ALUMINIUM TOLERANCE OF DIFFERENT *PAXILLUS INVOLUTUS* FR. STRAINS ORIGINATING FROM POLLUTED AND NONPOLLUTED SITES

MARIA RUDAWSKA, TOMASZ LESKI

Institute of Dendrology, Polish Academy of Sciences,
Parkowa 5, 62-035 Kórnik, Poland

(Received: April 11, 1997. Accepted: January 26, 1998)

ABSTRACT

The caps of the sporocarps of *P. involutus* originating from the polluted site (Luboń) and from the control site in Kórnik and Puszcza Nadnotecka accumulated high amounts of aluminium and revealed symptoms of bioconcentration. However in caps of the sporocarps from the control sites a lower amount of Al was accumulated than in caps from the polluted site (Luboń). A significantly lower concentration of Al was found in stems of sporocarps originating from the special control site in Puszcza Nadnotecka. Mycelia of 11 strains isolated from sporocarps collected at the polluted and control sites were cultivated on a liquid medium containing 100 mg L^{-1} Al. All strains showed high bioconcentration of Al despite of the place of origin. In a subsequent experiment 10 strains of *P. involutus* originating from polluted soil and 8 strains derived from the control sites were grown in agar media containing 10, 100, 500 and 1000 mg/L^{-1} of Al. The radial growth rates during culture, the final colony dry weight and the metal tolerance indices calculated on the basis of measured parameters were determined. *P. involutus* strains appeared to be very tolerant to the presence of Al in the medium and were able to grow even at the highest Al concentration. Increasing aluminium level in the medium to different extent influenced growth of tested strains, however the site of the origin did not influence the response of *P. involutus* to aluminium: among 18 strains tested, the most tolerant were selected both from the polluted and from the unpolluted sites. The results are discussed with reference to the high intraspecific variability of different physiological features of the ectomycorrhizal fungus *P. involutus*.

KEY WORDS: aluminium toxicity, ectomycorrhizal fungus, sporocarps intraspecific variation.

INTRODUCTION

Progressive soil acidity due to air pollution and an increase in mobile aluminium concentration in the soil solution are considered major causes of forest decline in Europe (Ulrich 1983, Godbold et al. 1988). Mechanisms of aluminium toxicity to plants including direct influences on root anatomy and morphology, competition between Al^{3+} and basic ions, and inhibition of important enzymatic systems were reported by many authors and reviewed by Boudot et al. (1994). Most forest tree species are ectomycorrhizal. Colonization of tree roots by ectomycorrhizal fungi may ameliorate the negative effects of aluminium to the plant.

Different mycorrhizal species have been shown to have various effects on the growth of coniferous seedlings in the presence of available aluminium. Ectomycorrhizal associations with *Paxillus involutus*, *Pisolithus tinctorius* and *Suillus bovinus* protected the seedlings of *Pinus rigida*, *Picea abies* and *Pinus sylvestris* against the toxic effects of Al (Cumming and Weinstein 1990, Hentschel et al. 1993, Wilkins and Hodson 1989, Granson and Eldhuset 1991). However, mycorrhizas developed by *P. abies* with *Lactarius rufus* and *L. theiogallus* did not prevent aluminium translocation to the shoot and the negative effects of Al toxicity (Jentschke et al. 1991 a, b).

Ectomycorrhizal fungi show a differential response to Al when cultivated in pure culture (Browning and Hutchinson 1991, Jongbloed and Borst-Pauwels 1992, Kieliszewska-Rokicka et al. 1996). Thompson and Medve (1984) and Hintikka (1988) have indicated that several mycorrhizal fungi displayed a tolerance to extremely high concentrations of aluminium in aseptic culture. Hinntika (1988) suggested that the high tolerance of ectomycorrhizal fungi to high Al concentrations in pure culture can be regarded as an adaptation to naturally aluminium rich conditions in acid forest soils. The isolation of metal tolerant mycorrhizal fungi from polluted sites has been well documented (Brown and Wilkins 1985a, Jones and Hutchinson 1986, Colpaert and Van Assche 1987). It has been suggested that the tolerance of the mycobiont may be an important factor in metal tolerance of the plants (Colpaert and Van Assche 1987). However, there may be no correlation between metal tolerance of mycorrhizal fungi and the toxicity of the soil of origin (Brown and Wilkins 1985b, Denny and Wilkins 1987, Darlington and Rauser 1988).

P. involutus is an ectomycorrhizal fungus with a relatively wide geographical distribution and a wide host range. It cohabits species of at least the genera *Pinus*, *Picea*, *Pseudotsuga*, *Tsuga* and *Betula*. *P. involutus* is counted among the ectomycorrhizal species which have not declined during this century in Europe (Arnolds 1991). It has been reported to produce for several seasons numerous fruitbodies and to form mycorrhizas in forest stands influenced by acid deposition and increased levels of available aluminium (Rudawska et al. 1996b) or by industrial dust containing heavy metals such as Pb, Zn, Cd, Cu (Turnau 1990, Turnau and Kozłowska 1991). Shaw et al. (1992) noted fruitbodies of *P. involutus* as the most common in the SO_2 treated plots. Dighton and Skeffington (1987) found, however, that acidic irrigation decreased number of ectomycorrhizal morphotypes, formed by *P. involutus*, on three-year-old Scots pine seedlings.

