4 g kg^{-1}, available P 50.5 mg kg^{-1}, soluble N 23.9 mg kg^{-1}, and initial DBP 2.3 mg kg^{-1}. Air-dried soil was passed through a 2 mm mesh, mixed, and artificially contaminated with increasing concentrations of DBP (0, 10, and 100 mg kg^{-1}). Soil moisture was adjusted to 60 % of its field capacity with sterile distilled water.

Arbuscular mycorrhiza (AM) fungus *Acaulospora laevis* was isolated from the Ecological Experimental Station of Red Soil (Yingtan, China) and propagated on white clover (*Trifolium repens* L) in a greenhouse for 4 months. Colonized clover roots and adhering soils were used as inoculum. Mycorrhizal inoculum was uniformly mixed with the soil in pots. Non-mycorrhizal treatments received the same amounts of autoclaved fungal agents.

Two 3-d-old cowpea seedlings were transferred to each pot containing 1 500 g soils. Pots were kept in a growth chamber with a temperature 22/18 °C (day/night), and a 16-h photoperiod at irradiance of 480 μmol m^{-2} s^{-1} (PAR; 400 - 700 nm). The plants were harvested 20, 40, and 60 d after transplanting, three replicates each time. The shoots were cut just above the soil surface. The roots were recovered by washing with distilled water. From

Received 27 January 2003, *accepted* 17 June 2003.

Abbreviations: DBP- di-*n*-butyl phthalate; PAEs - phthalate esters; AM - arbuscular mycorrhiza; NM - non-mycorrhizal; M - mycorrhizal; PAR - photosynthetically active radiation.

Acknowledgements: This study was supported by the National Key Basic Research Project of China (No. G1999011806). We appreciate the constructive comments from anonymous referee and Prof. Y.G. Zhu for improving the English manuscript.

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each pot a sample of *ca.* 1 g fresh root mass was taken for the measurement of mycorrhizal colonization. The shoots and other roots were oven dried at 70 °C and then ground for DBP analysis.

Mycorrhizal colonization rate was evaluated according to Koske and Gemma (1989). Analysis of DBP in shoot and root was performed using gas chromatography (Yin *et al.* 2003). Briefly, ground plant samples (2 g) were extracted in 60 cm^3 mixture consisting of acetone and petroleum ether (1:1, v/v). Splitting the extracts into two phases by adding 100 cm^3 6 % sodium sulfate solution, the organic layer was transferred to a Kuderna-Danish apparatus, and allowed to evaporate until the extract volume was reduced to 2 cm^3. The concentrated sample was cleaned on a column of activated *Florist PR 60/100* (*Silica Company*, Berkeley, USA). These samples were then analyzed by gas chromatography (*GC-9A* with a flame ionization detector, *Shimadzu*, Tokyo, Japan). Column material used was 10 % *SE-30* on chromosorb *WHP* packed in a stainless column (2 m, i.d. 4 mm). Conditions were as follows: oven 240 °C; detector 300 °C; nitrogen gas, 40 cm^3 min^{-1}. Two mm^3 of the solutions were injected and peak heights were compared with those standard solutions with known concentrations. The detection limit was 1 ng.

The percentage of mycorrhizal colonization decreased slightly at 10 mg kg^{-1} DBP compared with that at no addition of DBP, but it significantly decreased at 100 mg kg^{-1} DBP at the early growth stage (before 20 d) (Table 1). This could be due to the inhibition of spore germination. Also the length of hyphae produced by AM fungi in soil with 100 mg kg^{-1} DBP was less than that in soil with 0 or 10 mg kg^{-1} DBP (data not shown).

Table 1. Frequency [%] of non-mycorrhizal (NM) and mycorrhizal (M) roots of cowpea plants grown in soil artificially polluted with different concentrations of DBP (0, 10, and 100 mg kg^{-1}). Means ± SE, n = 3. Different letters in a column indicate significant difference between NM and M treatments at $P < 0.05$ determined by Duncan's test.

DBP [mg kg^{-1}]		20 d	40 d	60 d
0	NM	14.0 ± 2.00a	16.0 ± 1.76a	16.0 ± 0.88a
	M	32.5 ± 2.81b	34.0 ± 2.01b	36.0 ± 2.95b
10	NM	16.0 ± 1.46a	16.0 ± 0.85a	17.5 ± 1.62a
	M	30.0 ± 3.20b	30.0 ± 0.00c	35.0 ± 2.34b
100	NM	13.0 ± 1.47a	14.0 ± 1.09a	15.0 ± 0.85a
	M	24.5 ± 0.97c	31.5 ± 3.38bc	48.0 ± 4.12c

Table 2. Effects of mycorrhizal infection and applications of different concentrations of DBP (0, 10, and 100 mg kg^{-1}) on shoot and root biomass of non-mycorrhizal (NM) and mycorrhizal (M) cowpea plants. Means ± SE, n = 3. Different letters in a column indicate significant difference between NM and M treatments at $P < 0.05$.

