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SHORT NOTE

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Effects of cadmium on growth and glucose utilisation of ectomycorrhizal fungi in vitro

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Abstract Effects of Cd on growth and glucose utilisation of *Paxillus involutus*, *Rhizopogon subcaerulescens* and *Suillus bovinus* were investigated in vitro in liquid culture. *S. bovinus* was the species most sensitive to Cd in terms of dry matter production and *P. involutus* was less sensitive than *R. subcaerulescens*. Greater production of hyphae of *P. involutus* than the other fungi appeared to confer some degree of Cd resistance, possibly by binding Cd onto cell walls. Growth of the three fungi was increased by glucose addition. While Cd significantly reduced dry matter production of the fungi, there were no significant differences in glucose consumption caused by Cd treatment. This suggests that the use of glucose might have been diverted to detoxification and/or repair mechanisms. Further studies on respiration rates and energy metabolites of these fungi under Cd exposure are needed in order to clarify the results of the present study.

Keywords Cadmium · Dry matter production · Ectomycorrhizal fungi · Glucose

Introduction

Cd has been shown to be one of the most toxic heavy metals for ectomycorrhizal fungi (McCreight and Schroeder 1982; Tam 1995). However, a number of studies have also reported inter- and intraspecific differences in sensitivity of ectomycorrhizal fungi to Cd (Colpaert and Van Assche 1992; Colpaert et al. 2000). Differential magnitude of sensitivity to Cd toxicity in these fungi indicates that less sensitive species may confer a certain level of resistance to Cd on their host plants in contaminated soil (Jongbloed and Borst-Pauwels 1990). Colpaert and Van Assche (1992) and Colpaert et al. (2000) found greater Cd resistance in isolates of *Suillus bovinus* and *Suillus luteus* from Cd- and Zn-contaminated areas than those from uncontaminated areas in in vitro studies. However, Howe et al. (1997) and Blaudez et al. (2000c) did not observe any correlation between heavy metal-resistance of fungal isolates and the soil of origin.

Fungal requirement for C is greater than that for any other element. While some basidiomycetes have an ability to decompose organic matter, obligate ectomycorrhizal fungi which have no saprotrophic abilities, such as *Paxillus involutus* and *S. bovinus*, require a C source from their host plant (Haselwandter et al. 1990). Glucose is known to be the best C source for ectomycorrhizal fungi (Palmer and Hacskaylo 1970). Detoxification/repair mechanisms for heavy metals may be associated with a significant metabolic C cost (Andersen and Rygiewicz 1991). However, little is known about the C utilisation of ectomycorrhizal fungi in media containing Cd, a non-essential and toxic element.

We have investigated the responses of *Pinus sylvestris* to soil Cd and mycorrhizal infection (Kim 2001). Because *P. sylvestris* depends highly on ectomycorrhizal symbiosis for nutrient uptake (Kieliszewska-Rokicka et al. 1998), Cd effects on its fungal partner may also influence the growth and maintenance of *P. sylvestris* in forest ecosystems. Three ectomycorrhizal fungi, *Paxillus involutus*, *S. bovinus* and *Rhizopogon subcaerulescens*, which have been demonstrated to form ectomycorrhizas with *Pinus sylvestris*, were selected for the present study. *Paxillus* usually occurs in both deciduous and coniferous forests (Villeneuve et al. 1989) and has a broad host range, whereas *Suillus* and *Rhizopogon* seem to be ectomycorrhizal exclusively with members of the Pinaceae (Finlay 1989; Molina and Trappe 1994). These fungi were also chosen because of the ease of cultivation and fast growth rates. The objective of the present study was to investigate the effect of Cd on growth and glucose utilisation of the three species of ectomycorrhizal fungi.

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Materials and methods

Fungal culture

Cultures of *Paxillus involutus* (Batsch. ex Fr.) Fr. (isolate no. 87017), *Suillus bovinus* (Fr.) O. Kuntze (isolate no. 096) and *Rhizopogon subcaerulescens* Smith (isolate no. 379) were obtained from Professor D. J. Read at the University of Sheffield in April 1999. The stock cultures were grown in a 20°C incubator in the dark on modified Melin-Norkrans' (MMN) agar medium (Marx 1969) containing (g l^{-1}): KH_2PO_4 (0.55), $(NH_4)_2HPO_4$ (0.25), $MgSO_4.7H_2O$ (0.15), $CaCl_2.2H_2O$ (0.05), NaCl (0.025), $FeCl_3.6H_2O$ (0.012), glucose (2.5), malt extract (10) and agar (15). The medium was adjusted to pH 4.7 before sterilisation for 15 min at 121°C.

