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High aluminium tolerance among ectomycorrhizal fungi

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The aluminium tolerances of 12 ectomycorrhizal and 48 saprophytic fungi, mainly basidiomycetes were studied by cultivating aseptic cultures on MMN or ME solution to which aluminium sulphate, potassium aluminium sulphate or aluminium chloride was added. *Suillus luteus, S. variegatus, S. bovinus* and *Paxillus involutus* grew in concentrations over 10 g Al^{+++}/l. Species of *Amanita* and *Tricholoma* were more sensitive. Saprophytic species (*Mycena, Marasmius, Pleurotus*) had in general much lower tolerance, concentrations of 100–250 mg Al^{+++}/l being limiting. It is suggested that the high tolerance of mycorrhizal species can be regarded as an adaptation to naturally aluminium-rich conditions in acid forest soils.

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Introduction

The harmful effects of acidity and acid rains in forest soils are largely attributed to the toxicity of free aluminium (Al^{+++}) ions. Thompson and Medve (1984) have indicated that several mycorrhizal fungi display a high tolerance of aluminium in aseptic culture, and growth occurs in concentrations of 500 ppm Al^{+++}. In this paper, a similar and even higher tolerance of aluminium is reported among northern ectomycorrhizal fungi.

Material and methods

In the years 1984–1986 aseptic fungus cultures were isolated from fresh basidiocarps, collected in the vicinity of Helsinki, Finland, and kept on 1% malt extract (Difco) or MMN agar (Marx 1969) slants at ca. +5°C with transfers about twice a year. In the experiments, aluminium sulphate, $Al_2(SO_4)_3 \cdot 16H_2O$, Baker anal. reag. 0010, aluminium potassium sulphate, $KAl(SO_4)_2 \cdot 12H_2O$, Merck pro analysi, art. 1047, or aluminium chloride, $AlCl_3 \cdot 6H_2O$, Baker anal. reag. 002, was weighed into 100 or 250 ml Erlenmeyer flasks. The flasks were closed with cotton plugs and sterilized dry at 130°C for 1 h. Measured amounts of autoclaved hot MMN solution (Marx 1969) were pipetted aseptically onto the aluminium salt, and the salt was allowed to dissolve. In high concentrations $KAl(SO_4)_2$ sometimes later crystallized. An inoculum piece of MMN or ME agar with fungus mycelium was placed floating on the surface of the liquid. Saprophytic species were grown similarly on 1% malt extract (Difco) solution. Radial growth was measured after 14–52 days. The aluminium sulphate medium was very acid; in a concentration of 1 g Al^{+++}/l, the pH was 2.6, in 5 g/l it was 2.3 and in 10 g/l it was 2.1–2.2. Small amounts of precipitate were seen, evidently due to the malt extract. Analysis of the filtrate of the culture medium with an atomic absorption spectrophotometer indicated that the aluminium was in solution and not precipitated.

Results and discussion

The mycorrhizal fungi proved to be very tolerant of aluminium in both the aluminium sulphate and potassium aluminium sulphate solutions. Fig. 1 shows the growth of some species in MMN solution to which aluminium sulphate was added. Mycelial growth of *Suillus luteus* (L.) Gray, *S. variegatus* (Sw.) D. Kuntze, *S. bovinus* (L.) O. Kunze and *Paxillus invo-*

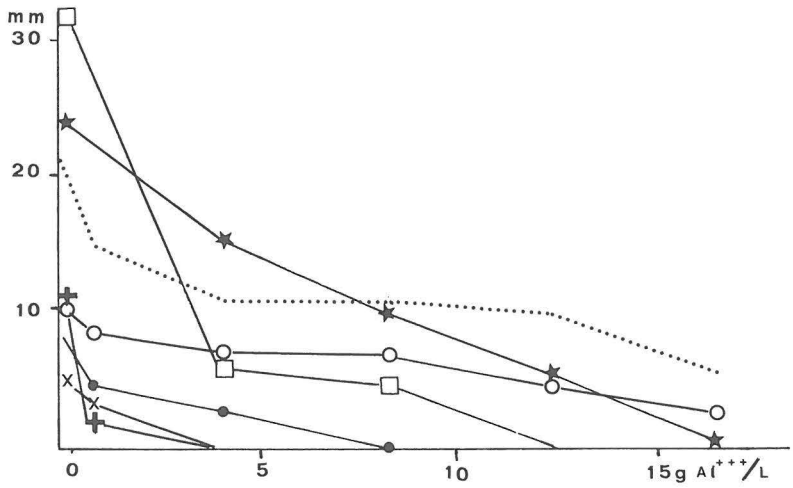

Fig. 1. Radial growth (vertical axis) of some ectomycorrhizal fungi in MMN solution to which $Al_2(SO_4)3 \cdot 16H_2O$ = was added. ..... = *Suillus variegatus*, o–o = *S. bovinus*, *–* = *S. luteus*, □–□ = *Paxillus involutus*, ·— = *Amanita muscaria*, x–x = *Leccinum testaceoscabrum*, and +–+ = *Lactarius rufus*.