DBP [mg kg^{-1}]		Shoot dry mass [g $plant^{-1}$]			Root dry mass [g $plant^{-1}$]		
		20 d	40 d	60 d	20 d	40 d	60 d
0	NM	1.24 ± 0.11a	3.06 ± 0.22a	4.60 ± 0.32a	0.63 ± 0.02a	0.80 ± 0.02a	1.09 ± 0.12a
	M	1.83 ± 0.05b	4.52 ± 0.19b	6.79 ± 0.60b	0.72 ± 0.05b	0.96 ± 0.05b	1.29 ± 0.06b
10	NM	1.17 ± 0.10a	2.96 ± 0.18a	4.02 ± 0.21c	0.56 ± 0.06ac	0.74 ± 0.03c	1.03 ± 0.04a
	M	1.70 ± 0.14b	4.36 ± 0.35b	6.38 ± 0.89b	0.68 ± 0.02b	0.88 ± 0.02a	1.14 ± 0.10ab
100	NM	1.02 ± 0.04c	2.60 ± 0.12d	4.42 ± 0.16a	0.47 ± 0.04c	0.75 ± 0.05c	0.93 ± 0.05c
	M	1.30 ± 0.18a	3.76 ± 0.11c	7.70 ± 0.29d	0.59 ± 0.05a	0.96 ± 0.04b	1.53 ± 0.15d

Table 3. Uptake of DBP by non-mycorrhizal (NM) and mycorrhizal (M) cowpea plants grown in soils added with different concentrations of DBP (0, 10, and 100 mg kg^{-1}). Means ± SE, n = 3. Different letters in a column indicate significant difference between NM and M treatments at $P < 0.01$. n.d. - not detectable, indicates DBP concentration was under detection limit (1 ng) or zero.

DBP [mg kg^{-1}]		Shoot DBP concentration [mg kg^{-1}(d.m.)]			Root DBP concentration [mg kg^{-1}(d.m.)]		
		20 d	40 d	60 d	20 d	40 d	60 d
0	NM	n.d*.	5.82 ± 0.43a	6.74 ± 0.21a	26.75 ± 2.12a	16.08 ± 1.19a	9.12 ± 0.29a
	M	3.68 ± 0.22 a	6.01 ± 0.62 a	6.20 ± 0.33 a	17.46 ± 0.66 b	10.44 ± 0.61 b	6.35 ± 0.38 b
10	NM	n.d.	11.43 ± 0.39 b	14.77 ± 1.05 b	70.69 ± 4.67 c	33.90 ± 1.32 c	20.98 ± 1.34 c
	M	7.25 ± 0.30 b	11.43 ± 0.59 b	11.26 ± 0.78 c	41.04 ± 2.56 d	24.60 ± 0.64 d	13.96 ± 0.88 d
100	NM	3.40 ± 0.18 a	16.30 ± 0.42 c	19.78 ± 1.43 d	123.67 ± 4.98 e	60.92 ± 2.85 e	35.04 ± 2.26 e
	M	8.31 ± 0.21 c	14.78 ± 0.37 d	14.35 ± 0.91 b	99.89 ± 5.02 f	38.74 ± 1.34 c	24.37 ± 1.05 c

Shoot and root biomass significantly decreased due to DBP treatment, especially at 100 mg kg^{-1} DBP (Table 2). The results did not agree with those of Shea *et al.* (1982), who found that maize heights and shoot mass were not significantly reduced at 200 mg kg^{-1} DBP in soil, and was significantly inhibited at 2000 mg kg^{-1} DBP. The difference in plants response to DBP concentrations was associated with difference in sensitivity of plants to DBP (Cai *et al.* 1994).

Inoculation with AM fungi significantly alleviated the inhibition effect of DBP on plant growth and increased shoot and root biomass compared with non-inoculated plants (Table 2), which indicated AM could promote plant growth in DBP-contaminated soil. Especially after 40 d, shoot and root biomass of mycorrhizal plants increased markedly, but shoot and root biomass of non-mycorrhizal plants increased slightly. Although shoot biomass of mycorrhizal plants was higher than that of non-mycorrhizal plants, DBP concentration in the shoot of mycorrhizal plants was higher than that of non-mycorrhizal plant at the early growth stage (before 20 d) (Table 3), which indicated that more DBP was uptake by the mycorrhizal seedlings. It was surprising, however, that the DBP translocation weakened after the mycorrhizal seedlings grew up (after 40 d). This is why DBP concentration in the mycorrhizal shoots increased only by 1.5 % from 40 d to 60 d, while by 29.2 % in the non-mycorrhizal shoots (Table 3). In roots, DBP concentrations in the mycorrhizal plants were always lower than that in the non-mycorrhizal plants. Tissue dilution also decreased DBP concentrations in the mycorrhizal shoot and root, because shoot and root biomass of mycorrhizal plants was significantly higher than that of non-mycorrhizal plants (Table 2).

In summary, mycorrhizal plants were less affected by high concentration of DBP, and had special mechanisms to control uptake and transformation of DBP, but the mechanisms need further investigations.

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