Bull. Environ. Contam. Toxicol. (1988) 40:626–632

Bulletin of Environmental Contamination and Toxicology

Effects of Phthalate Esters on Plant Seedlings and Reversal by a Soil Microorganism

Roderick Herring and Charles L. Bering

Department of Natural Sciences, State University of New York, College of Technology, Utica, New York 13504-3050

Phthalate esters have been widely used in industrial nations as plasticizers, in cosmetics, insect repellants, and munitions (Ribbons et al., 1984; Peakall, 1975). Due to the abundance of plastic materials in use, it has been estimated that 51 million pounds of phthalate esters leach into the environment annually (Ribbons et al., 1984). Phthalate esters have been found in many environmental samples and in tissues of higher animals (Peakall, 1975; Schneider, 1987), and the U.S. Environmental Protection Agency has classified the six most commonly occuring phthalate esters as Priority Pollutants (Keith and Telliard, 1979). The toxic effects of phthalate esters has been a topic of a recent symposium (Schneider, 1987). Although they do not appear to have significant acute toxicity or to be carcinogenic, there are reports of teratogenic effects in laboratory animals (Peakall, 1975). Phthalate esters are degraded by a number of soil and aquatic bacteria (Ribbons et al., 1984), but degradation is slow and may not keep pace with the increase in environmental contamination.

Very little has been reported concerning the effects of phthalate esters on green plants. Certain phthalate esters are known to be natural constituents of some plants (Peakall, 1975), but in significantly lower concentration than that which may be encountered due to contamination. We have examined the effects of four common phthalate esters- dimethylphthalate (DMP), diethylphthalate (DEP), dibutylphthalate (DBP), and di-(2-ethyl)hexylphthalate (DEHP)- added to the soil of developing spinach and pea seedlings. Two of the esters- DMP and DEP- caused significant inhibition of development.

Eaton and Ribbons (1982) have isolated and

Send reprint requests to C.L. Bering at the above address.

characterized a species of Micrococcus (sp 12B) from compost. This organism is capable of growth on several phthalate esters as sole carbon and energy source. When soil containing DMP or DEP was inoculated with Micrococcus sp 12B cells, some reversal of the inhibition was observed.

MATERIALS AND METHODS

Terra-Lite Potting Soil (W.R. Grace and Co., Cambridge, MA) was incubated at 170°C overnight to destroy indigenous microorganisms. One hundred grams of soil were added to each of several flats measuring 5" x 7" x 2". Phthalate esters were added to the soil by mixing the ester (100 µg) in 100 ml distilled water and pouring uniformly onto the soil. The moistened soil was thoroughly stirred. Five ml overnight cultures of Micrococcus sp 12B (kindly provided by D.W. Ribbons) grown to approximately 10^8 cells/ml in nutrient broth were added along with the ester to the appropriate flats. Thirty spinach seeds (Spinacia oleracea) were planted in each flat to a depth of 1". Fifteen to twenty pea seeds (Pisum sativum) were planted in appropriate flats. The plants were then grown under a bank of fluorescent lighting using a 12 hour light: 12 hour dark regime for 14-16 days at room temperature (23-25°C). At the end of the growth period the number of seedlings appearing and their heights were recorded. Photographs were taken of representative seedlings and their root structure. All of the experiments were repeated in triplicate.

The effects of phthalate esters on seed germination was tested by placing 15 pea or 25 spinach seeds in plastic Petri dishes. Each dish was filled with 20 ml of tap water and 0.01% to 0.1% phthalate ester. The phthalate esters were added as a methanol solution; a separate methanol control was also examined. The number of germinating seeds was recorded daily until no further change was observed (13 days).

RESULTS AND DISCUSSION

In initial screening experiments, spinach and pea seedlings were grown in the presence of 0.01% and 0.1% ester. These concentrations were similar to that found in digested municipal sludges (Inman et al., 1984). The four phthalate esters that were tested were DMP, DEP, DBP, and DEHP. At the end of the growth period, there were marked effects on the height of seedlings treated with DMP or DEP at the higher concentrations. Spinach seedlings also showed an altered physical appearance at the higher concentrations of DMP and DEP. However, neither spinach nor pea seedlings showed any

