INTRASPECIFIC ALUMINIUM RESPONSE IN *SUILLUS LUTEUS* (L.) S.F. GRAY., AN ECTOMYCORRHIZAL SYMBIONT OF SCOTS PINE

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ABSTRACT

Ten isolates of the ectomycorrhizal fungus *Suillus luteus* have been cultured on an aluminium containing growth medium in order to determine their *in vitro* aluminium tolerance. Five isolates originated from a site heavily polluted by acid rain with a high availability of aluminium in the soil. Five others were collected from a site free from direct pollution. Aluminium content in the sporocarps of *S. luteus* differed according to the site of origin and did not reveal symptoms of bioconcentration, although such phenomena were found when mycelium isolated from the sporocarps was submitted to 100 mg/L Al in liquid culture. A clear relationship between Al accumulation *in vitro* and the site of origin of the isolate was not observed, although the highest amount of Al was found in the mycelium derived from the polluted soil. In a second experiment all isolates were grown in agar media containing 10, 100, 500 and 1000 mg/L⁻¹ Al and the colony diameter during culture and the final colony dry weight determined. *S. luteus* appeared to be very tolerant to the presence of Al in the medium. Each of the parameters used to measure the metal tolerance of the fungus ranked the isolates in a slightly different order, but those originating from the polluted area showed some superiority over the others. In polluted soils this species seems to have been submitted to a selection for higher aluminium tolerance. The results are discussed with reference to the possibilities of transforming *in vitro* studies to situation in the forest ecosystem.

KEY WORDS: aluminium toxicity, ectomycorrhiza, intraspecific variation, *Pinus sylvestris*.

INTRODUCTION

Aluminium is one of the most abundant elements in soils and its solubility increases at pH values below 4.5. In sandy conifer forest soils with a low buffering capacity, acid rains may lead to extremely low soil pH values and, consequently to high levels of Al in the soil solution. Both acidification and a high Al concentration in the soil may be important factors in the decreasing vitality of forests (Ulrich 1981, Godbold et al. 1988). Under natural conditions, most forest tree species are heavily colonized by ectomycorrhizal fungi which may ameliorate the reaction patterns of the autobiont to heavy metal stress. However, when the soil solution is enriched with a toxic metal, the development of some mycorrhizal fungi may also be influenced. Recent reports indicate that many species of ectomycorrhizal fungi are declining in polluted forest soils (Arnolds 1988, Jansen and De Nie 1988, Kowalski et al. 1989). The effect of root colonization with different species of mycorrhizal fungi on the reaction of trees to aluminium has been the subject of recent studies. Wilkins and Hodson (1989) and Henschel et al. (1993) found a positive effect of *Paxillus involutus* on aluminium tolerance in spruce seedlings. Cumming and Weinstein (1990) exposed *Pinus rigida* seedlings to low concentrations of Al in sand culture and found that mycorrhizal colonization with *Pisolithus tinctorius* strongly alleviated the toxic effects of Al. Young Scots pine seedlings responded with a growth reduction at 6 mM Al but mycorrhizal

plants with *Suillus bovinus* did not react until the Al concentration reached 10 mM (Göranson and Eldhuset 1991). Norway spruce seedlings colonized with the mycorrhizal fungus *Lactarius rufus* were strongly affected by Al (Jentschke et al. 1991). The fungal sheath formed by *L. rufus* and *L. theiogallus* did not reduce Al uptake into the host tissues. On the basis of this observation the authors suggested that the hyphal sheath of other mycorrhizas might also be permeable to Al. Ectomycorrhizas formed on *Abies balsamea* roots with *Cenococcum geophilum*, *Hebeloma crustuliniforme* or *Laccaria laccata* were greatly reduced as the soil acidity and Al concentration increased (Entry et al. 1987). This findings suggest that in the influence of aluminium on the mycorrhizae and the mycorrhizal fungi both host and fungus specific effects is involved. Not all fungi are equally effective at protecting the host and thus there is a need to examine the effect of aluminium on mycorrhizal infections with particular mycobionts in greater detail. Al can influence the growth of mycorrhizal fungi *in vitro*. Their growth can be stimulated or inhibited, depending on the species and Al concentration (Thompson and Medve 1983, Zel et al. 1993, Kieliszewska-Rokicka et al. 1995).

