Bull. Environ. Contam. Toxicol. (1997) 59:125-131


Bulletin of Environmental Contamination and Toxicology

# Aluminum Toxicity and Nutrient Utilization in the Mycorrhizal Fungus *Hebeloma mesophacus*

F.-X. Kong, Y. Liu, F.-D. Cheng

Department of Environmental Science and Engineering, Nanjing University, Nanjing, 210093, People's Republic of China

Received: 13 October 1996/Accepted: 11 March 1997

Associated with acidic precipitation caused by atmospheric pollution is the increased availability of metals in soil. Aluminum is one of the most important growth-limiting factors in acidic soils (Taylor 1989). The combined toxicity of aluminum at low pH values has received considerable attention in acidic precipitation studies.

Mycorrhizae are important in forests and are essential for the survival and growth of most forest tree species. Poor stands of seedlings are encountered in the absence of the fungus. The formation of mycorrhizae is particularly pronounced in soil low in phosphorus and nitrogen. Because most fungi are able to proliferate under acidic conditions, one can expect minimal effects on survival or growth of mycorrhizal fungi and, consequently, their association with a host plant under acidic conditions. However, this ability is not unlimited. There are indications that some elements, such as Al, Cu, Ni and Mn may be toxic to mycorrhizal fungi. Termorshuizen and Schaffers (1988) found that the symbiotic association between roots and soil fungi were poorly developed or absent in areas exposed to metal contamination. Therefore, the sensitivity of mycorrhizal associations to acid rain may contribute to the decline of forests.

Variations in response of some mycorrhizal fungus species to aluminum concentration were demonstrated by Thompson and Medve (1984) and Oelbe-Farivar (1985). Their results showed that aluminum was toxic to the mycorrhizal fungi *Cenococcum*, *Pisolithus* and *Thelephora* at 4 mg/L. However. *Suillus luteus* showed no significant growth reduction until the concentration of aluminum reached 300 mg/L. In these studies, the radial growth of mycelia on the agar was measured at the final widest diameter. A number of possible mechanisms by which Al might disrupt cellular functions have been proposed; one of these is that Al may induce mineral deficiencies. It was demonstrated that P nutrition can be altered by Ca and Al activity in forest soil and that Ca has a protective effect on Al and pH toxicity (Truman et al. 1986). There is little information, however, on

*Correspondence to:* F. -X. Kong

the physiological and biochemical responses of mycorrhizal fungi to aluminum at low pH.

The objective of this study was to investigate the effects of Al and Ca/Al ratios at a low pH on the mycorrhizal fungus *H. mesophacus.* After exposure to various Al concentrations at different ratios of Ca to Al at a low pH, growth and protein concentrations of mycelia were measured. From measurements of acid phosphatase and nitrate reductase, the enzymes associated with the nutrient utilization of P and N, a possible mechanism of Al toxicity to mycorrhizal fungi at low pH values is discussed.

## MATERIALS AND METHODS

*Hebeloma mesophacus* isolated from mycorrhizae of pine in the field was obtained from the Institute of Applied Ecology of Shenyang Branch of the Chinese Academic of Science. Mycelia were cultured in 250mL flasks containing 100 mL liquid modified Melin Norkrans (MMN) medium (Guttenberger and Hampp 1992). The flasks were placed on a rotator shaker at 28°C in the dark with continuous shaking (90 rpm).

The pH treatments were done by adjusting the pH of MMN solution with 1 mol/L HCl to pH 6.8 and 4.3 before autoclaving. Aluminum [as $Al_2(SO_4)_3$] was incorporated into MMN solution for each pH treatment. The theoretical concentrations of Al were at 0.0, 50, 80, 120, 200 and 310 mg/L. The flasks were inoculated with 1 mL of fresh homogeneous mycelium and incubated on the shaker for 7 d. At the end of experiment, the mycelia contained in each flask were collected by filtration, frozen in liquid nitrogen, and then dried and kept under vacuum at -30°C for 14 d before each was weighed. Four replicates were set up for each treatment.

In another experiment, phosphate and nitrogen starvation were created by incubation of mycelia in MMN medium lacking P and N before the treatment. After P and N starvation (72 hr), the mycelia were exposed to MMN culture medium at the two pHs for each of the Ca/Al ratios. Calcium was added after the median effective concentration ($EC_{50}$) of Al (data not shown) was determined (Donald 1985). The Ca/Al ratios (mmol/L) were 0/0, 0/3, l/3, l/l, 2/l and 3/l. The mycelia were inoculated, incubated for 7 d, collected and dried as above. The mycelia were weighed, homogenized with an ultrasonic meter and extracted in 1 mL of Tris/borate (0.1/0.3 mol/L pH 7.5), EDTA (5 mmol/L) and β-mercaptoethanol (7 mmol/L) buffer at 4°C for 5 min. The extract was then centrifuged at 10,000 x g for 10 min at 4°C. The extracts were stored at -30°C until the enzymes were measured.