Glucose utilisation of fungi

Glucose and $CdCl_2$ were dissolved in distilled water and incorporated into the MMN basal liquid medium to give concentrations of 0, 1, 5, 10 or 20 g l^{-1} and 0 and 1 mg l^{-1}, respectively. Prior to inoculation in liquid MMN medium, agar plugs (7 mm diameter) from actively growing fungal colonies on MMN agar were removed and incubated on 15% (w/v) water agar. The plugs were allowed to remain until the mycelium projected around the upper periphery of the plugs sufficiently to enable them to float on a liquid medium (Palmer and Hacskaylo 1970). One 7-mm-diameter plug of inoculum was floated on the surface of the liquid medium and then incubated at 20°C in the dark. Four replicates were used for each treatment. After a 14-day incubation, the mycelium from a single replicate was collected on a Whatman no. 1 filter paper, washed, dried at 80°C for 48 h and weighed. Glucose concentrations of the medium were analysed by the Nelson (1944) and Somogyi (1945) procedure.

Statistical analysis

Data were analysed using the STATISTICA (version 5.5; Statsoft, USA) statistical package and were subjected to the Kolmogorov-Smirnov goodness of fit test of normality and Cochran's homogeneity of variance test. The interaction between glucose and Cd on the growth of each fungal species was evaluated by a two-way ANOVA. Statistically significant differences between means were identified by Tukey's honestly significant difference (HSD) test (P<0.05). Plots of dry matter production in the medium containing various concentrations of glucose were fitted by using Sigma Plot (version 7.0; SPSS, USA).

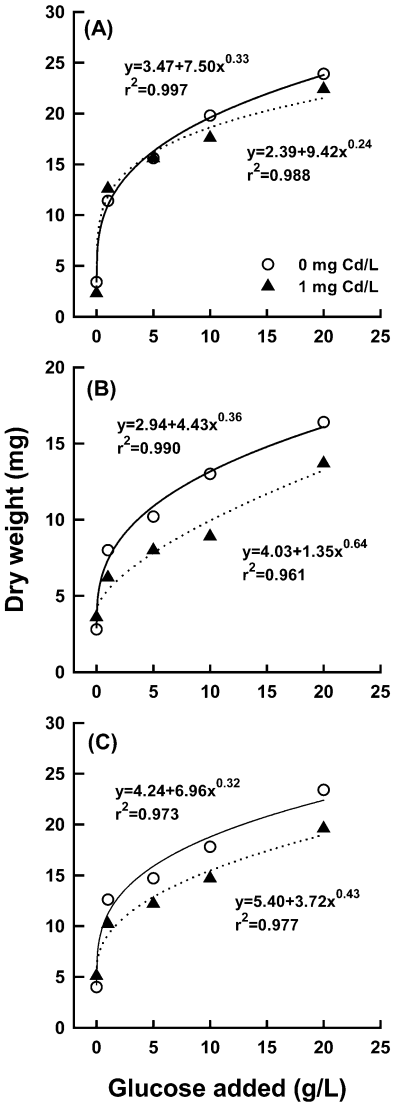

Fig. 1 Growth of **A** *Paxillus involutus*, **B** *Suillus bovinus* and **C** *Rhizopogon subcaerulescens* in liquid modified Melin-Norkrans' medium with various concentrations of glucose and Cd for 14 days

Results

Dry weight of the three fungi was increased (P<0.001) by glucose addition (Fig. 1). The results of the Tukey's HSD test showed differences(P<0.001) in dry weight between all levels of glucose treatments. Although Cd had an overall effect (P<0.05) on the growth of *Paxillus involutus*, Tukey's HSD test showed no differences (P>0.05) between the growth of the fungi in Cd-free or Cd-containing medium at a given level of glucose treatment. Cd also had an overall effect (P<0.001) on the growth of *S. bovinus* and *R. subcaerulescens*. Whilst *S. bovinus* was the species most negatively affected by Cd treatment, *P. involutus* was the least sensitive to Cd. Since the dry weight of the three fungi increased further at glucose concentrations of 10 and 20 g l^{-1} in the absence of Cd, relative to the l g l^{-1} treatment, an interaction (P<0.05) between glucose and Cd was observed. The mycelial dry weight was also significantly related to fungal species. The overall dry weight of *P. involutus* was greater than that of *S. bovinus* (P<0.001) and *R. subcaerulescens* (P<0.01). *R. subcaerulescens* also produced considerably greater dry mass than *S. bovinus* (P<0.001).