Our own studies have concerned mycorrhizas and mycorrhizal fungi of Scots pine grown on a heavily polluted area with low pH and a high aluminium content (Rudawska et al. 1995 a, b). Mycorrhizal infections on the roots of trees from the polluted area were lower than in a reference site, free from di-

rect pollution although a high abundance of fruitbodies of some mycorrhizal fungi, like *Suillus luteus*, *Paxillus involutus* and *Amanita muscaria*, was observed for several seasons in the polluted area. This feature presumably indicates the existence of metal tolerant mycorrhizal fungi. There is a controversy in the literature concerning the selection of mycorrhizal fungi for heavy metal tolerance. Brown and Wilkins (1985) found no relationship between the zinc tolerance of mycorrhizal fungi and the level of pollution at the collection site, neither did Jones and Hutchinson (1988) for nickel. Denny and Wilkins (1987) argued that there is little adaptation to toxic metals at the interspecific level. However, Rühling et al. (1984) and Rühling and Söderström (1990) found that the fungal biomass in the soil and the sporocarps production, as well as the species composition of mycorrhizal populations, are affected by heavy metal soil pollution. Colpaert and Van Assche (1987, 1992) showed that the fungal species isolated from a heavily metal-polluted soil had a higher tolerance than isolates obtained from uncontaminated sites.

In preliminary studies performed under axenic conditions *S. luteus* was characterized as one of the most resistant of the ectomycorrhizal symbionts of Scots pine to Al (Kieliszewska-Rokicka et al. 1995). The present study was designed to evaluate the intraspecific response of *S. luteus* to different Al concentration. Isolates with a potential tolerance to lower pH values and a high aluminium content were sought to test the hypothesis that more resistant strains of mycorrhizal fungus are selected on polluted areas.

MATERIALS AND METHODS

Sporocarps analysis

Sporocarps were divided into caps and stems, cleaned to remove any attached soil and oven dried at 60°C. Dry material was powdered and Al content analyzed after the method of Greweling and Peech (Nowosielski 1974).

Fungal material and site characteristics

Cultures of *Suillus luteus* (L.) S.F. Gray. were isolated from the sporocarps collected from polluted and non polluted stands of *Pinus sylvestris* (Tab. 1). The methods of isolation were as described by Rudawska (1986).

The polluted site (Luboń) is located about 2 km from a phosphate fertilizer factory (established in 1917), which until 1987, had a more or less continual increase in fertilizer production and in emission of pollutants (Hernik 1987). The main pollutant emitted was SO₂, which accounted for 78 % of the total emission of gaseous pollutants in 1989. In the past, average emission rates from the five 25 to 62 m tall chimneys were: oxides of S, calculated as SO₂ equivalents – about 445 t yr⁻¹, and F compounds, calculated as F equivalents – about 18.4 t yr⁻¹ (Anonymous 1990). In 1990 HF production was suspended, halting fluoride emissions (Anonymous 1991). Emission of other toxic pollutants was also high: H₂SO₄ mist – 8.6 t yr⁻¹, aromatic and aliphatic hydrocarbons – 1.5 t yr⁻¹ (Anonymous 1990). The control sites (Kórnik, Puszcza Nadnotecka) were not influenced by direct industrial pollution. The polluted site in Luboń and the reference site in Kórnik are situated in a region with an annual background SO₂ level of ca. 32 g m⁻³ (Godzik 1990). The second reference site situated in Puszcza Nadnotecka has a lower annual background of SO₂ contamination (20 g m⁻³) (Mazurski 1990). Soil at the polluted site was over 10-fold more acidic than at the control sites (mean pH_{water} = 4.2 vs. 5.8) and contained about 10-fold higher Al concentration (58.1 vs. 5.6 µg g⁻¹) (Reich et al. 1994).

Culture conditions

Liquid cultures

Aluminium accumulation in the mycelia of tested isolates was determined by growing the fungi in a modified liquid medium (Tomaszewski and Wojciechowska 1974) containing 100 mg L⁻¹ of aluminium in the form of Al₂(SO₄)₃ × 18 H₂O at pH 4.0. The nutrient medium contained: 55 mM glucose, 14 mM maltose, 6.25 NH₄NO₃, 3.7 mM KH₂PO₄, 2 mM MgSO₄, 0.15 M thiamine-HCl, 0.02 M biotine and 0.1% (v/v) of a trace-element stock solution as described previously (Tomaszewski and Wojciechowska 1974). Experiments were carried out in Roux bottles filled with 150 ml of nutrient solution. Each bottle was inoculated with 6 mm plugs removed from the edges of 3 week old agar plate cultures. After 6 weeks of cultivation in the darkness at 24°C, the mycelia were separated from the liquid media and dried at 60°C. The content of aluminium was determined in the dry fungal material according to the method of Greweling and Peech (Nowosielski 1974).