Protein concentration in each sample was determined by the method of Lowry et al. (1951), with bovine serum albumin (BSA) as the standard. Phosphorus concen-

tration in mycelia was measured according to Nicholas (1985). Acid phosphatase (EC. 3.1.3.2) in P-deficient mycelia exposed to the two pHs for each of the Ca/Al ratios was assayed by measuring the liberation of p-nitrophenyl from substrate PNP-phosphate at 405 nm (Boller and Kende 1979). Nitrate reductase (EC. 1.6.6.2) was determined as Hageman (1980). Enzyme activity was expressed in U per mg soluble protein. All biochemical compounds used in this experiment were from Sigma (USA). Two independent experiments with 4 parallels each were carried out for enzyme assay.

## RESULTS AND DISCUSSION

The growth of *H. mesophacus* was inhibited significantly by aluminum over the range of concentrations tested ($P < 0.05$). The dry weight of mycelia per unit volume of culture (100 mL) declined with the increase of aluminum in the medium (Fig. 1a) at pH 6.8 and 4.3. Compared with the control (0 mg/L), the growth was reduced to 47 % at pH 6.8 and 27 % at pH 4.3, respectively, at aluminum concentrations of 120 mg/L. No fungal growth occured at concentrations of 300 mg/L or higher. In addition, the mycelial growth was extremely sensitive to aluminum when the pH was low. For example, the growth at pH 4.3 was 30 % of that at pH 6.8 at an aluminum concentration of 120 mg/L. These results suggest that lower pH values and higher concentrations of aluminum were toxic to *H. mesophacus.* Compared with other mycorrhizal fungi investigated, *H. mesophacus* showed some tolerance to aluminum toxicity. Aluminum was toxic to the mycorrhizal fungi *Cenococcum, Pisolithus,* and *Thelephora* at 4 mg/L. However, growth of *Suillus luteus* at 500 mg/L showed at least 65 % of growth compared with the control (Thompson and Medve 1984).

When Ca was added to the culture medium, the response of the fungus to aluminum at the low pH values changed. Among the treatments, the biomass in the group treated with Ca/Al of 0/3 was the lowest (Fig. 1b). In other words, Ca at all concentrations tested in this study had a protective effect on fungal growth to aluminum toxicity. However, the growth was still significantly reduced at pH 4.3 compared with that at pH 6.8. This suggested that amelioration of Al toxicity by Ca was influenced by the pH of the soil solution.

The effects of aluminum at the low pH values on the protein concentration in the mycelia of *H. mesophacus* was not as dramatic as that on the mycelial dry weight (Fig. 1c). Although the mycelial protein concentration was at its lowest level at 0/3, the difference was not as significant as compared with the control ($P < 0.05$). The differences found in protein concentrations between pH 4.3 and 6.8 was also not as obvious. Previous reports on the effect of metals on the protein concentration vary. Our results were contrary to those obtained by Oelbe-Farivar (1985), who demonstrated suppression of protein synthesis in fungi exposed to aluminum. The experimental data obtained by Assche et al. (1988) showed that protein concentrations increased in roots and leaves of plants after application of