Variable results concerning tolerance of ectomycorrhizal fungi and ectomycorrhizal plants to mobile Al^{3+} suggest the existence of variability between strains of an ectomycorrhizal species. The present study was therefore undertaken to investigate the intraspecific variability of *P. involutus* in responses to Al and in order to select the most tolerant strains for further studies.

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 analysed by the method of Greweling and Peech (Nowosielski 1974).

Fungal material and site characteristics

Cultures of *P. involutus* were isolated from sporocarps collected from polluted and non polluted stands of *Pinus sylvestris* (Table 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 pollutants emission (Hernik 1987). The main pollutants emitted were SO_2, F compounds, H_2SO_4 mist, aromatic and aliphatic hydrocarbons. Up to 1989 the average emission of S, calculated as SO_2 equivalent was about 445 t yr^{-1}. The control sites 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 SO_2 level of ca. 32 g m^{-3} (Godzik 1990). The second reference site situated in Puszcza Nadnotecka has a lower annual background of SO_2 contamination (20 g m^{-3}) (Mazurski 1990). Soil at the polluted site in Luboń was about 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) (Reich et al. 1994).

More details about the stands were reported elsewhere (Leski et al. 1995, Rudawska et al. 1995, 1996a, b).

TABLE 1. Strains of the ectomycorrhizal fungus *P. involutus* used in the experiments.

Number	Origin
Strains from polluted sites	
Cz/1/92	Luboń
Cz/2/92	Luboń
Cz/4/92	Luboń
Cz/9/92	Luboń
Cz/11/92	Luboń
Cz/1/94	Luboń
Cz/2/94	Luboń
Cz/4/94	Luboń
Cz/10/94	Luboń
Cz/28/94	Luboń
Strains from non-polluted sites	
10-4	Kórnik
104-1	Kórnik
104-9	Kórnik
8	Kórnik
Zw/46/92	Kórnik
Zw/94/4	Kórnik
Zw/94/8	Kórnik
215-1	Puszcza Nadnotecka
PN/64/92	Puszcza Nadnotecka

Culture conditions

Liquid cultures

Aluminium accumulation in the mycelia of tested strains 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 $Al_2(SO_4)_3 \times 18\ H_2O$ at pH 4.0. The nutrient medium contained: 55 mM glucose, 14 mM maltose, 6.25 mM NH_4NO_3, 3.7 mM KH_2PO_4, 2 mM $MgSO_4$, 0.15 μM thiamine-HCl, 0.02 μM 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 darkness at 24°C, the mycelia were separated from the liquid media, washed with distiled water 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).

Agar cultures

The effects of different concentrations of aluminium on the growth of *P. involutus* strains 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 $Al_2(SO_4)_3 \times 18\ H_2O$ and pH adjusted to 4.0. To prevent the precipitation of insoluble aluminium compounds, $Al_2(SO_4)_3 \times 18\ H_2O$ 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 a 3 week old agar plate culture were placed in each dish. Cultures were placed in darkness at 24°C for 18 days. Radial growth of each inoculum of the fungal colony was measured 6 times at 3 day intervals. The dry weight of the mycelium was determined at the end of the experiment after oven drying at 60°C for 24 h.

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

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

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:

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

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

All data are means of 8 to 10 replicates ± SD. Statistical significance was evaluated using Student's *t*-test (Zgirski and Gondko, 1976) and Scheffe's test (Statistix 4.1).

RESULTS

The sporocarps of *P. involutus* originating from the polluted site in Luboń and from the control site in Kórnik accumulated rather similar amounts of aluminium (Tab. 2), however in caps of the sporocarps found in Kórnik, lower amount of Al was accumulated than in caps from the polluted site (Luboń). Significantly lower concentration of Al was found in stems of sporocarps originating from the additional control site in Puszcza Nadnotecka. The chemical properties of the soil only slightly influence the uptake of aluminium by fruitbodies of this species.

Concentrations of aluminium in mycelia of 11 *P. involutus* strains grown in a liquid culture containing 100 mg/L^{-1} Al are shown in Fig. 1. The highest concentration of aluminium was found in strains Cz/2/92, Cz/9/92, Cz/11/92 from the polluted site and strains 8, 215-1 from the control site. All strains accumulated high amounts of aluminium up to 4000 µg/g d.w., but no clear relationship between the site of origin and the ability to accumulate Al was observed (Scheffe pairwise comparisons of means). However among five strains with the highest bioconcentration of Al, three originated from the polluted site.

The effect of increasing concentrations of aluminium on fungal growth of 18 *P. involutus* strains was measured as radial growth of the colony (from which mean growth rates in mm/day was calculated) and as a final fungal dry weight. The radial growth rates of tested fungal strains on agar cultures at different aluminium concentrations are given in Table 3. It shows that *P. involutus* strains appeared to be very tolerant to the presence of Al in the medium and were able to grow even at the highest Al concentration (1000 mg/L^{-1}). Only some of the strains were limited in rates of their radial growth at the highest Al level. The metal tolerance indices for all 18 strains calculated on the basis of their radial growth rates are presented in Table 4. At 10 mg L^{-1} several strains were characterized by indices close or even higher than those of the control. A slight stimulation of growth of some strains (Cz/1/92, Cz/2/92/, 215-1) was observed also at 100 and even 500 mg L^{-1} Al. The tolerance index based on radial growth rates for strain No 8 (from the control site at Kórnik) was exeptionally high for all concentrations of aluminium and at 1000 mg L^{-1} Al T.I. reach value 126%. At the highest concentration of Al in the media (1000 mg L^{-1}) two strains from the polluted site (Cz/1/92, Cz/10/94) and two originating from the unpolluted area (215-1, Zw/94/4) were slightly inhibited but only by 20, 11, 9, 10%, respectively. The mean metal tolerance index at 1000 mg L^{-1} Al for strains of *P. involutus* from polluted soil is 57, and for strains from the control soils 67.