Table 1. Isolates of the ectomycorrhizal fungus *S. luteus* used in the experiments.

Number	Origin	Year of isolation
Isolates from polluted soils		
12	Luboń	1992
13	Luboń	1992
14	Luboń	1992
16	Luboń	1992
17	Luboń	1992
Isolates from unpolluted soils		
44	Kórnik	1992
45	Kórnik	1992
49	Kórnik	1992
62	Puszcza Nadnotecka	1992
63	Puszcza Nadnotecka	1992

Agar cultures

The effects of different concentration of aluminium on growth of isolates of *S. luteus* were determined on agar cultures. Mycelia were cultivated in Petri dishes on agar media as described above, containing various concentrations of aluminium (0, 10, 100, 500, 1000 mg L⁻¹) in the form of Al₂(SO₄)₃ × 18 H₂O and pH adjusted to 4.0. To prevent the precipitation of insoluble aluminium compounds, Al₂(SO₄)₃ × 18 H₂O was sterilized separately and added to the sterile culture medium under aseptic conditions, just before the medium was poured into the dishes.

The agar was covered with a sterile cellophane film. Two 6 mm inocula cut from the edge of 3 week old agar plate culture were placed in each dish. Cultures were placed in the darkness at 24°C for 18 days. Radial growth of each inoculum of the fungal colony was measured 6 times at 3 day intervall. The dry weight of the mycelium was determined at the end of the experiment after oven drying at 60°C for 48 h.

To compare the isolates used in the experiment, reduction of the radial growth rate was used as an index for the metal tolerance. This tolerance index (T.I.) was calculated as a percentage value from the ratio:

$$T.I._{rad.growth} = \frac{\text{radial growth rate in the metal treatment}}{\text{radial growth rate in the control (0 mg L}^{-1}\text{ Al)}}$$

At the end of the culture period the dry weights of the mycelia were measured. Reduction of the biomass was also used as an index of metal tolerance, expressed in the following formula:

$$T.I._{dry\ weight} = \frac{\text{mean biomass in the metal treatment}}{\text{mean biomass in the control (0 mg L}^{-1}\text{ Al)}}$$

This tolerance index is analogous to the one used by Colpaert and Van Assche (1987) for the determination of Zn and Cu tolerant strains of some ectomycorrhizal fungi.

All data are mean of 8 to 10 replicates ± SD. Statistical significance was evaluated using Student's *t*-test (Zgirski and Gondko 1976).

RESULTS

The chemical properties of the soil influence the uptake of aluminium by the sporocarps of *S. luteus* (Tab. 2). The highest amount of aluminium was found in caps and stems of *S. luteus* originating from the polluted area and the lowest from our special control site in Puszcza Nadnotecka.

Concentrations of aluminium in mycelia grown in liquid culture containing 100 mg L⁻¹ of Al are shown in Fig. 1. All isolates accumulated relatively high amounts of aluminium but no clear relationship between the site of origin and the ability to accumulate Al was observed. The highest aluminium amount was in isolates 14 and 17 from the polluted site and isolate 62 from the unpolluted one.

Since metal can affect several aspects of fungal growth, metal tolerance is difficult to measure using a single parameter. Thus the effect of increasing concentrations of aluminium on fungal growth was measured in two ways: 1. the effect on radial growth, from which the mean growth rate (mm/day) was calculated and 2. the effect on the final dry

Fig. 1. The content of aluminium in mycelium of different isolates of *Suillus luteus* grown on liquid media pH 4.0, containing 100 mg L⁻¹ Al. Origin of isolates as in Tab. I. Means within isolates not sharing a common letter are significantly different by Student's *t*-test at *p* = 0.05.

Table 2. Content of aluminium in sporocarps of *S. luteus* collected at different sites (composite sample).