Cd had no overall effect (P>0.05) on the glucose concentrations in the liquid MMN medium after the 14-day incubation of the three fungi (Table 1). Furthermore, there was no interaction (P>0.05) between Cd and glucose. *S. bovinus* consumed more glucose than the other fungi (P<0.05) in order to produce the same amount of dry matter.

The economic coefficient (the percentage of mycelium dry weight per amount of glucose consumed) was calculated in order to determine the efficiency of conversion of glucose to mycelium. Cd had no overall

Table 1 Glucose concentrations (g l^{-1}) of liquid modified Melin-Norkrans' medium after growth of ectomycorrhizal fungi with various concentrations of glucose and Cd for 14 days. Data are means±SE. *Values for each column* with the *same letters* are not significantly different (Tukey's honestly significant difference test, $P<0.05$). *F*-ratios and *P*-values are from the two-way ANOVA. *ND* Not detected

Glucose (g l^{-1})	Cd (mg l^{-1})	Glucose concentration (g l^{-1})		
		Paxillus involutus	*Suillus bovinus*	*Rhizopogon subcaerulescens*
0	0	ND	ND	ND
	1	ND	ND	ND
1	0	0.0±0.0 d	0.1±0.0 d	0.0±0.0 d
	1	0.0±0.0 d	0.1±0.0 d	0.0±0.0 d
5	0	1.4±0.2 cd	1.5±0.4 cd	1.8±0.1 cd
	1	1.5±0.3 cd	2.0±0.5 bcd	2.6±0.4 bcd
10	0	5.0±1.0 bc	5.1±1.0 bc	5.1±1.1 bc
	1	5.9±0.8 b	5.3±0.8 b	6.3±0.5 b
20	0	10.4±1.3 a	10.8±0.8 a	13.2±1.6 a
	1	10.7±1.2 a	10.4±2.1 a	14.7±1.5 a

ANOVA	*F*	*P*	*F*	*P*	*F*	*P*
Effect of Cd	0.3	0.567	0.1	0.779	2.2	0.150
Effect of glucose	73.2	<0.001	57.9	<0.001	103.1	<0.001
Cd×glucose	0.1	0.944	0.2	0.875	0.3	0.829

effect ($P>0.05$) on the economic coefficient of the three fungi. There was also no interaction between Cd and glucose. Overall, the economic coefficient was considerably decreased ($P<0.001$) at glucose levels above 5 g l^{-1} and the economic coefficient at 20 g l^{-1} glucose was lower ($P<0.05$) than that in the 10 g l^{-1} treatment. The economic coefficient was different ($P<0.001$) between fungal species. The overall economic coefficient of *S. bovinus* was 8% and 9% lower ($P<0.001$) than that of *P. involutus* and *R. subcaerulescens*, respectively. In the 1 g l^{-1} glucose treatment, the economic coefficient of Cd-free *S. bovinus* (34%) was lower ($P<0.05$) than that of Cd-free *P. involutus* (46%) and *R. subcaerulescens* (51%). In the same glucose treatment, the economic coefficient of Cd-treated *S. bovinus* (27%) was also lower ($P<0.05$) than that of Cd-treated *P. involutus* (50%); however, there were no differences ($P>0.05$) between the economic coefficient of Cd-treated *R. subcaerulescens* (42%) and that of other fungi.

