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Aluminium resistance in various isolates of two saprophytic Basidiomycetes from a coniferous forest in South Norway.

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Summary. A total number of 20 strains of the two saprobic Basidiomycetes, viz. *Mycena metata* and *Galerina atkinsoniana*, was isolated from spores or fruitbodies. The samples were collected in experimental plots with different amounts of aluminium in the humus layer. The plots are located in South Norway in a Norway spruce forest. *Mycena metata* was more resistant to aluminium than *G. atkinsoniana*. No correlation was found between the resistance of the isolates and the content of aluminium in the humus. Thus, the present study does not support the hypothesis that fungal species can develop ecotypic metal-tolerant populations.

Introduction

Acidification of forest soils caused by deposition of anthropogenic substances results in mobilisation of aluminium ions which may appear in high concentrations in soil-water. Several species of ectomycorrhizal and saprophytic fungi are found to be resistant to soluble aluminium (Thompson & Medve 1984, Hintikka 1988, Jongbloed & Borst-Pauwels 1988, Høiland & Dybdahl 1993). Typical for these species is that they are confined to acid soils and are usually tolerant to air pollution and acidification (Høiland & Dybdahl 1993). It is suggested that fungi are selected for acid soils on the basis of their tolerance to aluminium toxicity (Thompson & Medve 1984).

Little is known about ecotypic adaptation to high metal content among populations within saprophytic fungus species. It may be suggested that such mechanisms are working on saprophytic fungi like those recorded for ectomycorrhizal fungi to copper, zinc, or arsenic (Colpaert & Assche 1987, Evans & Sylvester 1987) and vascular plants to zinc, lead or copper (e. g. Bradshaw 1952, Gregory & Bradshaw 1965, Høiland & Oftedal 1980, Ergon 1993). Genetic tolerance to aluminium has been demonstrated for *Triticum aestivum* L. (Foy & al. 1973, Takagi & al. 1983).

The aim of the present study has been to investigate aluminium resistance in various isolates of two saprophytic Basidiomycetes inconiferous forest with different amounts of aluminium in the humus layer.

Material and methods

The field work was carried out (6.10.1992) within the Solhomfjell forest reserve, Gjerstad Municipality, Aust-Agder County, South Norway (8°58′E, 58°58′N). The area is situated in the part of South Norway receiving polluted precipitation from Central Europe and where changes in soils and vegetation have been reported (e.g. Bjørnstad 1991). Samples of fungi were taken in permanent plots, originally established to study vegetation-environmental relationships of boreal coniferous forests (Økland & Eilertsen 1993). A total number of 100 plots (4×4 m) have been lain with 10 m intervals in eight transects within the forest reserve. (For further details about the design, see Økland & Eilertsen 1993) The fungus samples were taken in ten plots in transects No. 1 and 2.

The vegetation in the actual plots is *Picea abies* (L.) Karst. forest with a field layer dominated by *Vaccinium myrtillus* L. and a bottom layer dominated by *Hylocomium splendens* (Hedw.) Bruch, Schimp. & Gümb., *Pleurozium schreberi* (Brid.) Mitt., and *Sphagnum girgensohnii* Russ. The bedrock consists of gneissic granites with pegmatite intrusions (Børset 1979). (For more details, see Økland & Eilertsen 1993).

Exchangeable aluminium in the humus layer was determined as described by Økland & Eilertsen (1993: 24) at Landbrukets Analysesenter, Ås, Norway. Since two humus samples were taken per plot, the aluminium concentration *(x)* was assessed as the average of these two samples and converted from ppm (mg/kg dry sample) to fraction of organic content by multiplication with 100/LI (LI = loss of ignition), following Økland (1988). The distribution is log-normal (Økland & Eilertsen 1993). Therefore, the converted aluminium concentration, $x \cdot 100/LI$, was transformed to $ln\,(1 + x \cdot 100/LI) = Al(humus)$.

Sporophores of two terricolous saprophytic Basidiomycetes, *Mycena metata* (Fr.) Kumm. and *Galerina atkinsoniana* A.H.Smith were collected. Samples of *M. metata* (denoted MM) were from plots No. 15, 16, 18, 19, and 21 in transect No. 1, and plot No. 43 in transect No. 2; *G. atkinsoniana* (denoted GA) was gathered in plots No. 10, 17, and 21 in transect No. 1, and plots No. 39, 42, and 43 in transect No. 2. In plots No. 19 and 10 two parallel samples of sporophores of *M. metata* and *G. atkinsoniana* respectively were taken. Each sporophore was put into a small plastic bag and sealed immediately.

