Accumulation of heavy metals into *Armillaria* rhizomorphs from contaminated soils

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Abstract
Armillaria sp. are widespread wood decomposing and pathogenic fungi that spread in the soil by root-like fungal structures named rhizomorphs. We investigated the effect of heavy metals on the production of *Armillaria* rhizomorphs and the extent of absorption of heavy metals into the rhizomorphs. *A. ostoyae* and *A. cepistipes* were inoculated into the soil of model ecosystems that were subjected to four treatments a) control without added heavy metals, irrigation pH 5.5. b) heavy metals dust resulting in: Cu, 640 mg/kg; Zn, 3000 mg/kg; Cd, 10 mg/kg; Pb, 90 mg/kg in the top soil, irrigation pH 5.5. c) acid irrigation pH 3.5 imitating acid rain. d) the combination of acid irrigation and heavy metals. The treatments had no effect on rhizomorph production, assessed by rhizomorphs length and dry weight in soil core samples. In the rhizomorphs produced by the *Armillaria* species over a period of three years, the concentration of Cu, Zn and Cd, but not Pb was significantly higher in soils with added heavy metals than those without. In the contaminated soils, the mean concentrations of heavy metals in the rhizomorphs were 228 mg/kg Cu, 6300 mg/kg Zn, 10.6 mg/kg Cd, and 12.1 mg/kg Pb. Energy dispersive X-ray (EDX) microanalysis showed that Zn was preferentially absorbed to the melanized cortex of the rhizomorphs. The results showed that *Armillaria* rhizomorphs can bind considerable amount of heavy metals and thus may contribute to the stabilization of heavy metals in the soil.

Keywords: fungi, *Armillaria,* rhizomorphs, heavy metals, Zn, phytostabilisation

1 Introduction

Species in the genus *Armillaria* are widespread soil borne fungi that play an important role in the decomposition of wood (KILE *et al.* 1991). Some species, however, behave also as plant pathogens that cause *Armillaria* root rot in woody plants (GUILLAUMIN *et al.* 1993, SHAW and KILE 1991). Most *Armillaria* species have the ability to spread in the soil by rhizomorphs to reach new food bases or potential hosts. Rhizomorphs are root-like fungal structures with diameters between 0.5 and 3 mm that consist of an outer melanised cortex and an inner core, named medulla (GARRAWAY *et al.* 1991). Due to the melanin content, the cortex protects the rhizomorphs from environmental stress including attacks by antagonistic fungi and bacteria. The medulla is the main active structure of the rhizomorphs responsible for transport of water, nutrients, and oxygen. Rhizomorphs normally are growing out from a woody food base and can produce extensive networks in the soil (SMITH *et al.* 1992; PROSPERO *et al.* 2003).

During growth through the soils rhizomorphs can uptake mineral nutrients at their growing tips (MORRISON 1974). The cortex of the rhizomorphs has the ability to adsorb metal ions from natural soils (RIZZO *et al.* 1992) but apparently there is no or only little translocation of these ions into the medulla (MORRISON 1974). Other wood decomposing fungi such as *Phanarochaete chrysosporium* Burdsall and *Polyporus versicolor* (L.) Fr. were found to absorb heavy metals from aqueous solutions and have the potential to be used in wastewater treatments (YETIS *et al.* 1998; BALDRIAN 2003).

The objectives of this study were to investigate the production of *Armillaria* rhizomorphs in heavy metals contaminated soils and to determine the extent of accumulation of heavy metals into the rhizomorphs.

2 Material and methods

Experimental design: In April 2000, model ecosystems were established in 16 open top chambers in the frame of the multidisciplinary research project "From Cell to Tree" (MENON *et al.* 2005; FREY *et al.* 2006; HERMLE *et al.* 2006). Each chamber was split into two ecosystem compartments one containing an acidic (pH 4.2) and one a calcareous subsoil (pH 7.4).