Discussion

The sensitivity to Cd in terms of dry mass production was highest in *S. bovinus*. This high sensitivity of *S. bovinus* to Cd has been demonstrated in both agar and liquid media elsewhere (Blaudez et al. 2000b). Although the growth of the three fungi tested in the present study was greatly inhibited by Cd, the magnitude of sensitivity was different, probably due to differences in Cd detoxification mechanisms. Higher growth rates of *P. involutus* in the present study appeared to contribute to reduced Cd toxicity. Faster growing fungi which produce more hyphae may be less sensitive to Cd due to the presence of more binding sites. Having observed significant transport of Cd into the vacuoles of *P. involutus*, Blaudez et al. (2000a) suggested that vacuolar compartmentation as well as binding of Cd on cell walls may be two essential detoxification mechanisms in *P. involutus*. *P. involutus* has also been shown to produce oxalic acid and secrete it into the surrounding environment (Lapeyrie et al. 1987). Formation of metal oxalate also might reduce Cd bioavailability in this species, as demonstrated by Sayer and Gadd (1997) for *Aspergillus niger* Tiegh.

Glucose is known to be the preferred C source of ectomycorrhizal fungi. Dry matter production of *S. bovinus* has been shown to be highest in medium containing glucose (Lamb 1974) compared with 25 other C compounds. Glucose also increased the dry mass of *P. involutus* 13 times more than the same concentration of sucrose (Hughes and Mitchell 1995). In agreement with the study by Jayko et al. (1962), which reported increased dry matter production of *Lactarius torminosus* (Schaeff.: Fr.) S.F. Gray, the present study showed that fungal dry mass production significantly increased with increasing concentrations of glucose. Adequate provision of carbohydrates is, therefore, crucial for the growth of ectomycorrhizal fungi. Glucose might affect Cd uptake by ectomycorrhizal fungi since Cd uptake involves both a non-metabolic and a metabolic process (Blaudez et al. 2000a).

Once Cd is taken up by ectomycorrhizal fungi using energy from glucose, Cd may affect glucose utilisation. Blaudez et al. (2000a) suggested metabolic Cd uptake via Ca carriers in *P. involutus*. Since Cd can compete with other cations for binding sites and transport mechanisms, Cd may be easily transported and accumulated with other solutes from the medium. Then, accumulated Cd in the cytoplasm might inhibit glucose uptake and carbohydrate metabolism by limiting fungal growth. The driving force for glucose transport in fungi is known to be the concentration gradient that is maintained by the rapid conversion of absorbed sugars to metabolic intermediates (Rothstein 1965). Inhibition of carbohydrate metabolism in a cell caused by Cd may decrease the glucose concentration gradient, which will in turn reduce glucose uptake. However, the present study showed no significant differences in glucose consumption in relation to Cd

treatment, whereas dry matter production decreased significantly. This result shows that Cd did not affect glucose uptake and suggests that C allocation might have been diverted to detoxification/repair mechanisms. This, therefore, indicates that a similar glucose concentration gradient could be maintained with or without Cd at the level used in the present study. Respiration rates of these fungi may be changed under Cd exposure, which can affect glucose utilisation. Therefore, further studies on respiration rates and energy metabolites of these fungi under Cd exposure are needed in order to clarify the results of the present study.

The present study showed that *S. bovinus* was the least efficient glucose user of the fungi examined. This result suggests that *S. bovinus* may be less beneficial to a host plant in terms of a C cost than the other two fungi since it may require greater amounts of carbohydrates from the plant to maintain a symbiotic association than *P. involutus* or *R. subcaerulescens*. However, it is important to consider the contribution of *S. bovinus* to the plant in return. Hilger and Krause (1989) suggested that an inefficient glucose user in pure culture may also benefit its host plant if it enhances P solubilisation in soil. If *S. bovinus* is able to take up more nutrients and/or protect its host plant better from adverse soil conditions than the other two fungi, infection by this species may be more beneficial.

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References

Andersen CP, Rygiewicz PT (1991) Stress interactions and mycorrhizal plant response: understanding C allocation priorities. Environ Pollut 73:217–244

Blaudez D, Botton B, Chalot M (2000a) Cadmium uptake and subcellular compartmentation in the ectomycorrhizal fungus *Paxillus involutus*. Microbiology 146:1109-1117

Blaudez D, Jacob C, Turnau K, Colpaert JV, Ahonen-Jonnarth U, Finlay R, Botton B, Chalot M (2000b) Differential responses of ectomycorrhizal fungi to heavy metals in vitro. Mycol Res 104:1366–1371

Blaudez D, Jacob C, Turnau K, Colpaert JV, Ahonen-Jonnarth U, Finlay R, Botton B, Chalot M (2000c) Differential responses of ectomycorrhizal fungi to heavy metals in vitro. Mycol Res 104:1366-1371