	Aluminium content (µg/g dry weight)		
	Polluted site	Nonpolluted sites	
	Luborń	Kórnik	Puszcza Nadnotecka
stem	25	21	trace
cap	175	52	12

weight. Both parameters were used to calculate the tolerance index of the isolates. The radial growth rates at different aluminium concentrations are presented in Table 3. There is no great variation in growth rates between the isolates originating from the sites of different degrees of pollution till 500 mg L⁻¹ Al in the medium. The only exception is isolate 17 being arrested in growth at 10 mg L⁻¹ Al in the culture medium. At 1000 mg L⁻¹ all strains from the unpolluted sites were slightly inhibited in their radial growth while virtually no reaction was observed for isolates from polluted site (with the exception of isolate 17).

The metal tolerance indices for all isolates calculated on basis of their growth rates are given in Table 4. At 10 mg L⁻¹ several isolates (12, 13, 14, 16, 44, 49) were characterized by indices very close or even higher than those of the control. This phenomenon awaits an explanation. At the highest concentration applied (1000 mg L⁻¹) four from five isolates originating from the polluted area (12, 13, 14, 16) were slightly inhibited by only 19, 19, 5 and 29 % respectively. Tolerance indices of mycelia coming from the unpolluted sites were low for isolates 44, 49 and 62 and comparable with isolates from the polluted site for two others, 45 and 63. Generally the lower the tolerance index, the more sensitive is isolate tested.

The final dry weight of the mycelia of isolates tested is given in Table 5. Dry weight production of *S. luteus* isolates originating from the polluted site (12, 13, 14, 16) was not significantly affected at the Al concentration range applied here. Only strain 17 differed significantly with a very low biomass of mycelium produced. On the contrary all isolates, except for 63, collected from non-polluted soils showed a large reduction in biomass production at the highest Al concentration (1000 mg L⁻¹). The aluminium tolerance indices, calculated on the basis of the dry weight, also reflect the same tendency (Table 6). The isolates from Al-polluted soils were strongly Al tolerant and isolates from the unpolluted soils were clearly inhibited at the highest Al concentration, with the exception of isolate 63 being extremely resistant.

Fig. 2 and 3 present examples of two extreme reactions of the mycelia tested to the aluminium content of the medium. In Fig. 2 "tolerant isolate" 14, originating from the polluted area shows a high degree of resistance to the range of aluminium concentrations used in the experiment and continued to grow even at the highest Al level. Fig. 3 presents an example of "sensitive isolate" 62 being gradually inhibited with increasing Al concentrations (10, 100, 500 mg L⁻¹) and completely arrested at 1000 mg L⁻¹.

Table 3. Mean growth rates on agar medium of 10 isolates of the ectomycorrhizal fungus *S. luteus*, treated with different aluminium concentrations.

No of isolate	Mean growth rates (mm/day) ± SD				
	Aluminium concentration (mg L ⁻¹)				
	0	10	100	500	1000
Isolates from polluted soils					
12	1.8 ± 0.5	2.2 ± 0.4	1.6 ± 0.2	1.3 ± 0.2	1.5 ± 0.2
13	2.6 ± 0.2	2.5 ± 0.1	2.0 ± 0.3	1.6 ± 0.1	2.1 ± 0.1
14	2.7 ± 0.7	3.1 ± 0.6	2.8 ± 0.8	2.7 ± 0.5	2.6 ± 0.3
16	2.5 ± 0.3	2.6 ± 0.3	2.6 ± 0.2	1.7 ± 0.2	1.8 ± 0.3
17	1.6 ± 1.4	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.0
Isolates from unpolluted soils					
44	3.3 ± 0.3	3.5 ± 0.5	3.1 ± 0.5	2.5 ± 0.7	0.8 ± 0.3
45	2.8 ± 0.8	2.6 ± 0.7	1.7 ± 1.2	1.8 ± 1.1	1.7 ± 1.0
49	3.8 ± 0.3	3.9 ± 0.2	3.5 ± 0.3	3.3 ± 0.3	1.5 ± 0.8
62	2.7 ± 0.2	2.4 ± 1.1	2.0 ± 1.0	1.6 ± 0.7	0.7 ± 0.2
63	2.3 ± 0.2	1.9 ± 0.1	2.0 ± 0.2	1.7 ± 0.0	1.8 ± 0.1

Table 4. Tolerance indices based on radial growth rates from 10 isolates of *S. luteus* treated with different aluminium concentrations.