Figure 1 shows the aluminium content of the humus layer of those plots from which the fungi were isolated.

Mycelium cultures were established either from vegetative hyphae in sporophore tissue, preferably from the interior of stipe, (denoted VE) or from detached spores (denoted SP). They were cultivated on plates containing 30 ml 0.2% malt extract (Moss maltekstrakt), 0.05% Bacto-Peptone (DIFCO Labora-

tories), and 1.5% agar in 14 cm diam. Petri dishes. They were stored in darkness at about 10 °C for two months in an incubator.

The stock liquid medium was prepared by adding 15 g malt extract and 5 g Bacto-Peptone to 1 l distilled water. Afterwards $Al_2(SO_4)_3 \cdot 16H_2O$ (AnalaR, BDH Chemicals Ltd.) was added to obtain 0, 3.1, 5.2, 8.6, 14.4, and 24 mM aluminium. The actual concentrations follows a 3/5 dilution series starting at 24 mM. The media were sterilized in an autoclave at 121 °C.

The amount of growth of the isolates was indicated by the absorbance of light (450 nm) through micro-wells. (For details about methods and the amount of growth of the isolates was indicated by the absorbance of light (450 nm) through micro-wells. (For details about methods and equipments, see Høiland & Dybdahl 1993).) In the present investigation the absorbance values were obtained from a DYNATECH MR 5000 microplate reader conveying the data to a microcomputer. The growing period for all isolates was 7 days.

For estimating the response to aluminium, two indices were calculated:

The first index, $In_{8.6}$, is the ratio between estimated growth in the 8.6 mM Al treatment and growth in media without Al.

The second index, $\Sigma\%RBR$, is the mean relative growth rate *(RBR)* for each treatment, expressed as the percentage of the mean relative growth rate of the treatment without aluminium *(%RBR)*, summed over all treatments except the zero concentration which was always 100% (Snowden & Wheeler 1993).

$x_{[f]i}$ is the growth estimate in micro-well i for an actual Al concentration *[f]* (denoted by a figure or formula). n is the number of micro-wells per concentration value per fungus isolate, altogether 12 wells. $c_{[f]j}$ is the absorbance of light measured in control micro-well j (containing media without fungus) for the same Al concentration. m is the number of control micro-wells per concentration value per fungus isolate, altogether 4. (For details about arrangement of micro-wells, see Høiland & Dybdahl 1993).) k is the number in the dilution series from k=0 (24 mM Al) to k=4 (3.1 mM Al).

To see whether there is a correlation between aluminium response and content of aluminium in soil, the two indices were compared with *Al(humus)* using Spearman's rank-order correlation. They were also correlated with each other. To see whether there is a difference in response between the two species, the Wilcoxon rank-sum test for two groups was employed for both indices.

Results

Figures 2 and 3 show the values of $In_{8.6}$ and $\Sigma\%RBR$. Looking at $In_{8.6}$, MM19-2SP is the most sensitive, MM21SP the most resistant of the *M. metata* isolates, and GA17SP is the most sensitive, GA42VE the most resistant of the *G. atkinsoniana* isolates. Looking at $\Sigma\%RBR$, MM15SP is the most sensitive, MM21SP