The final dry weight of mycelia of *P. involutus* strains tested is given in Table 5. All strains collected from the polluted and the unpolluted sites showed a reduction in biomass production at the highest aluminium concentration. Strains Cz/9/92, Cz/1/92 and Cz/11/92 originating from the polluted site (Luboń) were strongly inhibited at 1000 mg L^{-1} Al. The

TABLE 2. Content of aluminium in sporocarps of *P. involutus* collected at different sites (composite sample).

	Aluminium content (µg / g dry weight)		
	Polluted site	Nonpolluted sites	
	Luboń	Kórnik	Puszcza Nadnotecka
stem	121 a	121 a	36 c
cap	123 a	102 b	97 b

Means between sites not sharing a common letter are significantly different by Student's *t*-test at p = 0.05.

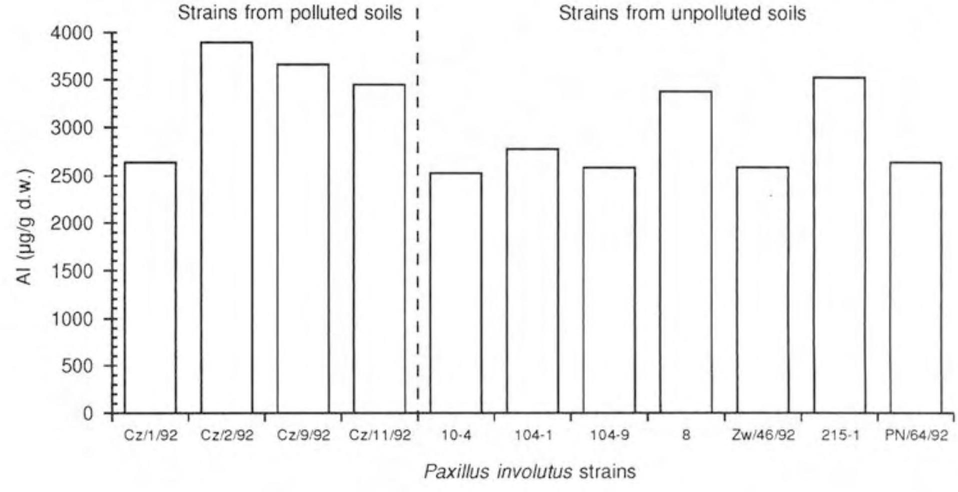

Fig. 1. The content of aluminium in mycelium of different strains of *P. involutus* grown on liquid media pH 4.0, containing 100 mg L^{-1} Al. There are no significant pairwise differences among the means (Scheffe pairwise comparisons of means, rejection level 0.05).

TABLE 3. Mean growth rates (calculated from radial growth) on agar medium of 18 strains of the ectomycorrhizal fungus *P. involutus*, treated with different aluminium concentrations.

No of strain	Mean growth rates (mm/day) ± SD				
	Aluminium concentration (mg L^{-1})				
	0	10	100	500	1000
Strains from polluted soils					
Cz/1/92	3.19±1.1	3.03±1.0	3.23±0.8	3.36±1.2	2.57±0.6
Cz/2/92	3.52±0.8	3.29±0.8	4.32±0.6	3.73±0.5	2.60±0.6
Cz/4/92	3.69±0.7	3.63±1.2	2.52±1.1	2.31±1.0	1.08±0.2
Cz/9/92	2.63±0.6	2.28±0.4	2.68±0.6	1.93±0.2	0.57±0.2
Cz/11/92	2.80±0.2	2.52±0.3	0.83±0.1	2.08±0.1	2.05±0.2
Cz/1/94	4.17±1.1	3.02±1.2	2.78±1.2	2.40±1.0	2.06±0.8
Cz/2/94	3.29±0.8	3.45±1.3	3.01±1.2	2.79±0.9	2.19±0.7
Cz/4/94	2.73±0.7	3.01±0.6	1.78±0.2	1.86±0.3	1.35±0.3
Cz/10/94	3.68±0.4	3.62±0.5	3.28±0.4	3.22±0.5	3.28±0.4
Cz/28/94	3.99±1.5	4.01±1.4	3.43±1.2	2.51±1.1	1.66±0.6
Mean value	3.37±0.8	3.18±0.9	2.79±0.7	2.62±0.7	1.94±0.5
Strains from unpolluted soils					
10-4	4.26±1.0	4.29±1.2	3.94±1.2	2.98±1.1	2.32±1.0
104-9	1.78±0.7	1.71±0.6	1.64±0.4	1.72±0.6	1.24±0.5
215-1	2.61±0.4	2.70±0.3	2.91±0.2	2.46±0.3	2.37±0.3
PN/64/92	2.44±0.6	2.50±1.2	1.78±0.5	1.56±0.4	1.06±0.2
8	2.47±0.3	2.68±0.2	2.59±1.2	2.82±0.3	3.11±0.2
Zw/46/92	2.54±1.2	1.82±0.8	2.17±1.1	1.39±0.6	0.54±0.3
Zw/94/4	3.34±0.7	3.22±1.1	3.06±1.0	3.28±1.2	3.00±1.2
Zw/94/8	2.61±1.1	2.72±1.1	2.22±1.0	1.22±0.7	1.11±0.4
Mean value	2.76±0.7	2.71±0.8	2.54±0.8	2.18±0.6	1.84±0.5