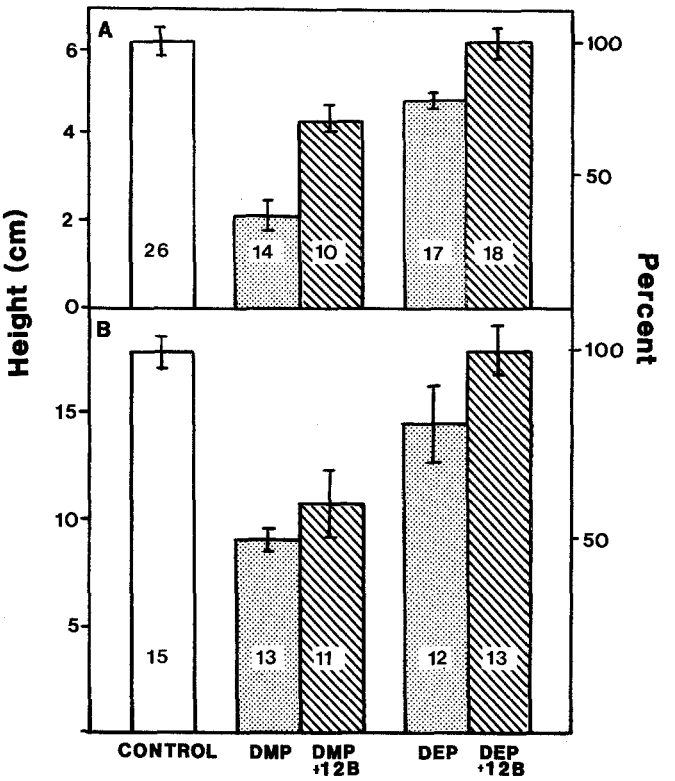

Figure 1. Inhibition of seedling growth with phthalate esters and reversal with Micrococcus sp 12B. Bars represent average height of seedlings in cm and expressed as percentage of control height. Standard error the the mean (SEM) is indicated as a line at the top of each bar. The number of seedlings (N) in each experiment is indicated by the number within each bar. A = spinach. B= peas.

significant effect when treated with DBP or DEHP even at 0.1%.

Figure 1 shows the results when seedlings are treated with 0.1% DMP or DEP. As can be seen, the average height of the spinach seedlings (Figure 1A) treated with DMP after 16 days was only 36% of that of the control. DEP-treated seedlings grew to 75% of the control value. Similar results were observed with the pea seedlings after 14 days. DMP-treated seedlings reached 50% of the control height, while the DEP-treated seedlings were 80% of the control. Although there was variation in these values from experiment to experiment, the same pattern was observed in repeats of

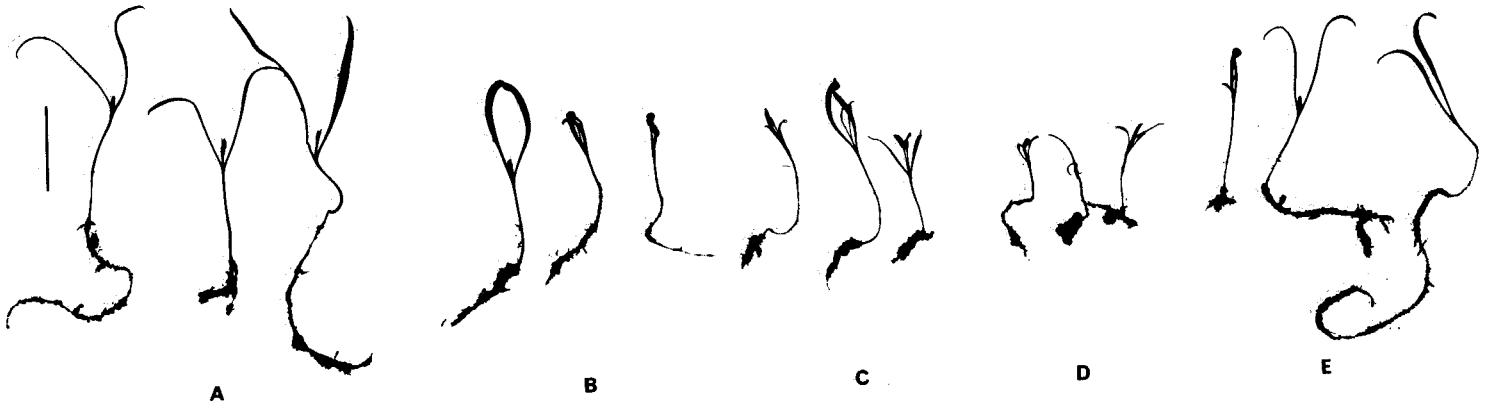

Figure 2. Representative spinach seedlings. 2A- Control. 2B- 0.1% DMP. 2C- 0.1% DMP + 12B cells. 2D- 0.1% DEP. 2E- 0.1% DEP + 12B cells. Bar on left represents 2 cm.

the experiment, using a total of 63 pea and 123 spinach seeds.