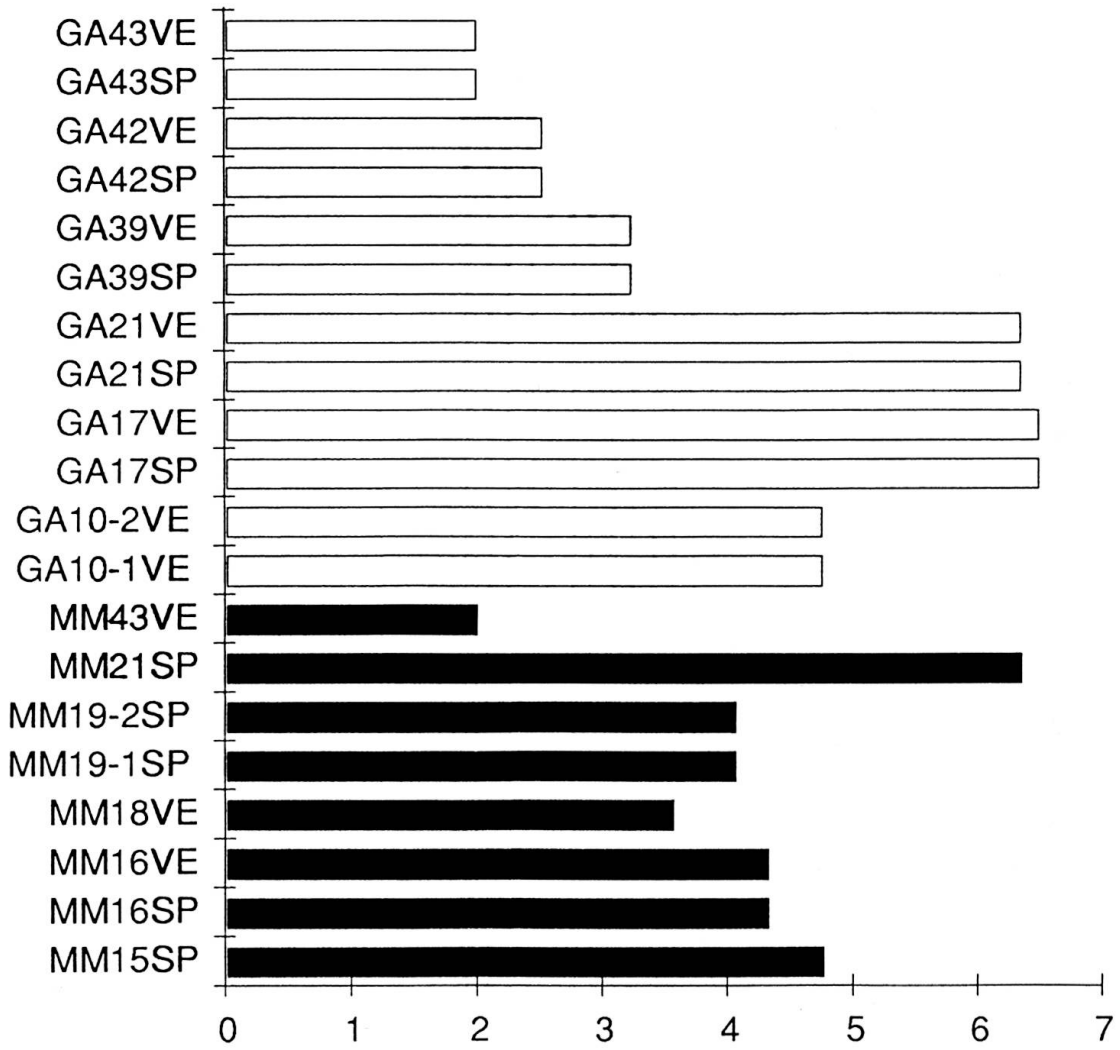

Figure 1. Aluminium concentration in the humus layer where the various isolates were taken. The bars represent the aluminium concentration given as $\ln(1+x\cdot 100/LI)$*, where* x *is ppm aluminium in the dry sample and* LI *is loss of ignition. – Solid bars represent* Mycena metata*, white bars* Galerina atkinsoniana.