aluminium tolerance indices, calculated on the basis of the *P. involutus* mycelia dry weight, also reflect the same tendency (Table 6). The mean T.I. for strains from polluted soils are 84 for 10 mg L^{-1} Al, 71 for 100 mg L^{-1} Al, 51 for 500 mg L^{-1} Al and 34 for 1000 mg L^{-1} Al. The values of this index for strains from control sites are higher (118, 110, 88 and 60 for Al concentration 10, 100, 500 and 1000 mg L^{-1}, respectively).

Fig. 2 and 3 present examples of two different growth responses of the mycelia to the aluminium content in the medium. The "tolerant strains" were selected from both polluted (Cz/10/94) and unpolluted sites (No. 8) and show a high degree of tolerance to increasing concentrations of Al. Both strains continued to grow even at the highest aluminium level (Fig. 2A, 3A). "Sensitive strains" were found both on the polluted (Cz/4/92) and control site (Zw/94/8) and their growth was reduced by increasing concentration of Al in the culture medium (Figs 2B and 3B).

DISCUSSION

The presented results show that sporocarps of *P. involutus* are able to accumulate aluminium, both from polluted and nonpolluted soils (Table 2). According to classification of Tyler (1980) this species may be counted among "bioconcentrators", which means to such a category of fungi that could contain 10 times more metal than the growth medium. The opposite category of fungi, according to this author, are "bioexcluders" which contain 10 times less metal than the growth medium. In this study, the phenomenon of Al bioconcentration by *P. involutus* was found at the control plot in Kórnik, where the Al concentration in *P. involutus* sporocarps (102-121 μg/g) was about 20 times higher than in the soil (Rudawska et al. 1995). At the polluted site in Luboń, however, Al level in the sporocarps was similar as at the control site in Kórnik (121-123 μg/g) and exceeded only twice the Al concentration in soil (58 μg/g), thus no bioconcentration of Al was observed. It indicates that only limited amounts of aluminium may be accumulated in sporocarps of *P. involutus* in spite of the high content of this compound in the soil. Tyler noted that the median concentration of aluminium in sporocarps of different fungi was 30 μg/g. This concentration is comparable to that in stems of sporocarps of *P. involutus* collected at the special control, unpolluted site in Puszcza Nadnotecka (Table 2). Other results have been obtained when the chemical properties of the soil was tested on the uptake of Al by the sporocarps of ectomycorrhizal fungus *S. luteus* (Leski et al. 1995). Significantly higher content of aluminium has been found in sporocarps of *S. luteus* grown at the polluted than at the nonpolluted sites, however no bioconcentration of Al was observed. In liquid culture, however, strains of *P. involutus* originated from the polluted and control sites behaved as typical bioconcentrators. In the medium containing 100 mg L^{-1} Al, all strains accumulated 25 to 40 times more of this metal in the mycelium (Fig. 1). There was no clear relation between the site of the origin of the strains and the uptake of Al from the culture medium, however the highest concentration of Al was found in mycelia of two strains from the polluted site. Bioconcentration of aluminium in the mycelia of *P. involutus* in liquid culture exceeded significantly that in sporocarps and pure cultures of *S. luteus* (Leski et al. 1995).

Since aluminium was detected in phosphate-rich granules within mycelia of ectomycorrhizal fungi, the immobilizing of Al by the fungi is considered the primary mechanizm for protec-

TABLE 4. Tolerance indices based on radial growth rates from 18 strains of *P. involutus* treated with different aluminium concentration.

No of strain	Tolerance indices			
	Aluminium concentration (mg L^{-1})			
	10	100	500	1000
Strains from polluted soils				
Cz/1/92	95	101	105	80
Cz/2/92	93	123	106	74
Cz/4/92	98	68	63	29
Cz/9/92	87	102	73	22
Cz/11/92	90	30	74	73
Cz/1/94	72	67	58	49
Cz/2/94	105	91	85	66
Cz/4/94	110	65	68	49
Cz/10/94	98	89	88	89
Cz/28/94	100	86	63	42
Mean value	95	82	78	57
Strains from unpolluted soils				
10-4	101	93	70	55
104-9	96	92	97	70
215-1	103	111	94	91
PN/64/92	102	73	64	43
8	109	105	114	126
Zw/46/92	65	85	55	21
Zw/94/4	97	92	98	90
Zw/94/8	104	85	47	43
Mean value	97	92	80	67

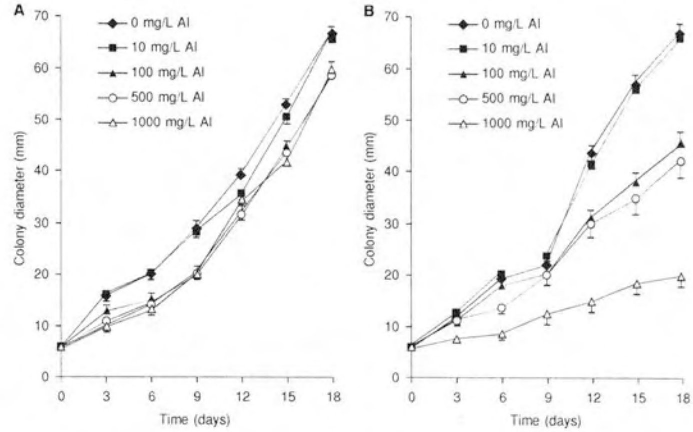

Fig. 2. Radial growth of a metal tolerant (Cz/10/94, Fig. 2A) and metal non-tolerant (Cz/4/92, Fig. 2B) strains of *P. involutus*, originating from polluted site, on agar media at different Al concentrations. Mean value Ě SD (n = 8).

