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Differential response of some ectomycorrhizal fungi to cadmium *in vitro*

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SUMMARY

Three ectomycorrhizal fungi originating from *Pseudotsuga menziesii* plantations were exposed to cadmium (Cd) concentrations up to 10 μM. *Laccaria bicolor* and *Lactarius hepaticus* showed a much higher sensitivity to Cd than *Lactarius rufus*. At 1 μM Cd, reductions in radial growth and in dry weight production were 91% and 95% in *L. bicolor*, 100% and 100% in *L. hepaticus* and 14% and 8% in *L. rufus*, respectively. Cadmium increased the lag time of radial growth in *L. bicolor* but not in the other fungi examined.

Key-words: cadmium; ectomycorrhizal fungi, growth, *Laccaria bicolor*, *Lactarius hepaticus*, *Lactarius rufus*.

INTRODUCTION

Heavy metal tolerance in mycorrhizal trees is influenced by the ectomycorrhizal fungi involved in the symbiotic association (Brown & Wilkins 1985a; Jones & Hutchinson 1986). Differential response among isolated ectomycorrhizal fungi is reported for several heavy metals (Brown & Wilkins 1985b; Colpaert & van Assche 1987; Jones & Hutchinson 1988) including cadmium (Cd) (McCreight & Schroeder 1982). Although the Cd content of forest soils is generally extremely low, accumulation of Cd occurs in the vicinity of traffic and certain industrial activity and may reach toxic concentrations in the future (Zöttl 1985), additionally, soil acidification will increase the solubility of Cd.

This work is part of a study on the effects of acid rain factors on ectomycorrhizal fungi of Douglas-firs and has the present objective of determining the effect of Cd on biomass growth and radial growth of three selected ectomycorrhizal fungi of the Douglas-fir.

MATERIALS AND METHODS

Three ectomycorrhizal fungi were isolated from fruitbodies in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) plantations in The Netherlands by A. E. Jansen (Department of Phytopathology, Agricultural University Wageningen). *Laccaria bicolor* (R. Mre.) Orton and *Lactarius rufus* (Scop.) Fr. originated from Kootwijk and *Lactarius hepaticus* Plowr. ap. Boud. originated from Amerongen. At these sites contamination by Cd was low. Determination of Cd content in the litter layer of soil samples from Kootwijk and

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Amerongen showed 0·81 and 0·87 mg Cd per kg dry matter, respectively (Kleijn *et al.* 1989). However, in the soil solution of the mineral layers beneath the litter Cd was not or was rarely detectable. Mycelial cultures of the fungi used as inoculum for the growth experiments were grown on MMN (modified Melin–Norkrans) agar (Marx 1969) which contained 10 g D-glucose, 3 g malt extract (Difco), 0·2 g NH_4Cl (instead of 0·25 g $(NH_4)_2HPO_4$), 0·5 g KH_2PO_4, 0·15 g $MgSO_4$, 50 mg $CaCl_2$, 12 mg $FeCl_3$, 25 mg NaCl, 0·1 mg thiamine–HCl, 15 g bacto-agar (Difco) and distilled water to 1 litre.

In the growth experiments a 10 mM succinic acid/Tris buffer (pH 4·0) was applied to the nutrient medium. We chose pH 4·0 for carrying out our experiments because at lower pH values growth of *L. bicolor* was reduced severely, whereas growth of the two other more acidophilic fungi was still appreciable at this pH. The pH of the habitat from which the fungi were originating ranged from pH 3 to 4 (Kleijn *et al.* 1989).

In the solid medium growth experiment, Petri dishes were filled with 20 ml of the MMN agar medium and $CdCl_2$ was added from a sterile stock solution to obtain Cd concentrations of 0, 0·1, 0·5, 1 and 10 μM. Each plate was inoculated with a 6-mm plug from the edge of a fungal colony and incubated at room temperature (±22°C) in the dark. Colony diameters were determined several times during the growth period. After that, the fungal dry weights were determined. The colonies were cut out of the media and autoclaved in water at 120°C in order to dissolve the agar. Then they were filtered on pre-weighed filters, dried at 105°C for 24 h and weighed.