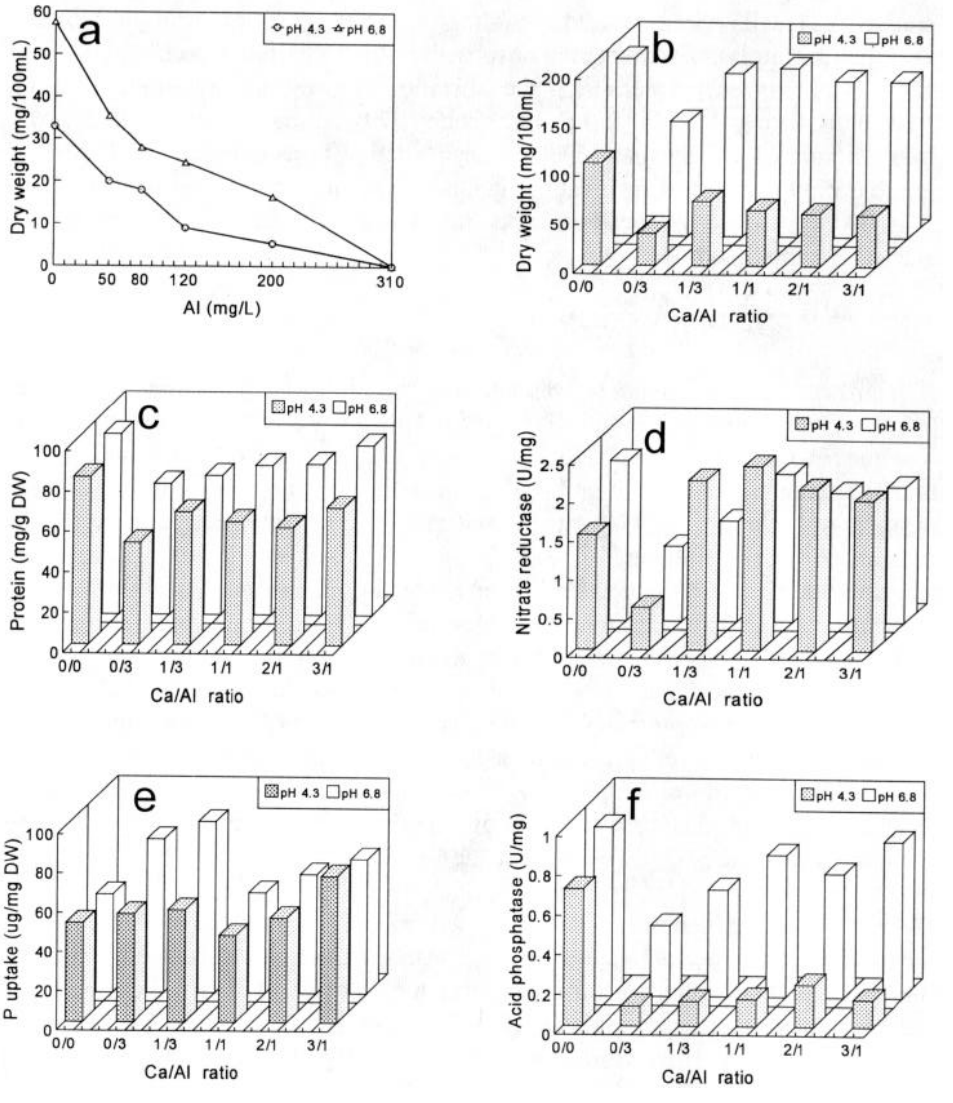


Figure 1. Effects of Al at two low pH values on the mycorrhizal fungus *H. mesophacus.*

Cd and Zn. In our experiment on the effects of Al, Cu and Mn on the mycorrhizal fungi *Cortinarius russus, Amanita muscaria,* and *Cenococcum geophilum* showed similar protein concentrations in mycelia to that in *H mesophacus* (Kong 1995).

Early studies suggested that low pH values and Al-stress disrupted $NO_3$- assimilation in plants. (Keltjens and Van Ulden 1987). Reduced nitrate reductase activity could be partially responsible for reduced $NO_3$- assimilation under Al stress. In our study, measurement of nitrate reductase indicated that enzyme activity was inhibited less at low pH values (Fig. 1d). There was no significantly difference between pH 4.3 and 6.8 ($P<0.05$). Zhou and Guan (1993) reported that the nitrate reductase was not affected at pH values higher than 3.0 in the mycorrhizal fungi *Pisolithus tinctorius, Lactarius insulsus,* and *Cortinarius russus,* but was reduced with acid rain below pH 2.0. The experimental data obtained in our study concurred with the results obtained from these mycorrhizal fungus species. However, nitrate reductase was sensitive to Al stress and showed the lowest activity with Ca/Al at 0/3 at both pH 4.3 and 6.8. Clearly, Ca had a protective effect here. With the increase of Ca/Al ratios, this enzyme was induced.

Measurement of P concentration in mycelia showed that there was no significantly difference between pH 4.3 and 6.8 ($P<0.05$), except at Ca/Al ratios of 0/3 and 1/3 (Fig. 1e). The P concentration in the control was the same as or lower than that in these two treatments with the addition of Al or Ca/Al. Calcium had no obvious effect on the Al toxicity. Taylor (1989) reported that an increase in soluble, inorganic P was observed in Al-stressed mycelia and possibly reflected the hydrolysis of P from phospholipids of the plasma membrane. Under acidic conditions and Al stress, this P pool decreased concurrently with a decline in acid phosphatase activity.

Acid phosphatase, an important enzyme associated with the P utilization in soil, was dramatically reduced by the application of Al at the low pH values (Fig. 1f). Obviously, this enzyme was injured by the Al. Early studies suggested that Al might induce mineral nutrient deficiencies that formed the basis of Al toxicity. Reduced acid phosphatase activity in Al-stressed plant roots has been reported (McCain and Davies 1984). Entry et al. (1987) hypothesized that such disruptions could arise from reduction in mycorrhizal associations. Some of the evidence was used to support the hypothesis that the toxic effects of Al could be the result of an Al-induced P deficiency. Al-stressed plants have shown increased concentrations of P in roots and decreased concentrations in shoots. The supply of P to cultural medium has a protective effect against Al injury (Bennet et al. 1987). The present experimental data provides direct evidence to support this hypothesis. Clearly, the variation in acid phosphatase activity and the concentration of P in plants roots may be related to the presence of the mycorrhizal fungus.