Each compartment was again split into two quarter-chambers of which one was used for the *Armillaria* experiment. The ecosystem in each quarter chamber consisted of three Norway spruce *(Picea abies),* two poplar *(Populus tremula),* one willow *(Salix viminalis),* and one birch *(Betula pendula)* and various understorey plants. All chambers had the same topsoil, 15 cm in depth, pH 6.5 (MENON *et al.* 2005). Before placing the sub- and topsoils into the chambers, roots and woody debris were removed. The treatments applied in each of four open top chambers were a) CO, control without added heavy metals, irrigation pH 5.5, b) AR, acid irrigation pH 3.5 imitating acid rain, c) HM, heavy metals dust resulting in: Cu, 640 mg/kg; Zn, 3000 mg/kg; Cd, 10 mg/kg; Pb, 90 mg/kg in the topsoil, irrigation pH 5.5, d) HMAR, the combination of acid irrigation and heavy metals (for details see GÜNTHARDT-GOERG and VOLLENWEIDER 2003; MENON *et al.* 2005; HERMLE *et al.* 2006). The concentrations of heavy metals in the non-contaminated topsoil were 28 mg/kg Cu, 97 mg/kg Zn, 0.1 mg/kg Cd, and 37 mg/kg Pb.

Inoculation of *Armillaria* sp. and quantification of rhizomorph production: In Mai 2000, an *Armillaria ostoyae* (Romagnesi) Herink and *Armillaria cepistipes* Velenovsky isolate was inoculated by inserting colonised hazelnut segments into the topsoil near each of the spruce trees. The inoculum was prepared as described by PROSPERO *et al.* (2004) with the modification that we used thicker hazelnut stem segments (diameter 7–8 cm), which had been colonised by the fungal isolates for five months at 25°C. Then, the stems were longitudinally split into four segments, each of which was used as a single inoculum. The *A. ostoyae* isolate used in this study was C18 and the *A. cepistipes* isolate B3 both described in PROSPERO *et al.* (2004 and 2005).

In October 2003 after four growing seasons, we assessed the rhizomorph production by systematically taking nine soil samples evenly distributed in each quarter chamber that had been inoculated with *Armillaria.* The soil core samples were removed to a depth of 10 cm using an aluminium cylinder (diameter 8 cm). The soil samples were brought to the laboratory and carefully examined for *Armillaria* rhizomorphs. The rhizomorphs were thoroughly washed with tap water using a sprinkler. The total length of the rhizomorphs in each core sample was determined from scanned images using the computer programme WinRHIZO (Régent Instruments, Quebec). Dry weight of the rhizomorphs was determined after they

were lyophilised for 24 h. The data of the nine soil core samples were used to calculate mean values of rhizomorph length and dry weight for each quarter chamber. After measuring the dry weight, one individual rhizomorph was randomly selected from each soil core sample for species identification using the PCR/RFLP methods described by HARRINGTON and WINGFIELD (1995). PCR products were digested with the restriction enzyme *Hin*fI that has a restriction site in the sequence of *A. cepistipes* but not in *A. ostoyae.*

To check whether the rhizomorphs were genetically identical to the *Armillaria* isolates inoculated into the chambers, cultures recovered from 34 rhizomorphs from six chambers were identified in somatic incompatibility pairings as described by PROSPERO *et al.* (2003). All cultures were either somatic compatible with the *A. ostoyae* isolate C18 (9 cultures) or the *A. cepistipes* isolate B3 (25 cultures) indicating that all rhizomorphs were produced by the two isolates inoculated into the soil. Inspection of the non-inoculated quarter chambers at the end of the experiment confirmed that there were no contaminating rhizomorphs in the soils.

Element analysis of the rhizomorphs: After taking the soil core samples, the topsoil was removed and all the remaining rhizomorphs were collected and pooled for each quarter chamber. The rhizomorphs were thoroughly washed and lyophilised as above. To determine the heavy metal content, the pooled rhizomorphs were cut into 1–2 cm segments and approx. 500 mg grinded to a fine powder (3 min at 90 % engine power) using a mixer Mill (Retsch).

The fine powder was solubilised by a microwave digestion system UltraCLAVE (MLS Milestone) in a mixture of HNO_3 (65 %) and HF (40 %) and the heavy metals were extracted by HNO_3 and analysed by multielement ICP-AES. Heavy metal contents of the rhizomorphs were only determined in the quarter chamber with the acidic subsoil.