For *L. bicolor* Cd sensitivity was also tested on liquid medium. This medium had the same composition as the solid one with omittance of the agar. After sterilization, 100 ml Erlenmeyer flasks with 40 ml of nutrient solution were cooled to room temperature. Then $CdCl_2$ was added to give the same concentrations as in the solid medium experiment. Each flask was inoculated with one mycelial plug and shaken continuously on a rotary shaker at 95 r.p.m. at room temperature in the dark. During the growth period of 31 days the nutrient solutions were not renewed. The colonies formed submerged spheres of mycelia and the dry weights were determined after rinsing in distilled water and drying at 105°C for 24 h.

Both the solid medium and the liquid medium experiments were carried out in triplicate.

RESULTS

The radial growth of the ectomycorrhizal fungi was clearly reduced by Cd (Fig. 1). Both *L. bicolor* and *L. hepaticus* appeared to be far more sensitive to Cd than *L. rufus*. *L. hepaticus* especially, was very intolerant to Cd, showing no growth at all above 0·5 μM Cd. This fungus showed almost constant radial growth after a lag period of 10 days. The lag period was defined as the time until growth was visible. The growth rates were reduced by 38 and 85% at 0·1 and 0·5 μM Cd, respectively. With *L. bicolor* a lag period in growth was also found. Contrary to *L. hepaticus* this lag period was small or even absent in the absence of Cd and increased with increasing Cd concentrations up to 10 μM. In the case of *L. bicolor* we stopped growth at 31 days instead of at 45 days, because in an orientating experiment it was found that radial growth in the absence of Cd became reduced after 31 days, whereas growth in the presence of Cd still persisted up to 45 days with a constant rate. Therefore, extending the experiment to 45 days would lead to an under-estimation of the effect of Cd on final growth. The radial growth rates were reduced relative to the control by 3, 30, 80 and 90% at 0·1, 0·5, 1 and 10 μM Cd, respectively. *L. rufus* was the most Cd-tolerant fungus examined. Cadmium did not affect radial growth during the

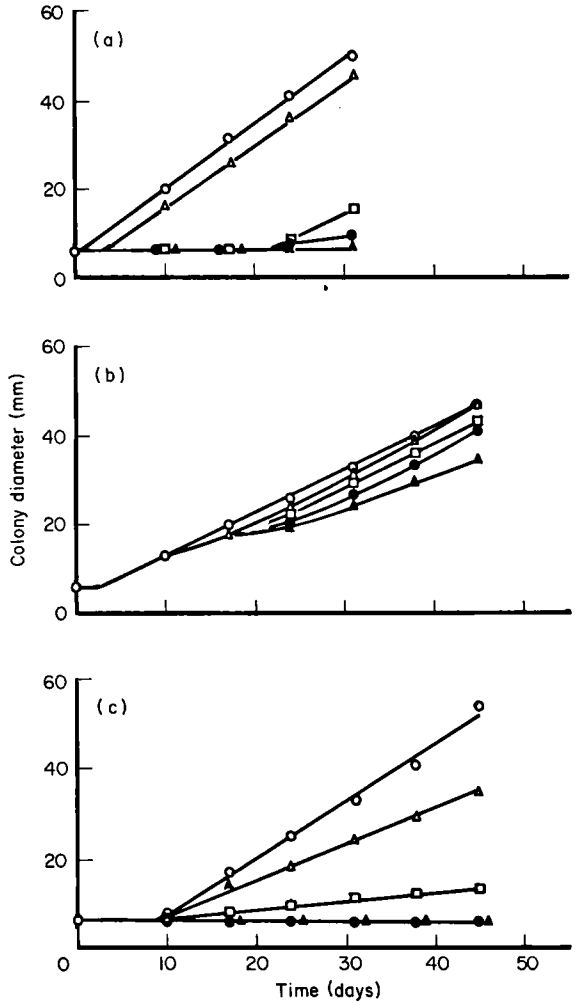

Fig. 1. Growth curves of *Laccaria bicolor* (a), *Lactarius rufus* (b) and *Lactarius hepaticus* (c) on buffered solid MMN medium at various cadmium concentrations: (○) 0 μM Cd, (△) 0·1 μM Cd, (□) 0·5 μM Cd, (●) 1 μM Cd, (▲) 10 μM Cd. The medium was buffered with 10 mM succinic acid/Tris at pH 4·0. Results are means of an experiment in triplicate. Standard errors of the mean are maximum at 1·2 and are not included in the figure.