Tolerance indices				
No of isolate	Aluminium concentration (mg L ⁻¹)			
	10	100	500	1000
Isolates from polluted soils				
12	121	89	73	81
13	97	78	62	81
14	111	100	97	95
16	103	101	65	71
17	43	24	28	24
Isolates from unpolluted soils				
44	105	94	73	23
45	93	60	63	79
49	104	92	87	38
62	89	74	59	25
63	85	90	77	80

DISCUSSION

From the present survey it can be concluded that the chemical properties of the site (Table 2) determine the aluminium uptake by the tested sporocarps of *S. luteus*. Aluminium concentrations in the sporocarps of different fungi have been investigated by Cromack et al. (1975) and Tyler (1980). According to Tyler, fungi may be divided in two behavioural categories in relation to some metal accumulation: "bioconcentrators" and "bioexcluders", containing 10 times more or 10

times less metal content respectively than the growth medium.

Our determinations indicate that in the area not influenced by major local sources of pollution (Puszcza Nadnotecka), *S. luteus* species (*Boletaceae*) neither accumulated elevated doses of aluminium nor excluded it from the fruitbodies. This is in accordance with Tyler (1980) who classified members of the *Boletaceae* to an indifferent group in terms of aluminium accumulation in the fruitbodies. Median concentrations of Al in the sporocarps of *Boletaceae* were determined by Tyler on

Table 5. Dry weight of 10 isolates of the ectomycorrhizal fungus *S. luteus* after 18 days of culture on agar media with different aluminium concentrations.

Colony dry weight (mg) ± SD					
No of isolate	Aluminium concentration (mg L ⁻¹)				
	0	10	100	500	1000
Isolates from polluted soils					
12	21 ± 5	21 ± 4	24 ± 2	22 ± 3	22 ± 4
13	23 ± 5	20 ± 4	30 ± 7	60 ± 5	23 ± 3
14	32 ± 3	35 ± 5	39 ± 6	29 ± 2	22 ± 2
16	32 ± 1	25 ± 4	25 ± 3	21 ± 2	26 ± 6
17	2 ± 0.3	2 ± 0.2	2 ± 0.03	2 ± 0.1	2 ± 0.2
Isolates from unpolluted soils					
44	28 ± 2	22 ± 3	25 ± 4	22 ± 4	2 ± 0.01
45	20 ± 3	16 ± 7	2 ± 0.9	4 ± 2	8 ± 3
49	17 ± 2	22 ± 2	16 ± 0	18 ± 3	3 ± 1
62	25 ± 5	19 ± 4	17 ± 5	14 ± 2	2 ± 0.1
63	28 ± 3	29 ± 2	32 ± 5	30 ± 4	31 ± 5

Table 6. Tolerance indices based on colony dry weights of 10 isolates of *S. luteus* treated with different aluminium concentrations.

Tolerance indices				
No of isolate	Aluminium concentration (mg L ⁻¹)			
	10	100	500	1000
Isolates from polluted soils				
12	99	113	106	105
13	89	131	119	99
14	108	123	91	67
16	77	79	66	81
17	113	100	106	95
Isolates from unpolluted soils				
44	77	88	79	8
45	77	12	20	39
49	134	98	114	18
62	75	69	58	7
63	101	111	106	107

30 µg/g. This is comparable with sporocarps of *S. luteus* collected at our control site situated in Kórník. However in highly polluted soils with a low pH and raised aluminium availability *S. luteus* sporocarps were able to accumulate aluminium to a higher degree (Table 2).

From this example we may say that accumulation of certain metals by sporocarps of mycorrhizal fungi is determined by their characteristics of bioaccumulation or bioexclusion or, de-

pends from the soil properties. In the case of *S. luteus* with higher aluminium availability in the soil, higher contents of aluminium in sporocarps was found. Obtained results are in line with concentration of Al that have been reported in sporocarps of *S. tomentosus* grown on acidic site with high level of Al in the soil solution (Browning and Hutchinson 1991).

It cannot be presupposed that the metal concentration in the sporocarps is similar to that in the mycelium, but after Tyler

Fig. 2. Radial growth of a metal tolerant isolate (No 14 originating from polluted site) of *Suillus luteus* on agar media at different Al concentrations.