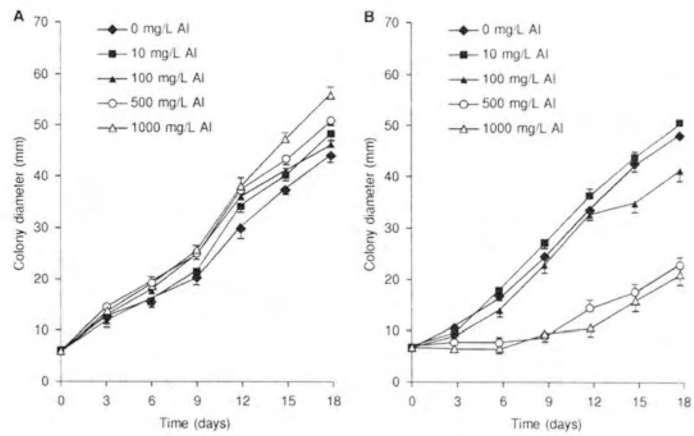

Fig. 3. Radial growth of a metal tolerant (8, Fig. 3A) and metal non-tolerant (Zw/94/8, Fig. 3B) strains of *P. involutus*, originating from non-polluted site, on agar media at different Al concentrations.Mean value ± SD (n = 8).

tion of the plant (Kottke 1991, Martin et al. 1994). In mycorrhizas of Scots pine seedlings with *P. involutus*, originating from a heavily polluted plot, polyphosphate granules containing aluminium were distributed in the fungal mantle and the Hartig net (Turnau et al. 1993).

Results shown in the present paper showed that the *P. involutus* mycelia are tolerant to high concentrations of Al in a pure culture condition. The purpose of using in agar tests concentrations of aluminium much higher than those we could find in the forests affected by acid deposition, was to select the strains particularly tolerant and sensitive to this metal. In the case of *P. involutus* the site of origin did not influence the tolerance of strains to aluminium. Leski et al. (1995), have found a relationship between the site of origin of a strain of *S. luteus* and its Al tolerance. Strains originating from the unpolluted soils were much less tolerant to Al than strains originating from soil heavily polluted with acid rain and containing mobile aluminium. Those results suggested an adaptation of *S. luteus* to environmental conditions and indicated that aluminium response of ectomycorrhizal fungi is species specific. Adaptation to toxic metals at the interspecific level seems not to be a universal phenomenon and in the case of *P. involutus*

TABLE 5. Dry weight of 18 strains of the ectomycorrhizal fungus *P. involutus* after 18 days of culture on agar media with different aluminium concentratins.

	Colony dry weight (mg) ± SD				
	Aluminium concentration (mg L^{-1})				
No of strain	0	10	100	500	1000
Strains from polluted soils					
Cz/1/92	76±21	48±19	55±17	26±10	5±2
Cz/2/92	66±13	35±14	28±7	17±3	8±1
Cz/4/92	92±22	77±19	45±19	40±11	20±13
Cz/9/92	21±7	18±4	29±4	7±1	1±0.3
Cz/11/92	40±9	35±5	3±1	7±1	6±1
Cz/1/94	49±9	35±4	31±3	23±4	20±3
Cz/2/94	74±19	75±17	68±17	61±14	53±10
Cz/4/94	52±12	56±13	37±9	35±7	22±7
Cz/10/94	70±17	62±14	55±12	53±12	55±9
Cz/28/94	68±21	67±22	66±12	53±14	32±7
Mean value	61±15	51±13	42±10	32±8	22±5
Strains from unpolluted soils					
10-4	21±3	28±3	26±4	16±2	9±1
104-9	36±4	39±3	65±12	52±10	34±7
215-1	89±12	111±19	85±10	72±8	66±8
PN/64/92	21±3	22±3	19±4	14±2	8±0.7
8	43±5	67±6	63±7	59±5	39±3
Zw/46/92	59±13	72±13	46±15	38±7	22±5
Zw/94/4	35±7	34±7	31±9	33±9	26±4
Zw/94/8	46±9	46±7	39±7	22±6	13±3
Mean value	44±7	53±8	47±8	38±6	27±4

do not support the hypothesis that more resistant strains of mycorrhizal fungi are selected on polluted areas (Colpaert and Van Assche J. A., 1987).

Aluminium tolerance of the *P. involutus* strains calculated on the basis of the radial growth and on dry weight are not always comparable. For instance strains Cz/1/92, Cz/2/92 and Cz/11/92 from Luboń had high tolerance indices based on radial growth rate and low tolerance indices based on colony dry weight. It was due to an expansive radial growth of a very loose fungal mat. Among 18 strains, tested the most tolerant originated both from the polluted and from the control sites (Cz/2/94 and Cz/10/94 – Luboń, 8 and Zw/94/4 – Kórnik). Similarly Brown and Wilkins (1985a) have found variations between *P. involutus* strains in response to Zn. The ability of each strain to grow in metal-enriched agar was independent of its origin from soils with low or high metal content.