first 17 days; then radial growth showed a transient reduction, but recovered completely at concentrations up to 1 μM Cd. Only at 10 μM Cd did a small reduction in the growth rate persist, amounting to 30% of the control value.

Figure 2 shows that dry weight production of *L. bicolor* at the end of the growth period was slightly but significantly increased by addition of 0·1 μM Cd without a concomitant stimulation of radial growth. Further increase in the Cd concentration led to a sharp reduction in both radial growth and dry weight production. The reductions in dry weight production exceeded that of radial growth. On the other hand, reductions in dry weight found with the two *Lactarius* spp. were less severe than those found for radial growth. This was especially apparent for *L. hepaticus*. *L. rufus* showed a slight stimulation of dry weight production at 0·1 μM Cd just as was found for *L. bicolor*.

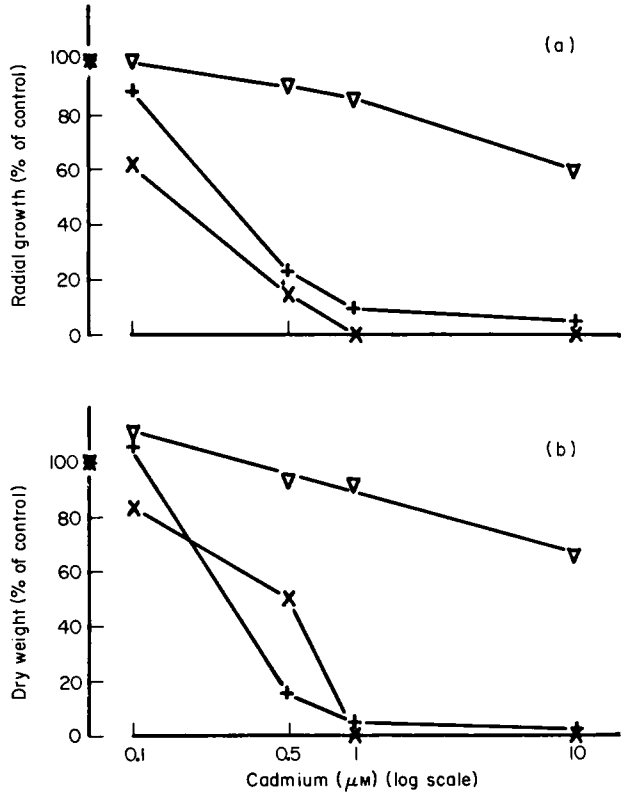

Fig. 2. Radial growth (a) and biomass production (b) of *Laccaria bicolor* (+), *Lactarius rufus* (▽) and *Lactarius hepaticus* (×), after growth on buffered solid MMN medium at various cadmium concentrations. Growth period was 31 days for *Laccaria bicolor* and 45 days for both *Lactarius* spp. Results are means of an experiment in triplicate. Standard errors of the mean are maximum at 3% for both radial growth and dry weight production and are not included in the figure.

To examine whether the presence of agar in the solid medium reduced the free Cd concentration appreciably we made a comparison between the effect of Cd in buffered liquid medium and buffered solid medium on the growth of *L. bicolor*. Table 1 shows that the reproducibility in liquid medium is lower than in the solid medium. The average SEM are five times higher in the liquid medium than in the solid medium. This may be the reason that no significant stimulation of growth was found at 0·1 μM Cd in the liquid medium. At both 0·5 and 1 μM Cd, growth was reduced significantly less in liquid medium than in solid medium, whereas at 10 μM Cd the reverse was found.

