EFFECT OF BENZOTRIAZOLES ON SUN-FLOWERS AND FESCUE

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ABSTRACT

Benzotriazoles are recalcitrant molecules used as corrosion inhibitors in antifreeze and deicing formulations. Remediation of deicing solution runoff to prevent damage to aquatic organisms has recently been recognized as a need at airports. Both the propylene glycol and the corrosion inhibitors are of concern, but this work is focused on the corrosion inhibitors. Plants that we are testing as remediation agents include fescue, a grass commonly planted at airports, and sunflowers, a rapidly growing annual with high levels of peroxidatic activity. Both species are negatively affected by water-applied benzotriazole and tolyltriazole (5-methylbenzotriazole). Severe cumulative toxicity is quickly observed at 0.1 mg/mL in the water, a level present in ~10 % deicer solution. Both species are somewhat more tolerant of lower applied levels of contaminant. Long-term experiments are in progress to determine the cumulative toxicity or contaminant degradation with different plant species.

Key words: *benzotriazole, tolyltriazole, deicer, sunflower, fescue*

INTRODUCTION

Tolyltriazole (MBz), one of several methyl substitution isomers, primarily at the 4 and 5 positions on the benzene ring, is commonly used in antifreeze and deicer formulations at levels of about 0.1% (1 g/L or 1 mg/mL) as applied to aircraft. The parent compound benzotriazole (Bz) is found in some formulations and is a by-product of hydroxybenzotriazole (HBz) oxidative reactions in a recently proposed paper-pulping process (Call and Mucke, 1997). In the process described, which is intended to be more environmentally benign than the usual alkali delignification of wood pulp, HBz would serve as a laccase free-radical mediator to facilitate breakdown of lignin. However, the environmental fate of the HBz and Bz has not been examined. There are no reports of bacterial degradation of the benzotriazoles in waste treatment processes, and benzotriazoles show toxicity to microbes at fairly low concentrations (USEPA, 1977). Aquatic organisms are known to be sensitive to low levels of benzotriazoles, for instance for fish, LC50 is around 30 mg/L (~3% deicer level).

We have reported that a white-rot fungus, *Phanerochaete chrysosporium,* is able to degrade both Bz and MBz (Wu et al., 1998). The mechanism of degradation is likely related to a Fenton oxidation, which is known to rapidly degrade Bz and MBz (unpubl. obs.).

For use *in situ* at airports, a plant-based remediation process would be desirable. Plants contain peroxidases essential for lignin formation and other metabolic processes and so may be able to attack benzotriazoles. Little is known of the relative toxicity of Bz, MBz, or HBz to higher plants (Klingensmith, 1961; USEPA, 1977), although derivitives have been developed as herbicides active at 1 kg/ha (Barton, 1990) and the parent compound Bz was identified early on as a plant growth regulator for tomatoes at levels ~0.1 mg/kg (2.5 mg/pot) (Davis, 1954). Male sterility in wheat has been induced by a single treatment with Bz at 50 mg/kg of soil, most likely via copper chelation (Graham, 1986). Thus there is known to be toxicity to plants at <5% deicer equivalent, similar to the threshold for toxicity to fish. When considering the potential environmental impact of benzotriazoles (Betts, 1999), one cannot ignore their effect on either terrestrial or aquatic plant life. Here we describe some efforts to determine the extent of plant tolerance and possible metabolism of benzotriazoles.

METHODS

Sunflowers (large-seeded cultivar of *Helianthus annuus*) were germinated in 100 g vermiculite with 0.5 L 1/4 strength Hoagland's solution and grown until two pairs of true leaves had emerged. They were then transplanted to provide five plants per pot, watered daily with 50 mL of the indicated concentrations of triazoles in the same nutrient solution for 20 days, under continuous light at 25°C.

Hybrid poplar (*Populus deltoides x nigra*, cv Imperial Carolina) cuttings were rooted in vermiculite and then tested with the contaminants at 0.1 mg/mL. In another experiment, 10 sunflower seeds were germinated in that same vermiculite with 0.1 mg/mL of the triazoles present, and their rate and extent of growth was determined with a 12-hr light/dark cycle at 23°C.

Fescue (*Festuca arundinacea* cv. K31) was grown with 12-hr light/dark cycles at 23°C for three weeks in vermiculite with one-quarter strength Hoagland's solution and then 12 seedlings were transplanted to individual pots, each with vermiculite and with 0.5 L of the solutions at various contaminant concentrations. Evaporative loss was ~50 mL/day. Plants were watered with the contaminant solution in one-quarter strength Hoagland's solution until the desired level of triazole input was obtained (10 days). Then plants were given distilled water for several weeks.

Chemicals were obtained from Fisher Scientific, except the benzotriazoles which were from Sigma/Aldrich. Analyses of benzotriazoles were done with 60% MeOH (ACS certified) as solvent on a 150 mm long x 4.1 mm diam. Hamilton PRP-1 column. Standards of contaminant at 0.1 mg/mL were prepared in water. Detection was by uV absorption at 275 nm. Flow rate was 1 mL/min, controlled by a Beckman HPLC. Samples (20 µL) of plant extract or watering solution were analyzed following a two-min centrifugation at 13,000 x g. Detection limits were below 0.01 mg/mL. Reducing the MeOH concentration improves detection sensitivity in the presence of plant phenolics which elute before the contaminants, but lengthens the analysis. At 40% MeOH, MBz takes about 20 min to elute.