The intraspecific variability of *P. involutus* in Al tolerance shown in this paper may explain different results concerning responses of *P. involutus* to Al reported by various authors. Hintikka (1988) has reported that mycelia of *P. involutus* grew in liquid media (pH 2.1-2.6) containing over 10 g L^{-1} Al^{3+} and was fairly good at concentrations up to 8 g L^{-1} Al^{3+}. However Kieliszewska-Rokicka et al. (1996) noted that 1 g L^{-1} Al inhibited growth of *P. involutus* mycelium. *P. involutus* has been shown to be an ectomycorrhizal fungus with a high variability. Other physiological features also indicate high intraspecific variability of this species. Laiho (1970) found that pH optimum could differ by three pH units between different strains of *P. involutus* and that some strains grew well over a span of a full pH unit, but others grew well over only a few tenths of a unit. Strains of *P. involutus* varied also widely in response to nitrogen nutrition and acid phosphatase activity (Kieliszewska-Rokicka 1992) and in the activity of IAA – oxidizing enzymes (Kieliszewska-Rokicka and Rudawska 1996). *P. involutus* belongs to fungi which produce an abundant loose mycelial mat when cultured on the agar medium. Under natural conditions the ability of *P. involutus* to produce an extensive fungal hyphae could probably influence the colonization of soil by the extramatrical mycelium.

High tolerance of *P. involutus* to Al and relatively high rate of radial growth suggest a predisposition of this fungus to be an efficient protector of host roots against the metal. Howe-

TABLE 6. Tolerance indices based on colony dry weights of 18 strains of *P. involutus* treated with different aluminium concentration.

No of strain	Tolerance indices			
	Aluminium concentration (mg L^{-1})			
	10	100	500	1000
Strains from polluted soils				
Cz/1/92	63	72	34	7
Cz/2/92	52	43	26	12
Cz/4/92	83	49	43	21
Cz/9/92	89	141	35	6
Cz/11/92	90	8	18	15
Cz/1/94	70	63	47	40
Cz/2/94	101	92	82	72
Cz/4/94	108	72	68	42
Cz/10/94	88	78	76	79
Cz/28/94	98	96	77	47
Mean value	84	71	51	34
Strains from unpolluted soils				
10-4	132	120	74	45
104-9	109	180	143	93
215-1	124	96	81	74
PN/64/92	104	87	64	37
8	155	146	136	90
Zw/46/92	123	78	65	38
Zw/94/4	99	90	95	74
Zw/94/8	100	86	48	28
Mean value	118	110	88	60

ver there need not necessarily be a relationship between tolerance of ectomycorrhizal fungi, in pure culture, and their ability to increase metal tolerance of the host plant (Jones and Hutchinson 1988). Therefore studies are under way to check the response of selected "tolerant" and "sensitive" mycelia in Scots pine seedlings as protectors against increased aluminium content in the soil.

LITERATURE CITED

ARNOLDS E. J. M., 1991. Decline of ectomycorrhizal fungi in Europe. Agriculture Ecosystems and Environment 35: 209-244.

BOUDOT J. P., BECQUER T., MERLET D., ROUILLER J., 1994. Aluminium toxicity in declining forests: a general overview with a seasonal assessment in a silver fir forest in the Vosges mountains (France). Ann. Sci For. 51: 27-51.

BROWN M. T., WILKINS D. A., 1985a. Zinc tolerance of *Amanita* and *Paxillus*. Trans. Br. Mycol. Soc. 84: 367-369.

BROWN M. T., WILKINS D. A., 1985b. Zinc tolerance in *Betula*. New Phytol. 99: 101-106.

BROWNING M. H. R., HUTCHINSON T. C., 1991. The effects of aluminium and calcium on the growth and nutrition of selected ectomycorrhizal fungi of jack pine. Can. J. Bot. 69: 1691-1699.

COLPAERT J. V., VAN ASSCHE J. A., 1987. Heavy metal tolerance in some ectomycorrhizal fungi. Funct. Ecol. 1: 415-421.

CUMMING J. R., WEISTEIN L. H., 1990. Aluminium-mycorrhizal interactions in the physiology of pitch pine seedlings. Plant and Soil 125: 7-18.

DARLINGTON A. B., RAUSER W. E., 1988. Cadmium alters the growth of the ectomycorrhizal fungus *Paxillus involutus*: A new growth model accounts for changes in branching. Can. J. Bot. 66: 225-229.

DENNY H. J., WILKINS D. A., 1987. Zinc tolerance in *Betula* spp. III. Variation in response to zinc among ectomycorrhizal associates. New Phytol. 106: 535-544.

DIGHTON J., SKEFFINGTON R. A., 1987. Effects of artificial acid precipitation on the mycorrhizas of Scots pine seedlings. New Phytol. 107: 191-202.

GODBOLD D. L., FRITZ E., HUTTERMAN A., 1988. Aluminium toxicity and forest decline. Proceedings of the National Academy of Science. USA 85: 3888-3892.