Fig. 3. Radial growth of a non-metal tolerant isolate (No 62 originating from nonpolluted site) of *Suillus luteus* on agar media at different Al concentrations.

(1980) it should reflect the capacity of the mycelium to absorb the metal from the substrate. Our *in vitro* studies do not confirm this assumption, at least for *S. luteus*. This fungus, which in comparison with other species (e.g. *Amanita* sp.) (Rudawska, unpublished data) was characterized by relatively low content of aluminium in its sporocarps, behaved under *in vitro* studies as a typical member of the "bioconcentration group". At aluminium contents in the medium of 100 mg L^{-1} , mycelia of all isolates accumulated at least 10 times more of this metal. Although there was a great variation in Al accumulation between the isolates used, the highest content was reached by two isolates originating from the heavily polluted area.

The question arises as to what extent aluminium accumulation in the mycelium cultured under *in vitro* conditions is reflected in a similar response when the mycelium is growing in the soil and in a symbiotic association? *S. luteus* is known to form an extensive mycelium in the soil and to develop large mycorrhizal clusters with thick mantles. Also under *in vitro* culture this fungus produces an abundant mycelium in a short time. If accumulation of aluminium by the mycelium in the forest soil responds in the same manner as in the *in vitro* cultures, *S. luteus* could play an important role in decreasing the phytotoxic effects of Al on the growth of the phytobiont. Jones and Hutchinson (1988) also emphasized that the amount of tissue available may be the characteristic which distinguishes fungi in their ability to increase metal tolerance, regardless of the mechanism involved in the binding of the metal. A variety of mechanisms occur in both partners of symbiosis enabling the toxic elements to be removed. These mechanisms range from physicochemical interactions, such as absorption, to processes that depend on cell metabolism, such as intracellular metal accumulation or extracellular precipitation of metals, as a result of excreted metabolites (e.g. malic or oxalic acid). Aluminium was detected in ectomycorrhizal fungi in P-rich granules within the vacuoles (Kottke 1991, Turnau et al. 1993), cytoplasm (Turnau et al. 1992) and cell walls (Väre 1990) and it has been suggested that exclusion at

the cell wall is the primary mechanism for protection of fungal hyphae (Väre 1990). Recently Martin et al. (1994) in a ^{27}Al – nuclear magnetic resonance (NMR) study have shown that aluminium was rapidly taken up and accumulated into polyphosphate complexes in the vacuole of the mycorrhizal fungus *Laccaria bicolor*. The Al-polyphosphate complexes in the mycelium of fungus tested are stable and are not remobilized in an Al-free nutrient solution. Such findings make a significant contribution to the potential role of ectomycorrhizal fungi in the protection of trees against aluminium toxicity.

Modulation of the Al effect by mycorrhizas depends on the fungal species involved in this symbiotic association; a high degree of variation in Al sensitivity was found among three ectomycorrhizal fungi isolated from Douglas-fir (Jongbloed and Borst-Pauwels 1992). Both *Lactarius rufus* and *L. hepaticus* appeared to be very sensitive to Al, whereas *Laccaria bicolor* exhibited a high Al tolerance. High aluminium tolerance among ectomycorrhizal fungi was also shown by Hintikka (1988). In this latter study *S. luteus*, *S. variegatus*, *S. bovinus* and *Paxillus involutus* grew in concentrations up to 10 g/l Al, while species of *Amanita* and *Tricholoma* were more sensitive. The author suggested that the high tolerance of certain mycorrhizal species may be interpreted as a natural adaptation to Al-rich conditions in highly acid soils. The only study dealing with the problem of the relationship between the occurrence of some ectomycorrhizal fungi on acid soils with high Al concentrations and their Al tolerance under *in vitro* conditions is paper of Thompson and Medve (1984). In this study strains of *S. luteus* appeared to be indifferent to Al concentration ranging from 0 to 500 ppm while other species evidenced a high variation in Al tolerance although no correlation was found between the occurrence of these species on acid soils and high Al concentrations.