Energy dispersive X-ray (EDX) microanalysis: Elemental cell composition was determined for six rhizomorphs from contaminated soils and four from non-contaminated soils. The fresh rhizomorphs were washed and blotted dry. The rhizomorphs were cut in small pieces (length 3–4 mm) using a razor blade. For the analysis of freeze-fractures, the rhizomorphs were mounted vertically on an scanning electron microscrope (SEM) stub using a cryo-adhesive (Biorad) and the samples were rapidly frozen by plunging into liquid propane for the analysis of freeze-fractures. EDX microanalysis was performed in a Philips SEM 515 equipped with an SEM cryo unit (SCU 020, Bal-Tec, Balzers, Liechtenstein) and a Tracor Northern energy dispersive X-ray analysis system interfaced with a Voyager software package as previously described (FREY *et al.* 2000; FREY *et al.* 1996; BRUNNER and FREY 2000). Electron-induced X-rays were detected by a Si(Li) spectrometer detector (Tracor Northern 30 mm^2 Microtrace) with an ultra-thin beryllium window. The microscope was operated at an acceleration voltage of 18 kV with a beam current of 80nA and the stage-tilt was adjusted to obtain a take-off angle of 44 degrees. Working distance was 12 mm. The temperature on the SEM cold stage was kept below –120 °C. Single spot analyses of selected cells in the rhizomorphs were carried out to determine the distribution of zinc.

Statistical analysis: Data were analysed using the program DataDesk Version 6 (VELLEMAN 1997). Analysis of variance (ANOVA) was performed to test for differences in rhizomorph production and heavy metal content of the rhizomorphs among treatments (CO, AR, HM, HMAR). Pairwise comparisons of means were done using Scheffe Post-Hoc tests. Differences were considered statistically significant at $p \leq 0.05$. The rhizomorph length and dry weight were used to quantify rhizomorph production in each quarter chamber.

3 Results

Rhizomorphs were found in the topsoil of all quarter chambers inoculated with *Armillaria* at the end of the third growing season. In the systematic survey, 189 out of 288 soil core samples were positive for rhizomorphs. At average 5.9 of the nine soil core samples taken in each quarter chamber contained rhizomorphs. There were no significant differences in rhizomorph production (assessed as rhizomorph length and dry weight) among the four treatments (Table 1). The type of subsoil (acidic vs. calcareous) and the interaction soil type x treatment had also no effect on rhizomorph production (not shown). Species identification of one individual rhizomorph per soil core sample was successful for 176 out of 189 rhizomorphs. Thirteen rhizomorphs could not be identified due to failure of PCR amplification. In all treatments, the majority of the rhizomorphs were produced by *A. cepistipes* (Table 1). The relative portion of *A. ostoyae* rhizomorphs, however, was significantly higher (Chi-square test) in the heavy metal contaminated soils (HM + HMAR treatments) than in the not-contaminated soils (CO + AR treatments).

Table 1. Mean length and dry weight of rhizomorphs produced by *Armillaria* sp. in model forest ecosystems subjected to four treatments. [1] Means within a column followed by the same letter are statistically not different (Scheffe Post-Hoc tests). N = 8.

Treatment	Length (cm/dm^3 soil)	Dry weight (mg/dm^3 soil)	Ratio of rhizomorphs in the soil *(A. cep/A. ost)*
Control (CO)	38.4 a[1]	58.2 a	42 / 6
Acid rain (AR)	24.2 a	42.3 a	30 / 7
Heavy metal (HM)	30.2 a	58.9 a	33 / 19
Combination (HMAR)	29.4 a	59.6 a	29 / 10

The concentrations of Zn in the rhizomorphs were significantly higher in the contaminated soils (HM, HMAR) than in the soil without heavy metals added (CO, AR) (Table 2). Cd and Cu contents were also higher in the contaminated than non-contaminated soils, but the differences were statistically significant only for the HM treatment. No differences among the treatments were observed for the Pb concentrations in the rhizomorphs. When comparing the concentrations of heavy metals in the rhizomorphs with those in the soil, we found higher bioconcentration factors in the soils without heavy metal added (Table 3). Particularly, Zn and Cu accumulated at high levels into the rhizomorphs in these soils. In the contaminated soils, only Zn had a concentration factor significantly greater than one.

Table 2. Concentrations of heavy metals (mg/kg dry weight) in the *Armillaria* rhizomorphs. Cu, Zn, Cd, and Pb were added to the topsoil in the treatments HM and HMAR. [1] Means within a column followed by the same letter are statistically not different (Scheffe Post-Hoc tests). N = 4, only determined in quarter chambers with acidic subsoil.

Treatment	Cu	Zn	Cd	Pb
Control (CO)	44.8 a[1]	771.0 a	1.4 a	9.8 a
Acid rain (AR)	62.5 a	800.2 a	2.0 a	10.2 a
Heavy metal (HM)	228.4 b	6337.2 b	10.6 b	12.2 a
Combination (HMAR)	144.8 ab	5448.4 b	9.3 ab	8.1 a

Table 3. Bioconcentration factors (= metal content in rhizomorphs / metal content in soil) of heavy metals in the *Armillaria* rhizomorphs.