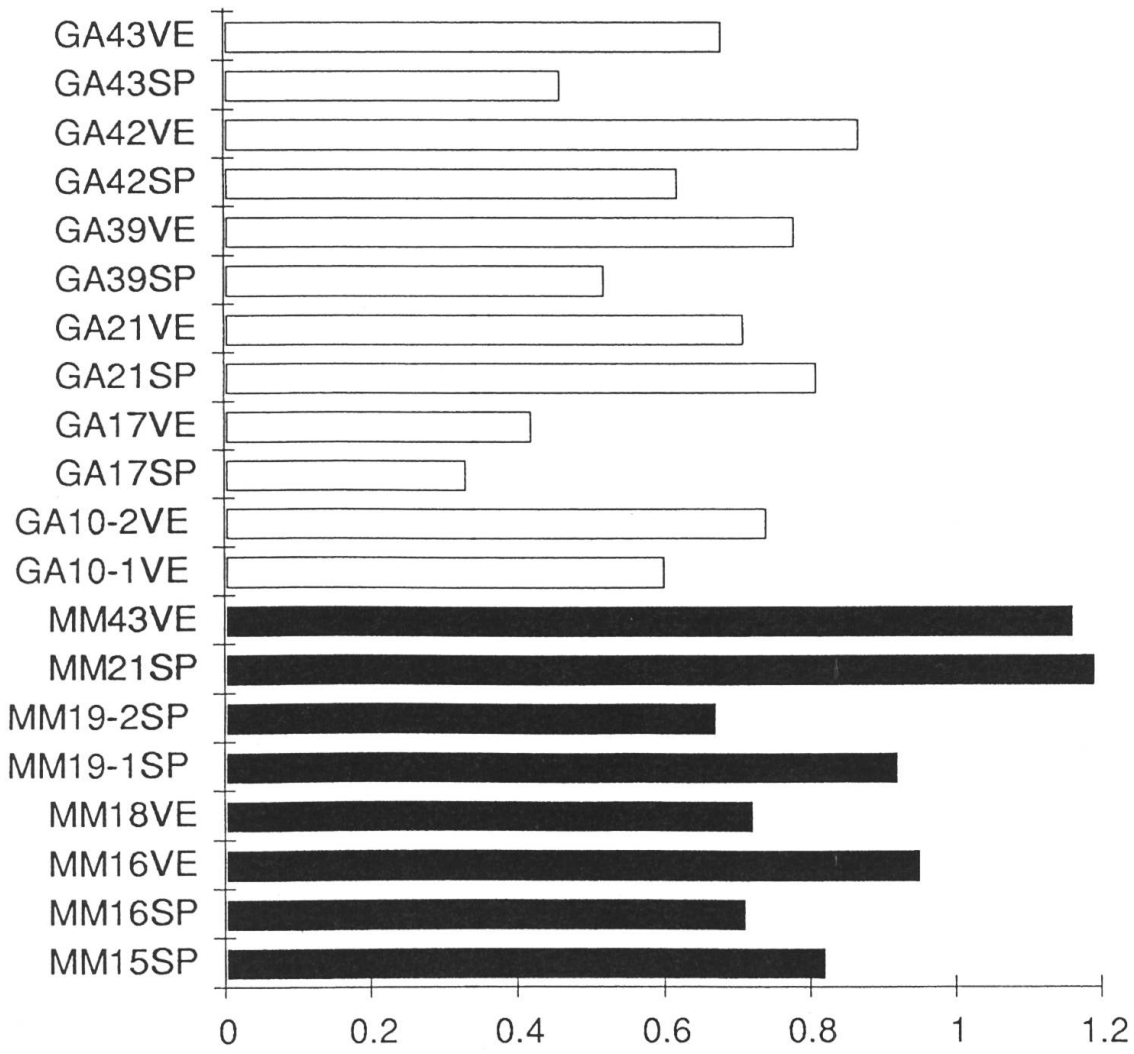

Figure 2. Aluminium resistance given as the index $In_{8.6}$ *for the various isolates. – Solid bars represent* Mycena metata, *white bars* Galerina atkinsoniana.