lutus (Batsch) Fr. occurred in solutions with an Al^{+++} concentration of 15 g Al^{+++}/l, and was fairly good in a concentration of 5 g Al^{+++}/l. The latter solution contains about 100 g $Al_2(SO_4)_3 \cdot 16H_2O$ per litre (10%). *Cenococcum geophilum* Fr. formed new side colonies in this concentration. More sensitive fungi were *Amanita muscaria* (L.) Pers., *A. rubescens* Pers., *Tricholoma albobrunneum* (Pers.) Kumm., *T. pessundatum* (Fr.) Quél., *Lactarius torminosus* (Schaeff.) Pers., *L. deterrimus* Gröger and *L. rufus* (Scop.) Fr., their tolerances being 1–5 g Al^{+++}/l. On media containing potassium aluminium sulphate (Fig. 2) growth was approximately the same. Aluminium chloride proved to be more toxic, and growth was obtained for mycorrhizal species in concentrations of 0.5 g Al^{+++}/l, some growth also occurring in a concentration of 1 g Al^{+++}/l in cultures of the tolerant species.

Compared with the mycorrhizal species, the saprophytic basidiomycetes were much more sensitive (Fig. 3). A few species (*Pleurotus ostreatus*) did not grow in concetrations of 10 mg Al^{+++}/l, and the maximum concentration for the majority of the 48 species tested was below 100 mg Al^{+++}/l.

In the following saprophytic species mycelial growth did not occur in a concentration of 100 mg Al/l. The pH of this medium was 3.2–3.5.

Agaricus abruptibulbus Peck
Cerrena unicolor (Bull.) Murr.
Clavariadelphus ligula (Schoeff.) Donk
Coprinus atramentarius (Bull.) Fr.
Cudonia confusa Bres.
Flammulina velutipes (Curt.) Karst.
Fomes fomentarius (L.) Fr.
Fomitopsis pinicola (Sw.) Karst.

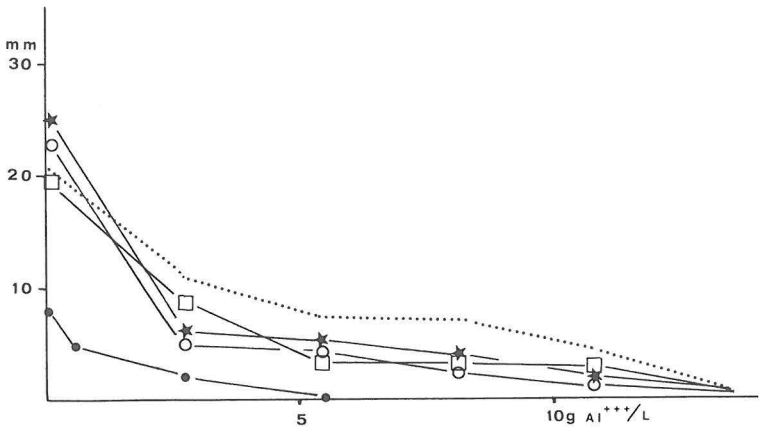

Fig. 2. Growth of some ectomycorrhizal fungi in MMN solution to which $KAlSO_4 \cdot 12H_2O$ was added. Symbols as in Fig. 1.