Treatment	Cu	Zn	Cd	Pb
Control (CO)	1.60	7.95	13.81	0.27
Acid rain (AR)	2.23	8.25	20.13	0.27
Heavy metal (HM)	0.36	2.11	1.06	0.14
Combination (HMAR)	0.23	1.82	0.93	0.09

Rhizomorph concentrations of other metals, which were not supplied with the metal-containing filter dust are shown in Table 4. Several of these metal ions (Al, Cr, Fe, Ni, Ba) showed significant lower concentrations in the soils with heavy metals than without heavy metals added.

EDX microanalysis of the rhizomorphs from contaminated soils indicated that high concentrations of Zn were located in the outer cortex of the rhizomorphs. Table 5 shows a representative exemple of an *A. cepistipes* rhizomorph from the HM treatment, acidic subsoil. High Zn counts ranging from 2500 to 9700 were recorded throughout the thickness of the cortex.

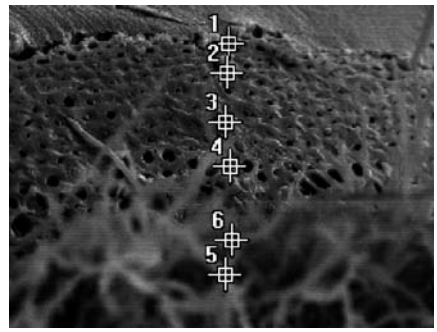
Rhizomorphs from non-contaminated soils showed low net counts for Zn (average 72 ± 47), which were often at the detection limit of the method. In these rhizomorphs, no significant differences among measurement locations (outer or inner cortex, medulla) were observed. Cu, Cd, and Pb could not be detected by EDX analysis because their concentrations in the rhizomorphs were below detection limit.

Table 4. Concentrations of metal ions and other elements (mg/kg dry weight) in the *Armillaria* rhizomorphs. The content of these elements in the soil was not increased in the HM and HMAR treatments. * indicates an element with significant decreased concentration in the rhizomorphs from the heavy metal contaminated soils.

Treatment	Na	Mg	Al *	P *	S	K	Ca
Control (CO)	210	1065	2630	5370	649	9463	32479
Acid rain (AR)	243	1041	2602	5165	584	8708	30748
Heavy metal (HM)	208	947	1795	4499	432	8557	35211
Combination (HMAR)	210	1017	1875	4626	482	9297	34272

Treatment	Cr *	Mn	Fe *	Co	Ni *	Ba *
Control (CO)	4.2	129	1370	3.5	31	78
Acid rain (AR)	4.4	203	1233	6.8	34	96
Heavy metal (HM)	3.1	94	958	5.1	22	57
Combination (HMAR)	3.5	337	1044	4.6	19	51

Table 5. Detection of Zn by EDX microanalysis in a cross section of an *Armillaria cepistipes* rhizomorph grown in a contaminated soil containing 3000 mg Zn /kg dry soil.

No.	Tissue	Distance from surface (µm)	Zn counts	EDX measurements locations in the cross section
1	Cortex	1	2585 ± 243	
2	Cortex	6	9703 ± 308	
3	Cortex	14	6222 ± 156	
4	Cortex	21	6599 ± 164	
6	Medulla	33	1822 ± 122	
5	Medulla	36	542 ± 113	

4 Discussion

The production of *Armillaria* rhizomorphs from woody inoculum was not affected by the heavy metal dust added to the topsoil. This result suggests that *Armillaria* is quite resistant to moderate levels of heavy metal pollution. WARGO and CAREY (2001) reported that rhizomorph production by *A. ostoyae* was inhibited in natural soils containing high concentrations of heavy metals, particularly Pb. We did not observe an adverse effect of heavy metals on the production of *A. ostoyae* rhizomorphs in our experiment. The species ratios of the identified rhizomorphs rather suggest that *A. ostoyae* produced more rhizomorphs in the contaminated than in the non-contaminated soils. These contradictory results may be explained by the use of different *A. ostoyae* isolates and different experimental conditions. The *A. ostoyae* isolate used in our study is a good rhizomorph producer compared to other *A. ostoyae* isolates (PROSPERO *et al.* 2004) and may be less susceptible to heavy metals than other isolates. In addition, the Pb concentrations in most soils used by WARGO and CAREY (2001) were up to two fold higher than in our contaminated soil, which could also account for the different results observed.