GODZIK S., 1990. Impact of air pollution on agriculture and horticulture in Poland. In: Ecological Risks. Pesperctives from Poland and the United States. Grodzińki W., Cowling E. B., Breymeyer A. I. (ed.). National Academy Press, Washington DC. pp. 196-214.

GÖRANSON A., ELDHUSET T. D., 1991. Effects of aluminium on growth and nutrient uptake of small *Picea abies* and *Pinus sylvestris* plants. Trees 5: 136-142.

HENTSCHEL E., GODBOLD D.L., MARSCHNER P., SCHLEGEL H., JENTSCHKE G., 1993. The effects of *Paxillus involutus* Fr. on aluminium sensitivity of Norway spruce seedlings. Tree Physiol. 12: 379-390

HERNIK I., 1987. The danger of industrial pollution to Wielkopolska forests (in Polish). In: Biological Reaction of Trees to Industrial Pollution. Materials from the 2nd National Symposium. Siwecki R. (ed). Adam Mickiewicz University Press, Poznań, pp. 111-119.

HINTIKKA V., 1988. High aluminium tolerance among ectomycorrhizal fungi. Karstenia, 28: 41-44.

JENTSCHKE G., GODBOLD D. L., HÜTTERMAN A., 1991a. Culture of mycorrhizal tree seedlings under controlled conditions: effects of nitrogen and aluminium. Physiol. Plant. 81: 408-416.

JENTSCHKE G., SCHLEGEL H. I., GODBOLD D. L., 1991b. The effect of uptake and distribution of magnesium and calcicum in roots of mycorrhizal Norway spruce seedlings. Physiol. Plant. 82: 266-270.

JONES M. D., HUTCHINSON T. C., 1986. The effect of mycorrhizal infection on the response of *Betula papyrifera* to nickel and cooper. New Phytol. 102: 429-442.

JONES M. D., HUTCHINSON T. C., 1988. Nickel toxicity in birch seedlings infected with *Lactarius rufus* or *Scleroderma flavidum* I. Effects on growth, photosynthesis, respiration and transpiration. New Phytol. 108: 451-459.

JONGBLOED R. H., BORST-PAUWELS G. W. F. H., 1992. Effects of aluminium and pH on growth and potassium uptake by three ectomycorrhizal fungi in liquid culture. Plant and Soil 140: 157-165.

KIELISZEWSKA-ROKICKA B., 1992. Effect of nitrogen level on acid phosphatase activity of eight strains of ectomycorrhizal fungus *Paxillus involutus* cultured in vitro. Plant and Soil 139: 229-238.

KIELISZEWSKA-ROKICKA B., RUDAWSKA M., 1996. The variability of *Paxillus involutus* strains in IAA-synthesizing activity. In: Mycorrhizas in integrated systems from genes to plant development. Proceed. Fourth European Symposium On Mycorrhizas, Granada, 11-14 July 1994. Azcon-Aguilar C. Barea J. M. (ed.). pp. 345-348.

KIELISZEWSKA-ROKICKA B., RUDAWSKA M., LESKI T., 1996. Influence of aluminium on growth of mycelia and acid phosphatase activity of ectomycorrhizal symbionts of Scots pine (in Polish). In: Biological Reactions of Trees to Industrial Pollution. Material from the 3rd National Symposium, Poznań-Kórnik, 23–26.05.1994. Siwecki R. (ed.). Sorus, Poznań, pp. 471-479.

KOTTKE I., 1991. Electron energy loss spectroscopy and imaging techniques for subcellular localization of elements in mycorrhizas. Methods Microbiol. 23: 369-382.

LAIHO O., 1970. *Paxillus involutus* as a mycorrhizal symbiont of forest trees. Acta For. Fenn. 106: 1-72.

LESKI T., RUDAWSKA M., KIELISZEWSKA-ROKICKA B., 1995. Intraspecific aluminium response in *Suillus luteus*, an ectomycorrhizal symbiont of Scots pine. Acta Soc. Bot. Pol. 64: 97-105.

MARTIN F., RUBINI P., CÔTE R., KOTTKE I., 1994. Aluminium polyphosphate complexes in the mycorrhizal basidomycete *Laccaria bicolor*: A ^{27}Al-nuclear magnetic resonanse study. Planta 194: 241-246.

MAZURSKI K.R., 1990. Industrial Pollution: The Threat to Polish Forest. Ambio 19: 70-74.

NOWOSIELSKI O., 1974. Methods of necessity of fertilization. PWRiL, Warszawa.

REICH P. B., OLEKSYN J., TJOELKER M. G., 1994. Relationship of aluminium and calcium to net CO_2 exchange among diverse Scots pine provenances under pollution stress in Polland. Oecologia 97: 82-92.

RUDAWSKA M., 1986. Studies on the symbiotic properties of mycorrhizal fungi of Scots pine (*Pinus sylvestris* L.) as affected by age of the fungal culture. Arboretum Kórnickie 31: 269-280.

RUDAWSKA M., KIELISZEWSKA-ROKICKA B., LESKI T., OLEKSYN J., 1995. Mycorrhizal status of a Scots pine (*Pinus sylvestris* L.) plantation affected by pollution from a phosphate fertilizer plant. Water, Air and Soil Pollut. 85: 1281-1286.