DISCUSSION

Comparison of the various fungi as far as their sensitivity to Cd is concerned is complicated by the fact that the time dependence of the effect of Cd upon radial growth is quite different for the three species examined. With *L. hepaticus* the lag period of growth is independent of the Cd concentration whereas with *L. bicolor* the lag period increases with Cd concentrations up to 10 μM. The latter may indicate that *L. bicolor* is very sensitive to

Table 1. Comparison of biomass production of *Laccaria bicolor* after 31 days of growth in buffered liquid MMN medium and in buffered solid MMN medium at various cadmium concentrations

	Dry weight (% of control)	
Cd (μM)	Liquid medium	Solid medium (agar)
0	100·0 ± 4·0	100·0 ± 0·6
0·1	100·0 ± 7·5	105·2 ± 1·9
0·5	28·6 ± 5·5	15·2 ± 1·0
1	8·7 ± 2·2	5·0 ± 0·4
10	0·9 ± 0·5	2·1 ± 0·1

Results are means ± SEM of an experiment in triplicate.

Cd and that initially at Cd concentrations of 0·5 μM and higher, growth is impaired completely. After about 24 days, growth is resumed though still with a reduced rate, the extent of which depends upon the Cd concentration. This recovery of growth may be due to an adaption of the cells to Cd developed during the lag period (Trevors *et al.* 1986). With *L. rufus* yet another time dependence is found. Apparently growth is initially unhampered by Cd. Only after a lag period of 17 days is a small but significant, more or less transient, inhibition of radial growth by Cd found.

In accordance with the findings of McCreight & Schroeder (1982) a great variation in Cd sensitivity among ectomycorrhizal fungi was found. Our experiments showed that the growth of two of the three ectomycorrhizal fungi in nutrient media was already reduced more than 50% at the relatively low Cd concentration of 0·5 μM.

It has to be considered that Cd toxicity is influenced by the physicochemical characteristics of any environment (Gadd & Griffiths 1978). In our media a Tris/succinate buffer was applied. The buffer anion binds some Cd. This binding, however, is relatively small, as shown by B.G.F. Kessels in our laboratory using a Cd selectrode. At pH 4·0 binding of Cd by succinate amounts to approximately 5%.

There were no great differences between the effect of Cd on biomass production in solid medium and liquid medium. This indicates that eventual binding of Cd to the solid medium (Gadd 1983) is small and is not affecting the apparent sensitivity to Cd appreciably. The absence of an appreciable binding of Cd to the agar is probably due to the low pH applied by us.

In the solid medium at 0·1 μM Cd, a small stimulatory effect on the dry weight production of *L. bicolor* occurs. Growth stimulation at sub-inhibitory concentrations of toxic agents including Cd, so called 'hormesis', is a well known phenomenon (Stebbing 1982).

Interaction of Cd with fungi not only depends upon the free Cd concentration but also upon the presence of other cations or anions in the medium (Trevors *et al.* 1986). This is a complication in extrapolating *in-vitro* growth response to field situations. As far as cations are concerned, Ca generally decreases the Cd toxicity, whereas Mg has no or less effect (Babich & Stotzky 1981; Abel & Barlocher 1984; Kessels *et al.* 1985).

The high level of Cd sensitivity of *L. bicolor* and *L. hepaticus* may be a consequence of the low Cd content of the site from which they originate. Verkleij & Prast (1989) reported Cd tolerance in populations of *Silene vulgaris* (Moench.) Garke originating from heavily

Cd contaminated sites and Cd intolerance in a population from an uncontaminated site. The same phenomenon was also observed for several ectomycorrhizal fungi with respect to zinc and copper tolerance (Colpaert & van Assche 1987).

In Cd-contaminated soils the vitality of the host tree and the ectomycorrhizal formation may be affected more or less, depending on the fungal species involved. This may lead to a selection for the most resistant species. However, prediction of Cd toxicity on natural sites is difficult, even for soils with a high content of Cd, due to the variation in fixation capacity for heavy metals among different soil types (Zöttl 1985).

Our study shows that ectomycorrhizal fungi may be very sensitive to Cd and that large differences exist between this sensitivity for various species. *L. rufus* may be well suited as an ectomycorrhizal fungus for trees in highly Cd-contaminated soil.

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