Sunflower tops in the first experiment were harvested at the end of the treatment period, dried, and weighed. They were ground in a Wiley Mill to pass a 20-mesh sieve and portions were extracted with 60% MeOH.

Spiking experiments were done to determine the threshold for contaminant detection in plant tissue. Sorption to vermiculite was checked by long-term incubation of the triazoles with vermiculite in closed tubes at the same levels used in watering experiments.

RESULTS

Table 1 shows the effect of several levels of triazoles in the watering solution for sunflowers. Plants survived but those treated with Bz or MBz had their vertical growth severely inhibited shortly after application of the highest level of contaminant (0.1 mg/mL) was begun. At harvest they had relatively few, short roots. The dominant fraction of water loss was via the plants rather than evaporation for most treatments, and the final concentration of contaminant in the water of the pot was close to that of the input water. This suggests that contaminant was being taken up by the plants, or else it would have accumulated in the residual water. Control experiments showed very little sorption to vermiculite over a period of a month.

Attempts to detect the contaminants in the plants were unsuccessful. Spiking experiments with the dried plant material indicated that free contaminant within the plant, at a level found in the watering solution, would have been measurable.

Other experiments, not shown here, were done with horseradish or sunflowers grown with contaminant and extracted fresh, rather than dried. No contaminant was detected in these cases either. It appears that only very low levels of free contaminant are present in plants.

The effect of watering fescue with several levels of contaminant was similar to that with sunflowers. The concentrations used were one-half those used with sunflowers. Growth inhibition was similar with the plants treated at the highest levels of Bz and MBz (0.05 mg/mL) showing little new leaf growth after treatment was initiated.

With such small plants and just 12 seedlings per pot, evaporation was the dominant mode of water loss in most treatments for the first several weeks. The contaminant concentrations measured in the water 10 days after switching back to pure water as the watering solution were close to those expected from the input amount (nearly two-fold higher than the input concentration), but lower for those treatments with larger amounts of plant material present. At four weeks half of the HBz was gone from the solution while only one-third of the Bz and one-fourth of the MBz was gone. This is presumably because the contaminant was taken up into the plants. Plant material has not yet been analyzed for contaminant content. The plants continued to survive and those dosed with higher levels recovered to some extent as the contaminant disappeared from the soil solution.

Rooted hybrid poplar cuttings treated with 0.1 mg/mL of contaminant, either Bz or MBz, died. When sunflower seeds were germinated with 0.1 mg/mL level of contaminants, there was strong inhibition of growth. After 10 days, those treated with HBz appeared about like the controls, while the Bz and MBz seeds germinated but failed to elongate roots. After a month, the control and HBz treatments had seven plants 10-15 cm high with six true leaves, while in the Bz treatment the seven emerging plants had four leaves and were 5-8 cm high. The three emerging MBz-treated plants were only 3-5 cm high, with 2-4 leaves that were dark green and only 1-2 cm long. Several more seeds were alive but without elongated roots.

CONCLUSIONS

Both sunflower and fescue plants, once established, are able to survive treatment with triazole contaminants and appear to sequester it to a non-extractable form. Inhibition of growth is stronger for smaller plants.

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REFERENCES

Barton, J.E.D., 1990. Preparation of benzotriazole derivitives as herbicides, Braz. Pedido PI BR89 04,147 (cited in Chem Abs 114:#6515).

Betts, K.S., 1999. Airport pollution prevention takes off, Environ. Sci. Technol. May 1999, pp. 210A-212A.

Call, H.P., and I. Mucke, 1997. History, overview and applications of mediated ligninolytic systems, expecially laccase-mediator systems (lignozyme process), J. Biotechnol. 53, pp. 163-202.

Davis, D., 1954. Benzotriazole, a plant growth regulator, Science 128, p. 989.

Graham, R.D., 1986. Induction of male sterility in wheat using organic ligands with high specificity for binding copper, Euphytica 35, pp. 621-9.

Klingensmith, M.J., 1961. Effect of certain benzazole compounds on plant growth and development, Amer. J. Bot. 48, pp. 40-45.

USEPA, 1977. Investigation of selected potential environmental contaminants: benzotriazoles, EPA 560/2-77-001.

Wu, X., N. Chou, D. Lupher, and L.C. Davis, 1998. Benzotriazoles: toxicity and biodegradation, Proceedings of the Conference on Hazardous Waste Research, Snow Bird, UT pp. 374-382.

Table 1. Effect of benzotriazole treatment on sunflower growth[1].

Treatment(mg/mL)	Height (cm)	Weight (g)
Control	21	4.7
0.05 Bz	16	3.4
0.075 Bz	14	3.1
0.1 Bz	12	1.7
0.05 MBz	16	4.0
0.075 MBz	15	2.3
0.1 MBz	14	1.5
0.05 HBz	17	3.5
0.075 HBz	17	3.5
0.1 HBz	18	2.5

[1]Five plants per 600 mL pot were watered for 20 days with the indicated concentrations of benzotriazole (Bz), 5-methylbenzotriazole (MBz), or 1-hydroxybenzotriazole (HBz). Heights shown are the average for each pot. Weights are for the top portions only of the five plants in each pot.