Colpaert JV, Van Assche JA (1992) The effects of cadmium and the cadmium-zinc interaction on the axenic growth of ectomycorrhizal fungi. Plant Soil 145:237–243

Colpaert JV, Vandenkoornhuyse P, Adriaensen K, Vangronsveld J (2000) Genetic variation and heavy metal tolerance in the ectomycorrhizal basidiomycete *Suillus luteus*. New Phytol 147:367–379

Finlay D (1989) Functional aspects of phosphorus uptake and carbon translocation in incompatible ectomycorrhizal associations between *Pinus sylevstris* and *Suillus grevillei* and *Boletinus cavipes*. New Phytol 103:185–192

Haselwandter K, Bobleter O, Read DJ (1990) Degradation of ^{14}C-labelled lignin and dehydropolymer of coniferyl alcohol by ericoid and ectomycorrhizal fungi. Arch Microbiol 153:352–354

Hilger AB, Krause HH (1989) Growth characteristics of *Laccaria laccata* and *Paxillus involutus* in liquid culture media with inorganic and organic phosphorus sources. Can J Bot 67:1782–1789

Howe R, Evans RL, Ketteridge SW (1997) Copper-binding proteins in ectomycorrhizal fungi. New Phytol 135:123-131

Hughes E, Mitchell DT (1995) Utilization of sucrose by *Hymenoscyphus ericae* (an ericoid endomycorrhizal fungus) and ectomycorrhizal fungi. Mycol Res 99:1233–1238

Jayko LG, Baker TI, Stubblefield RD, Anderson RF (1962) Nutrition and metabolic products of *Lactarius* species. Can J Microbiol 8:361–371

Jongbloed RH, Borst-Pauwels GWFH (1990) Differential response of some ectomycorrhizal fungi to cadmium in vitro. Acta Bot Neerl 39:241–246

Kieliszewska-Rokicka B, Rudawska M, Leski T, Kurczyńska EU (1998) Effect of low pH and aluminium on growth of *Pinus sylvestris* L. seedlings mycorrhizal with *Suillus luteus* (L. ex Fr.) S.F. Gray. Chemosphere 36:751–756

Kim C-G (2001) Effects of Cd on Scots pine (*Pinus sylvestris* L.) and its mycorrhizas. PhD thesis. University of London, London

Lamb RJ (1974) Effect of D-glucose on utilization of single carbon sources by ectomycorrhizal fungi. Trans Br Mycol Soc 63:295–306

Lapeyrie F, Chilvers GA, Bhem CA (1987) Oxalic acid synthesis by the mycorrhizal fungus *Paxillus involutus* (Batsch. ex Fr.) Fr. New Phytol 106:139–146

Marx DH (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59:153–163

McCreight JD, Schroeder DB (1982) Inhibition of growth of nine ectomycorrhizal fungi by cadmium, lead, and nickel in vitro. Environ Exp Bot 22:1–7

Molina R, Trappe JM (1994) Biology of the ectomycorrhizal genus, *Rhizopogon*. I. Host associations, host-specificity and pure culture syntheses. New Phytol 126:653–675

Nelson N (1944) A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 153:375–380

Palmer JG, Hacskaylo E (1970) Ectomycorrhizal fungi in pure culture. I. Growth on single carbon sources. Physiol Plant 23:1187–1197

Rothstein A (1965) Uptake and translocation. 1. Uptake. In: Ainsworth GC, Sussman AS (eds) The fungal cell. The fungi. An advanced treatise, vol I. Academic Press, New York, pp 429–455

Sayer JA, Gadd GM (1997) Solubilization and transformation of insoluble inorganic metal compounds to insoluble metal oxalates by *Aspergillus niger*. Mycol Res 101:653–661

Somogyi M (1945) A new reagent for the determination of sugars. J Biol Chem 160:61-68

Tam PCF (1995) Heavy metal tolerance by ectomycorrhizal fungi and metal amelioration by *Pisolithus tinctorius*. Mycorrhiza 5:181–187

Villeneuve N, Grandtner MM, Fortin JA (1989) Frequency and diversity of ectomycorrhizal and saprophytic macrofungi in the Laurentide Mountains of Quebec. Can J Bot 67:2616–2629