In our studies, *S. luteus*, a mycorrhizal symbiont of young stands of *Pinus sylvestris*, proved to be very tolerant to aluminium when tested in pure culture conditions. The aluminium concentrations used in our experiments are high when com-

pared with those normally found in acidic forest soils and much higher than those in soils polluted by industrial acid rain. However the limiting concentration, which could distinguish between the more resistant and the less resistant isolates was being sought. In several cases the highest concentration employed, only slightly arrested the mycelial growth of the isolates tested. Each of the parameters used to measure the metal tolerance of the fungi ranked the isolates in a slightly different order, but those originating from the polluted areas showed a distinct superiority over the others. As an example of very resistant isolate we choose isolate 14 and very sensitive one 62. Thus we may say with caution about some relationship between the site of origin of an isolate and its Al tolerance. However there are some exceptions. For example strain 63 from unpolluted forest of Puszcza Nadnotecka revealed resistance even to highest aluminium concentration when consider its tolerance index calculated on the basis of radial growth and biomass production. Such findings suggest that the genetic adaptation for metal tolerance in mycorrhizal fungi is highly complicated. A non-homogenous soil composition may be one explanation for such a phenomenon. Availability of metal ions in different soils (polluted and unpolluted) is not always constant over the whole area and differences at the micro-scale are possible; organic compounds can bind metals strongly and so retain the metals from the soil solution (Gadd and Griffiths 1978). Also the metals are not dispersed equally along the soil profile and it may be possible that some non-tolerant species or strains can survive in soil horizons which contain high metal concentration or some tolerant clones may be selected in soil profiles containing only locally high metal contents.

However, caution should be applied in extrapolating results from the axenic screening of ectomycorrhizal fungi to the situation of the symbiotic organism under field conditions. Jones and Hutchinson (1986,1988) showed that the copper tolerance of *Betula papyrifera* seedlings was increased by the presence of copper-tolerant ectomycorrhizal fungi, but that nickel tolerance was not increased by nickel tolerant fungi.

To date no reports have appeared describing whether differential responses of mycorrhizal fungi to Al in axenic culture is reflected in similar responses in the symbiotic condition. Experiments in progress, testing some isolates selected in the present study in mycorrhizal association with *P. sylvestris* will be the subject of a forthcoming paper.

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WEWNĄTRZGATUNKOWE ZRÓŻNICOWANIE REAKCJI NA GLIN U *SUILLUS LUTEUS* (L.) S.F. GRAY., EKTOMIKORYZOWEGO SYMBIONTA SOSNY

STRESZCZENIE

Dziesięć izolatów ektomikoryzowego grzyba *Suillus luteus* hodowano na pożywkach zawierających glin. Pięć izolatów pochodziło z terenu skażonego kwaśnymi deszczami i dużej zawartości glinu w glebie, natomiast pozostałe wyizolowano z owocników zebranych na terenie wolnym od bezpośrednich zanieczyszczeń. Zawartość glinu w owocnikach *S. luteus* zebranych z terenów o różnym stopniu skażenia nie wykazywała symptomów biokoncentracji, natomiast takie zjawisko wystąpiło, kiedy sterylną grzybnie hodowano na pożywkach płynnych zawierających 100 mg Al / L. Nie wykazano wyraźnej zależności pomiędzy akumulacją glinu w grzybni w hodowli *in vitro* a miejscem zebrania owocnika z którego pochodzi dany izolat. Jednakże najwyższą akumulację glinu stwierdzono w grzybni pochodzącej z terenu skażonego, natomiast najniższą z terenu kontrolnego. Testowano także wpływ różnych stężeń glinu (10, 100, 500, 1000 mg L⁻¹) na wzrost radialny oraz końcową suchą masę grzybni i wyliczono na tej podstawie współczynniki tolerancyjności. Każdy z badanych parametrów szeregowal badane izolaty w nieco innym porządku, ale te pochodzące z terenów skażonych okazały się bardziej tolerancyjne na najwyższe stężenie glinu (1000 mg L⁻¹). Wydaje się, że na terenach skażonych kwaśnymi deszczami, o podwyższonej dostępności glinu w glebie ten gatunek grzyba mikoryzowego poddany jest selekcji ukierunkowanej na tolerancję podwyższonych stężeń glinu. Uzyskane wyniki dyskutowane są na tle aktualnej wiedzy nad wpływem glinu na mikoryzę oraz możliwości przenoszenia wyników z kultur *in vitro* do sytuacji w naturalnych zespołach leśnych.

SŁOWA KLUCZOWE: ektomikoryza, toksyczność jonów glinu, kwaśne deszcze, *Pinus sylvestris*.