RUDAWSKA M., KIELISZEWSKA-ROKICKA B., LESKI T., 1996a. Effect of acid rain and aluminium on the mycorrhizas of *Pinus sylvestris*. In: Mycorrhizas in integrated systems from genes to plant development. Proceed. Fourth European Symposium On Mycorrhizas, Granada, 11-14 July 1994. Azcon-Aguilar C., Barea J. M. (ed.), pp: 469-471.

RUDAWSKA M., KIELISZEWSKA-ROKICKA B., LESKI T., 1996b. Mycorrhizal status as an indicator of stress of Scots pine (*Pinus sylvestris* L.), grown under influence of industrial pollutants (in Polish). In: Biological Reactions of Trees to Industrial Pollution. Material from the 3rd National Symposium, Poznań–Kórnik, 23–26.05.1994. Siwecki R. (ed.). Sorus, Poznań, pp. 611-623.

SHAW P. J. A., DIGHTON J., POSKITT J., 1992. Studies on the effects of SO_2 and O_3 on the mycorrhizas of Scots pine by observations above and below ground. In: Mycorrhizas in Ecosystems. Read D. J., Lewis D. H., Fitter A. H., Alexander I. J. (ed.). CAB International, pp. 208-213.

STATISTIX. Version 4.1 Analytical Software.

THOMPSON G. W., MEDVE R. J., 1984. Effects of aluminium and magnanese on growth of ectomycorrhizal fungi. Appl. Environ. Microbiol. 48: 556-560.

TOMASZEWSKI M., WOJCIECHOWSKA B., 1974. The role of regulators released by fungi in pine mycorrhizae. In: Plant Growth Substances. Hirokawa Publishing Co., Tokyo, Japan, pp. 217-227.

TURNAU K., 1990. The influence of industrial dust on macromycetes in a Pino-Quercetum forest. Zeszyty Naukowe Uniwersytetu Jagielońskiego. Prace Botaniczne 21: 173-191.

TURNAU K., KOZŁOWSKA A., 1991. Influence of cadmium dust on heavy metal concentration in macromycetes. Zeszyty Naukowe Uniwersytetu Jagielońskiego. Prace Botaniczne 22: 135-144.

TURNAU K., KOTTKE I., OBERWINKLER F., 1993. Element localization in *Paxillus involutus* – *Pinus sylvestris* mycorrhizas using electron energy loss spectroscopy and imaging. Bot. Acta 245-249.

TYLER G., 1980. Metals in sporophores of *Basidiomycetes*. Trans. Br. mycol. Soc. 74: 41-49.

ULRICH B., 1983. Soil acidity and its relation to acid deposition. In: Effects of Accumulation of Air Pollutants in Forest Ecosystems. Ulrich B., Pankrath J. (ed.) Reidel Publishing Company, Dordrecht, Boston, London, pp. 127-146.

WILKINS D. A., HODSON M. J., 1989. The effects of aluminium and *Paxillus involutus* Fr. on the growth of Norway spruce [*Picea abies* (L.) Karst.]. New Phytol. 113: 225-232.

ZGIRSKI A., GONDKO R., 1976. Biochemical calculation (in Polish). PWN, Warszawa, pp. 23-28.

TOLERANCJA NA GLIN SZCZEPÓW EKTOMIKORYZOWEGO GRZYBA *PAXILLUS INVOLUTUS* FR. POCHODZĄCYCH Z TERENÓW SKAŻONYCH ORAZ KONTROLNYCH

STRESZCZENIE

Owocniki ektomikoryzowego grzyba *Paxillus involutus* zebrano na stanowiskach sosny *Pinus sylvestris*, rosnącej na terenie skażonym kwaśnymi deszczami o podwyższonej zawartości glinu w glebie (Luboń) oraz na terenach wolnych od bezpośrednich zanieczyszczeń (Kórnik, Puszcza Nadnotecka). Zawartość glinu w kapeluszach owocników pochodzących z terenu skażonego oraz terenów kontrolnych była podobna i wykazywała symptomy biokoncentracji. W trzonach owocników *P. involutus* zebranych na terenie Puszczy Nadnoteckiej stwierdzono niższą zawartość glinu niż na pozostałych dwu stanowiskach. Kiedy sterylne kultury testowanych szczepów hodowano na pożywce płynnej zawierającej 100 mg Al/L^{-1} stwierdzono wyraźną tendencję do biokoncentracji glinu w grzybni. Nie wykazano wyraźnej zaleności pomiędzy ilością akumulowanego glinu w grzybni w hodowli *in vitro* a miejcem zbioru owocnika z którego pochodził dany szczep. 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 współczynnik tolerancyjności na ten pierwiastek. Większość szczepów wykazywała wyraźną tolerancję na glin i rosła dobrze nawet przy najwyższym stężeniu tego pierwiastka w podłożu (1000 mg/L^{-1}). Nie stwierdzono zależności pomiędzy miejscem zbioru owocnika a jego wrażliwością na glin w kulturze *in vitro*. Tolerancyjne oraz wrażliwe na glin i szczepy *P. involutus* przeznaczone do dalszych badań pochodziły zarówno z terenów skażonych jak i kontrolnych. Uzyskane wyniki dyskutowane są na tle danych z literatury dotyczących znacznego wewnątrzgatunkowego zróżnicowania cech u ektomikoryzowego symbionta *P. involutus*.

SŁOWA KLUCZOWE: grzyby ektomikoryzowe, owocniki, wewnątrzgatunkowe zróżnicowanie, toksyczność jonów glinu, kwaśne deszcze, *Pinus sylvestris*.