Fernandez (1989) proposed that N cycles altered by acidic deposition in most N-limited ecosystems had the potential to increase forest growth, but the increased N

availability in the soil solution could create element imbalances. Kong and Chen (1995) suggested that the balance between the availability of P and N that was optimum for plant growth was possibly disturbed when some metals were present. The measurement of the activity of enzymes associated with P and N utilization in mycorrhizal fungi may support these hypotheses.

Acknowledgment. This investigation was supported by National Natural Science Foundation of P.R.China

## REFERENCES

Assche FV, Cardinaels C, Clijsters H (1988) Induction of enzyme capacity in plants as a result of heavy metal toxicity: Dose-response relations in *Phaseolus vulgaris* L., treated with zinc and cadmium. Environ Pollut 52:103-115

Bennet RJ, Breen CM, Fey MV (1987) The effects of aluminum on root cap function and root development in *Zea mays* L. Environ Exp Bot 27:91-104

Boller T, Kende H (1979) Hydrolytic enzymes in the central vacuole of plant cells. Plant Physio 63 : 1123- 1132

Donald BP (1985) Toxicity test methods for aquatic organisms. In: Arnold EG, Trussell RR, Lenore SC (eds) Standard methods for the examination of water and wastewater. 16th ed. American Public Health Association, Washington DC, 689-823

Entry JA, Cromack J, Stafford SG, Castellano MA (1987) Effect of pH, aluminum, and sulfate on a high-elevation Armillaria isolate cultured in vitro. Can J For Res 17:865-871

Fernandez IJ (1989) Effects of acidic precipitation on soil productivity. In: Adriano DC, Johnson AH (eds). Acidic Precipitation, Vol 2. Springer-Verlag, New York, 6-83

Guttenberger M, Hampp R (1992) Ectomycorrhizins-symbiosis specific or artifactual polypeptides from ectomycorrhizas? Planta 188:129-136

Hageman RH, Reed AJ (1980) Nitrate reductase from higher plants. Methods Enzymol 69:270-280

Keltjens WG, Van Ulden PSR (1987) Effects of aluminum on nitrogen (ammonium and nitrate) uptake, nitrate reductase activity and proton release in two sorghum cultivars differing in aluminum tolerance. Plant Soil 104:227-234

Kong FX (1995) Influence of copper, manganese and pH on the growth and several enzyme activities in mycorrhizal fungus *Amanita muscaria*. Chemosphere 30:199-207

Kong FX, Chen Y (1995) Effect of aluminum and zinc on enzyme activities in the green alga *Selenastrum capricornutum.* Bull Environ Contam Toxicol 55:759-765

Lowry OH, Rosenbrough NR, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:263-275

McCain S, Davies MS (1984) Effects of pretreatment with phosphate in natural populations of *Agrostis capillaris* L.. II. Interactions with aluminum on the acid

phosphatase activity and potassium leakage of intact roots. New Phyto 196:589-599

Nicholas NC (1985) Determination of inorganic nonmetallic concentrations. In: Arnold EG, Trussell RR, Lenore SC (eds) Standard methods for the examination of water and wastewater. 16th ed. American Public Health Association, Washington DC, 437-453

Olelbe-Farivar M (1985) Physiologiche Reaktionen von Mykorrhizapilzen auf simuliierte saure Bodenbedingungen. Ph. D. thesis, University of Gottingen, Germany

Taylor GJ (1989) Aluminum toxicity and tolerance in plants. In: Adriano DC, Johnson AH (eds) Acidic Precipitation, Vol 2. Springer-Verlag, New York, 327-361

Termorshuizen AJ, Schaffers AP (1988) Factors causing the decline of fruit bodies of mycorrhizal fungi in the Netherlands. In: Mejstrik R (ed) Abstracts of the Second' European Symposium on Mycorrhizae, Praha, Czechoslovakia, 105-110

Thompson GW, Medve R (1984) Effects of aluminum and manganese on the growth of ectomycorrhizal fungi. Appl Environ Microbio 48: 556-560

Truman RA, Humphreys FR, Ryan PJ (1986) Effect of varying solution ratios of aluminum to calcium and magnesium on the uptake of phosphorus by *Pinus radiata.* Plant Soil 96:109-123

Zhou CL, Guan LD (1993) Effects of acidic rain on the enzyme activity in mycorrhizal fungi isolated from *Pinus massoniana*. In: Feng ZW (ed) Effects of Acidic Rain on the Ecosystem, Science and Technology Press of China, Beijing, 68-78