When the phthalate ester-degrading bacterium Micrococcus sp 12B was added to soil, some reversal of these effects was seen. Both pea and spinach seedlings showed a significant increase in average height in the presence of 12B. In the DMP-treated spinach seedlings Figure 1A), some reversal was observed. Average seedling height increased from 36% to 73% of the control value. The effect with DEP-treated seedlings was more dramatic in this experiment (75% to 100%). In the pea seedlings, reversal was also observed (Figure 1B). DMP-treated pea seedlings increased in average height from 50% to 59% of the control. In the DEP-treated pea seedlings, height increased from 80% to 100% of the control. Complete reversal was not always observed, but in every experiment, average peak height did increase when 12B cells were added to the soil.

Certain morphological changes were also observed in spinach seedlings when grown in the presence of DMP or DEP. Figure 2 shows representative seedlings after 16 days growth under various conditions. Figure 2A shows control seedlings, while 2B and 2D show seedlings treated with DMP and DEP respectively. In addition to the effect of the esters on seedling height, the spinach seedlings also exhibited several other physical differences from the control. First in every experiment a significant decrease in root length was observed. Second, many of the seedlings appeared desiccated during development, showing dried, yellow tips. Finally, the main leaves of several seedlings failed to bifurcate, as can be seen in Figures 2B and 2D.

Figures 2C and 2E show the effect of adding Micrococcus sp 12B to the soil with pea seedlings treated with DMP (2C) or DEP (2E). The increase in average height is apparent in the presence of 12B, as well as some increase in the size of the roots. Also, bifurcation of the growing tips is noted.

Table 1 shows the results of an experiment in which we soaked spinach or pea seeds in water or water plus 0.1% phthalate ester. Over a 13 day period, we recorded the percentage of seedlings which germinated. A reduced percentage of germinated seeds is observed in the phthalate ester-treated cases, but is far more pronounced with the pea seeds than with the spinach seeds. Only about half of the control spinach seeds germinated while 100% germination was observed with the pea seeds. Methanol did not appear to affect the percent germination. In addition, the seeds appeared

Table 1. Germination of seeds in water containing phthalate esters. Seeds were placed in water plus methanol (carrier solvent, control) or water plus 0.1% phthalate ester. The number of seeds which germinated was recorded daily until no further change was observed (13 days). Dishes contained 15 pea seeds or 25 spinach seeds.

Sample	% of seeds germinated
Peas	
Control + Methanol	100
0.1% DMP	67
0.1% DEP	48
0.1% DBP	52
0.1% DEHP	60
Spinach	
Control + Methanol	52
0.1% DMP	44
0.1% DEP	38
0.1% DBP	42
0.1% DEHP	25

to germinate at about the same rate over the course of the experiment. Although DBP and DEHP have some effect on germination, those seeds which do germinate are unaffected in later development, as we observed no effects of DBP and DEHP on seedling development. Seeds treated with DMP or DEP however not only show a reduced percent germination, but their development is also affected as seen by seedling morphology and average height (Figure 1). In these cases, the phthalate esters may also affect some later physiological process such as photosynthesis or transport of photosynthetically-produced materials. We are currently examining the effect of phthalate esters on photosynthetic parameters in spinach chloroplasts.

Our results clearly indicate an inhibitory effect on spinach and pea seedling development when grown in soil contaminated with two common phthalate esters. The concentrations which we used are not unlike those which may be found in the environment particularly near landfills or other areas where the esters may leach from plastic products. Our results with soil inoculated with *Micrococcus* sp 12B indicate possible reversal of this effect in the laboratory. However,

optimal conditions for complete reversal by 12B in the soil are difficult to determine and control. Nevertheless it does indicate that phthalate ester degradation may modify some of the more severe effects on seedlings which we observed.

It is unclear why DBP and DEHP showed no effects on seedling development. It is known that one environmental source of phthalate esters is plants (Peakall, 1975). One possibility might be that spinach and pea plants synthesize analogous phthalate esters, and are more tolerant to exogenous esters with higher alkyl chains.

REFERENCES

Eaton RW, Ribbons DW (1982) Metabolism of dibutylphthalate and phthalate by Micrococcus sp. strain 12B. J Bacteriol 151:48-57

Inman JC, Strachan SD, Sommers LE, Nelson DW (1984) The decomposition of phthalate esters in soil. J. Environ Sci Health B19:245-257

Keith LH, Telliard WA (1979) Priority pollutants. I- A perspective view. Environ Sci Technol 13:416-423

Peakall DB (1975) Phthalate esters: Occurence and biological effects. Residue Reviews 54:1-41

Ribbons DW, Keyser P, Kunz DA, Taylor BF, Eaton RW, Anderson BN (1984) Microbial degradation of phthalates. In: Gibson DT (ed) Microbial degradation of organic compounds. Marcel Dekker, New York. p 371-397

Schneider BF (1987) Proceedings of a symposium on recent advances in phthalate ester research. Toxicol Ind Health 3:1-227

Received November 2, 1987; accepted December 12, 1987.