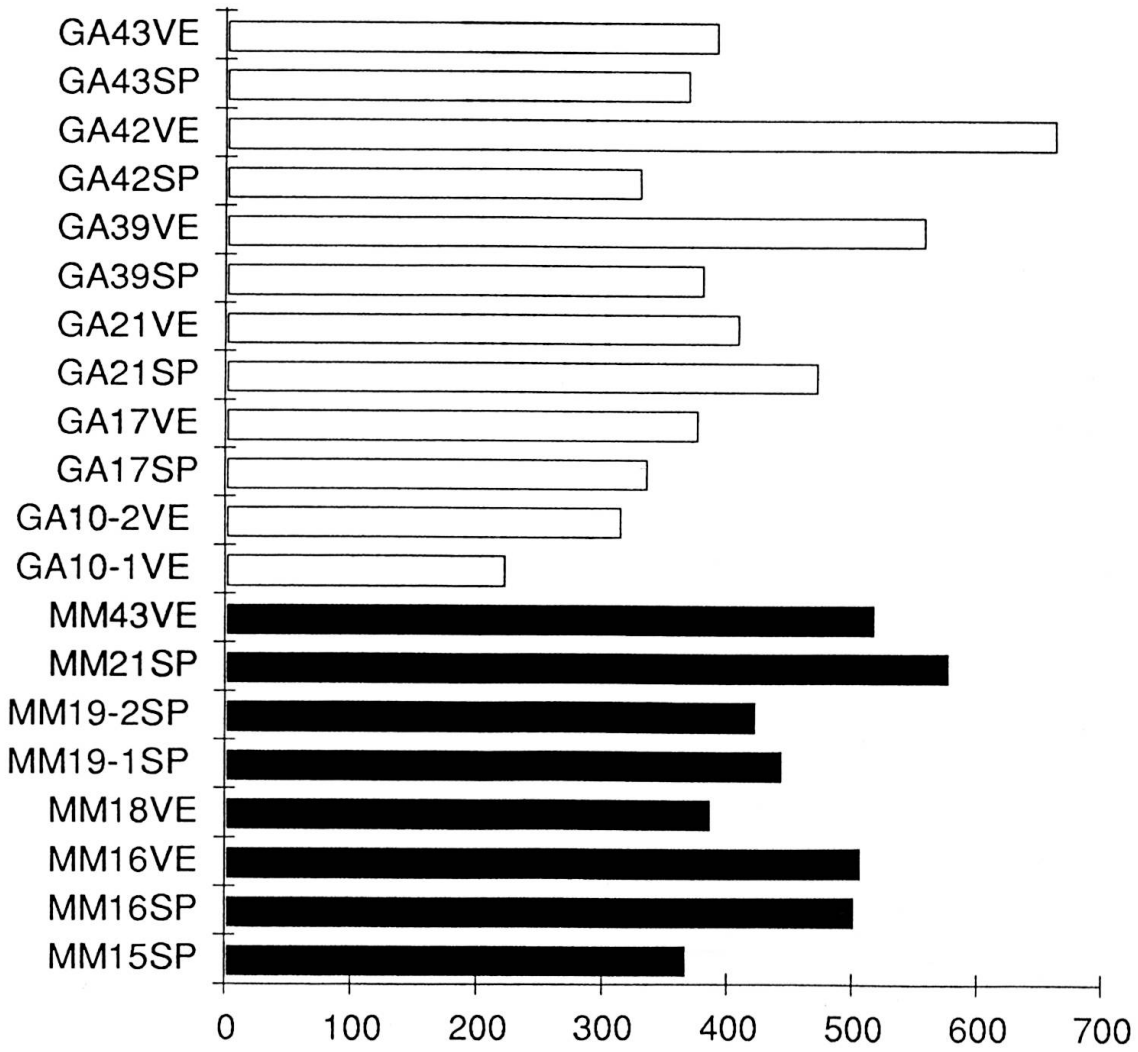

Figure 3. Aluminium resistance given as the index Σ%RBR *for the various isolates. – Solid bars represent* Mycena metata, *white bars* Galerina atkinsoniana.

the most resistant of the *M. metata* isolates, and GA10-1VE the most sensitive, GA42VE the most resistant of the *G. atkinsoniana* isolates. The two indices were positively correlated, $r = 0.69$ ($p < 0.001$).

The correlation between the indices and *Al(humus)* gave the following results: *Mycena metata*, $r = 0.19$ (n. s.) for $In_{8.6}$ and $r = 0.12$ (n. s.) for $\Sigma\%RBR$. *Galerina atkinsoniana*, $r = -0.23$ (n. s.) for $In_{8.6}$ and -0.13 (n. s.) for $\Sigma\%RBR$. Neither $In_{8.6}$ nor $\Sigma\%RBR$ were correlated with *Al(humus)*.

The mean indices are $In_{8.6}(avg) = 0.89$ and $\Sigma\%RBR(avg) = 465.17$ for *M. metata*, and $In_{8.6}(avg) = 0.63$ and $\Sigma\%RBR(avg) = 402.41$ for *G. atkinsoniana*. The Wilcoxon rank-sum test showed that *M. metata* is significantly more Al-resistant than *G. atkinsoniana* ($p = 0.006$ for $In_{8.6}$ and $p = 0.045$ for $\Sigma\%RBR$) – although the $\Sigma\%RBR$ value for GA42VE is higher than any value for *M. metata*).

Neither any similarity in resistance was seen between samples originating from spores or tissue taken from the same sporophore, nor any similarity in resistance between two different samples from the same plot.

Discussion

A possible selection mechanism towards metal resistance in fungi has been suggested by Jordan & Lechevalier (1975), Ross (1975), and Thompson & Medve (1984). Colpaert & Assche (1987) demonstrated that strains of ectomycorrhizal fungi derived from polluted soils were more tolerant to zinc and copper than strains from unpolluted soils. A selection mechanism to these metals was suggested. Evans & Sylvester (1988) found that isolates of *Paxillus involutus* (Batsch: Fr.) Fr. from contaminated sites were more resistant to arsenic than those from clean soils. On the other hand, Denny & Wilkins (1987) in their study of zinc tolerance in mycorrhizae between *Betula* spp. and *Paxillus involutus* suggested that the ameliorating influence of mycorrhizal fungi on zinc toxicity to birch depends more on the adaptation of the fungal strain to the higher plant associate than it does on adaptation of the fungus to zinc. Arnebrant & al. (1987) found little evidence for adaptation to copper among isolates of microfungi from soils with short or long history of pollution.

The above investigation could not prove any relationship between aluminium resistance in samples of *Mycena metata* and *Galerina atkinsoniana* and the content of aluminium in humus from the particular sample plots. The statements by Arnebrant & al. (1987) and Wainwright (1988) that fungal populations seem to be genetically static when confronted with toxic metals, are possibly also valid for saprophytic Basidiomycetes and aluminium.

This contribution is dedicated to Prof. Dr. Moser (Innsbruck, Austria), on occasion of his 70th birthday.

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