Effects of aluminium and pH on growth and potassium uptake by three ectomycorrhizal fungi in liquid culture

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Abstract

Soil acidification and Al toxicity may be important factors in the decline in vitality of many forest trees and the associated ectomycorrhizal fungal flora. In this study, effects of low pH and high Al concentrations were investigated on both growth and K⁺ uptake by three Douglas-fir ectomycorrhizal fungi in solution culture. In growth experiments, Lactarius rufus and Lactarius hepaticus appeared to be tolerant to low pH but sensitive to Al. In contrast, Laccaria bicolor exhibited high Al tolerance and high sensitivity to acidification. Al toxicity in both Lactarius isolates was alleviated by an increase in the orthophosphate concentration from 20 to 120 μM, whereas it was not influenced by an increase in Ca concentration. Elevation of the Mg concentration alleviated Al toxicity in Lactarius rufus, but not in Lactarius hepaticus, although growth was stimulated in this fungus.

Net K⁺ uptake by a 2-week-old (log phase) mycelium was determined as function of both Al and medium pH, at a K⁺ concentration of 100 μM. The fungal species each exhibited specific pH optima for K⁺ uptake. At pHs below 4, K⁺ uptake rate was decreased in each species. High Al concentrations severely inhibited K⁺ uptake in Lactarius hepaticus, but not in the other species.

Introduction

High deposition rates of NH₃ and NH₄⁺ on poorly buffered sandy soils may induce soil acidification and thereby high concentrations of dissolved Al (Van Breemen and Van Dijk, 1988). Both acidification and high Al³⁺ concentrations in the soil solution may be important factors in the decreasing vitality of forests. Many species of ectomycorrhizal fungi in these forests are also declining (Arnolds, 1988). Since Ulrich (1981) postulated that Al in the soil solution may be a primary factor in forest decline, many forest tree species have been screened for Al sensitivity in solution culture (Andersson, 1988).

Ectomycorrhizal trees often show better growth and improved resistance against unfavourable conditions than non-mycorrhizal trees (Harley and Smith, 1983). Information about the influence of mycorrhizas on the response of trees to low pH and high Al concentrations is scarce and can not be generalized (Cumming and Weinstein, 1990; Entry et al., 1987; Jentschke et al. 1991; Jones et al., 1986). This could be partly dependent on the fungal partner involved in the association. Ectomycorrhizal fungi exhibit differential response to Al (Thompson and Medve, 1984) which was also found with heavy metals (Jones and Hutchinson, 1988; Jongbloed and Borst-Pauwels, 1990a).

Al may induce deficiency of nutrients like Ca, Mg, P and K in plants (Andersson 1988). High concentrations of these nutrients are reported to reduce Al toxicity (Alva et al., 1986a, b; Kin-
raide and Parker, 1987). Both stimulated (Cumming et al., 1985; Lindberg, 1990) and reduced (Pettersson and Strid, 1989) K⁺ uptake in the presence of Al has been reported. Uptake of K⁺ by trees is enhanced by inoculation with ectomycorrhizal fungi (Boxman and Roelofs, 1988; Rygiewicz and Bledsoe, 1984) but information about the influence of mycorrhizas on the effects of Al and pH upon K⁺ uptake is lacking.

The first objective of this paper was to investigate the effect of various Al concentrations and pH in the nutrient medium on the growth of three ectomycorrhizal fungi, *Laccaria bicolor*, *Lactarius rufus* and *Lactarius hepaticus*, originating from Douglas-fir stands. Whether raised concentrations of Ca, Mg and phosphate could alleviate Al toxicity was also examined. The second objective was to investigate whether K⁺ uptake by these fungi is affected by both ‘acid rain’ factors.

**Materials and methods**

**Ectomycorrhizal fungi and growth conditions**

*Laccaria bicolor* (Maire) P.D. Orton, *Lactarius rufus* (Scop.) Fr. and *Lactarius hepaticus* Plowr. ap. Boud. were obtained from Dr. A.E. Jansen (Wageningen Agricultural University, The Netherlands). These isolates originated from Douglas-fir stands. Whether raised concentrations of Ca, Mg and phosphate could alleviate Al toxicity was also examined. The second objective was to investigate whether K⁺ uptake by these fungi is affected by both ‘acid rain’ factors.

**Growth experiments**

The effect of the pH on growth was determined by growing the fungi for 4 weeks in liquid 1/4-strength MMN medium containing 3 g L⁻¹ of malt extract at different pHs. The pH was kept constant by a 10 mM citric acid/Tris buffer at pH values ranging from 2.0 to 7.0. Erlenmeyers were filled with 80 mL nutrient medium and inoculated with 2 mycelial plugs from actively growing fungal cultures on agar plates. The liquid cultures were incubated at about 22°C on a rotary shaker at 95 r.p.m. in the dark.

For the Al growth experiments liquid 1/4-strength MMN medium was also used. However, the solution was not buffered in order to avoid a reduction in Al-ions by formation of complexes between citrate and Al. Furthermore, the phosphate concentration was lowered in order to prevent precipitation of insoluble aluminium phosphate and to reduce the complexing of phosphate with Al (Alva et al., 1986b; Lindsay, 1979). KH₂PO₄ was replaced by an equivalent amount of KCl. Furthermore, 1.5 g L⁻¹ instead of 3 g L⁻¹ of the malt extract (Difco), which contains some orthophosphate, was applied. The nutrient medium contained (μM): Ca²⁺ 85; Mg²⁺ 152; Fe³⁺ 11; NH₄⁺ 946; K⁺ 918; Na⁺ 107; SO₄²⁻ 152; Cl⁻ 2174 and 1.5 g L⁻¹ glucose and 1.5 g L⁻¹ malt extract containing 20 μM H₂PO₄⁻ (after sterilisation). AlCl₃ was added from a sterile stock solution at appropriate Al concentrations (0, 30, 100, 300 and 1000 μM). In studies of the effect of Ca, Mg and phosphate upon Al toxicity, CaCl₂, MgCl₂ or KH₂PO₄ were added from sterile stock solutions to the nutrient solution at final concentrations of 1 mM Ca, 1 mM Mg and 120 μM H₂PO₄⁻, respectively. The pH was adjusted to 3.5 with sterile diluted HCl. This pH value is near the average pH value in the upper 20 cm soil layer of a Douglas-fir stand at Kootwijk, The Netherlands, where *Laccaria bicolor* and *Lactarius rufus* originated from (Van der Maas and Pape, 1991). The 500-mL Erlenmeyers containing 200 mL of the appropriate solution were inoculated with three mycelial plugs of 6-mm in diameter and grown for 28 days at 22°C. On day 15 and 22 the solutions were renewed in order to prevent extreme medium acidification and phosphate depletion. The maximal acidification was 0.52, 0.31, 0.38 pH units in *Laccaria bicolor*, *Lactarius rufus* and *Lactarius hepaticus*, respectively. This acidification was independent of the Al concentration. In none of the treatments did depletion of H₂PO₄⁻ occur. The minimal H₂PO₄⁻ medium concentrations amounted to 3, 6 and 6 μM in cultures of *Laccaria bicolor*, *Lactarius rufus* and *Lactarius hepaticus*, respectively. In order to be sure that no Al was precipitated total Al concentrations in the liquid media were analyzed by inductively coupled plasma emission spectroscopy (ICP). They were found to be within 10% of intended levels, indicating that eventual precipitation was