Geopyxis carbonaria (Pers.) Sacc.
Hohenbuehelia serotina (Schrad.) Sing.
Hypholoma capnoides (Fr.) Kumm.
H. fasciculare (Huds.) Kumm.
Ischnoderma benzoinum (Wahlenb.) Karst.
Kuehneromyces mutabilis (Schaeff.)
Marasmius bulliardii Quél.
M. scorodonius (Fr.) Fr.
M. urens Fr.
Mycena aurantiomarginata (Fr.) Quél.
M. clavicularis (Fr.) Gill.
M. galopus (Pers.) Kumm.
M. iodiolens Lund.
M. laevigata (Lasch) Quél.
M. metata (Fr.) Kumm.
M. niveipes Murr.
M. sanguinolenta (Alb. & Schw.) Kumm.
Phaeolepiota aurea (Matt.) Konr. & Maubl.
Phellinus chrysoloma (Fr.) Donk
Phlebiopsis gigantea (Fr.) Jül.
Pholiota heteroclita (Fr.) Quél.
Piptoporus betulinus (Bull.) Karst.
Pleurotus pulmonarius (Fr.) Quél.
Pycnoporus cinnabarinus (Jacq.) Karst.
Stereum hirsutum (Willd.) Pers.
S. sanguinolentum (Alb. & Schw.) Fr.
Tephrocybe tylicolor (Fr.) Moser
Trametes zonatella Ryv.

In the following saprophytic species the maximum concentration was 100–250(–500) mg/l:

Collybia asema (Fr.) Kumm.
C. dryophila (Bull.) Kumm.
Gymnopilus penetrans (Fr.) Murr.
Hapalopilus nidulans (Fr.) Karst.
Hygrophoropsis aurantiaca (Wulf.) Maire
Hypholoma sublateritium (Fr.) Quél.
Marasmius androsaceus (L.) Fr.
Micromphale perforans (Hoffm.) Gray
Mycena megaspora Kauffm.
Pholiota alnicola (Fr.) Sing.
Stropharia hornemannii (Fr.) Lund. & Nannf.
Tephrocybe palustris (Peck) Donk

There is evidently a clear difference in aluminium tolerance between saprophytic and mycorrhizal basidiomycetes, the maximum concentrations being about 100 mg/l and 10 g Al^{+++}/l, respectively. The difference might in certain cases be useful for separating mycorrhizal species from contaminating bacteria and fungal mycelia.

Comparison with the harmful concentrations of aluminium reported for other organisms shows that the saprophytic species tolerate about the same level as tree roots (Shier 1984, Simon & Rothe 1985, Arovaara & Ilvesniemi 1986, Göransson & Eldhuset 1987) and some other fungi (Firestone et al. 1983, Orellana et al. 1985) and about the level reported to allow mycorrhizal formation (Entry et al. 1987). Entry et al. (1987) give higher values for *Armillaria*. The aluminium tolerance of ectomycorrhizal species seems to be exceptionally high, at least among eukaryotic organisms. However, in the present experiments a mould, *Penicillium citrinum* Thom. (determined at CBS, Baarn), grew in the same concentrations of aluminium as mycorrhizal species. In view of the above results, aluminium cannot be regarded as an efficient poison for mycorrhizal species.

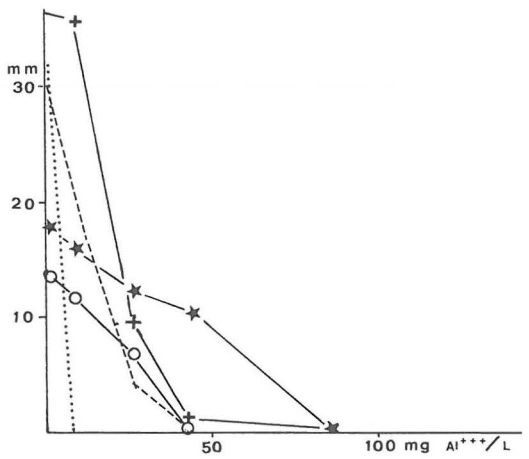

Fig. 3. Growth of some saprophytic basidiomycetes in ME solution to which aluminium sulphate was added. ..... = *Pleurotus pulmonarius*, +–+ = *Cerrena unicolor*, - - - - - = *Ischnoderma resinosa*, o–o = *Marasmius urens*, ★–★–★ = *Mycena galopus*.

The most tolerant mycorrhizal species (*Suillus luteus, S. variegatus* and *S. bovinus*) are characteristic of podzolic soils, the pH of which may be below 4. *Cenococcum* is known to be a species of extreme habitats (Meyer 1987). In acid soils aluminium becomes soluble naturally. Although the aluminium concentrations reported for soil solutions of forest soils are considerably lower (0.4 mM, Ulrich 1980, 0.2 mM, Nilsson & Bergkvist 1983), during dry periods the aluminium level may rise. Thus the high tolerance of mycorrhizal species may be interpreted as a natural adaptation to Al-rich conditions in highly acid soils.

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