The effect of heavy metals on *A. cepistipes* has not been studied so far. In all the treatments, this species consistently produced more rhizomorphs than *A. ostoyae.* This finding supports previous research on the rhizomorph production of the two species (PROSPERO *et al.* 2005) and shows that also in heavy metal contaminated soils *A. cepistipes* is a better rhizomorph producer than *A. ostoyae.* Generally, less pathogenic *Armillaria* species, such as *A. cepistipes,* are superior to pathogenic species in respect to rhizomorph production (RISHBETH 1982; GUILLAUMIN *et al.* 1993).

The rhizomorph concentrations of Zn, Cu, and Cd measured in our study demonstrate that *Armillaria* rhizomorphs have much higher absorption capacity for these elements than previously found in natural soils (RIZZO *et al.* 1992). The low Pb content of the rhizomorphs in both, the control and the contaminated soils could be attributed to the relatively low Pb concentration in the contaminated soil (Pb content was only about 3 times higher than in the control) and to the generally low availability of Pb (KABATA-PENDIAS and PENDIAS 2001).

Several metal ions such as Al, Cr, Fe, Ni, and Ba (not enhanced by the HM treatments) showed lower concentrations in rhizomorphs from the contaminated soils compared to the controls. Possibly, the high concentrations of Zn, Cu, and Cd in the contaminated soils have inhibited the absorption of these metal ions because of competition for binding sites in the cortex of the rhizomorphs.

EDX microanalysis allowed us to localise Zn in cross sections of the rhizomorphs. High amounts of Zn were found in the cortex but not in the inner medulla of the rhizomorphs. These results are consistent with reports that metal ions from natural soils are absorbed to the melanised cortex of the rhizomorphs (RIZZO *et al.* 1992). In addition, our study shows that Zn is not only located at the surface but throughout the entire cortex. Although not detectable by EDX microanalysis, we can assume that Cu, Cd, and Pb are also preferentially absorbed into the cortex. Like Zn these elements are known to be absorbed to fungal melanins (FOGARTY and TOBIN 1996). By binding of heavy metals, the melanised cortex might function as filter protecting the inner medulla from high concentrations of heavy metals (RIZZO *et al.* 1992). We can anticipate that the capacity of the rhizomorphs to adsorb heavy metals would increase with the thickness of the cortex and hence with age of the rhizomorphs. The rhizomorphs in our experiment had a maximum age of three years and it is likely that they can adsorb higher amounts of heavy metals when exposed for a longer period of time.

Through their ability to bind metal ions and to produce extensive networks in the soil (SMITH *et al.* 1992; PROSPERO *et al.* 2003) *Armillaria* rhizomorphs may contribute to the stabilization of heavy metals in contaminated soils. Fungal melanins contain various functional (chemical) groups, which provide binding sites for metal ions and are rather difficult to decompose by microorganisms (FOGARTY and TOBIN 1996; BUTLER and DAY 1998). Therefore, rhizomorphic melanin might still bind heavy metals as part of the soil organic matter after the death of the rhizomorphs.

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5 References

BALDRIAN, P., 2003: Interactions of heavy metals with white-rot fungi. Enzym. Microb.Technol. 32: 78–91.

BRUNNER, I.; FREY, B., 2000: Detection and localization of aluminium and heavy metals in ectomycorrhizal Norway spruce seedlings. Environ. Pollut. 108: 121–128.

BUTLER, M.J.; DAY, A.W., 1998: Fungal melanins: a review. Can. J. Microbiol. 44: 1115–1136.

FOGARTY, R.V.; TOBIN, J.M., 1996: Fungal melanins and their interactions with metals. Enzym. Microb. Technol. 19: 311–317.

FREY, B.; STEMMER, M.; WIDMER, F.; LUSTER, J.; SPERISEN, C., 2006: Microbial characterization of a heavy metal-contaminated soil in a model forest ecosystem. Soil Biol. Biochem. 38: 1745–1756.

FREY, B.; KELLER, C.; ZIEROLD, K.; SCHULIN, R., 2000: Distribution of Zn in functionally different leaf epidermal cells of the hyperaccumulator *Thlaspi caerulescens*. Plant Cell Environ. 23: 675–687.

FREY, B.; SCHEIDEGGER, C.; GÜNTHARDT-GOERG, M.S.; MATYSSEK, R., 1996: The effects of ozone and nutrient supply on stomatal response in birch leaves *(Betula pendula)* as determined by digital image analysis and X-ray microanalysis. New Phytol. 132: 135–143.

GARRAWAY, M.O.; HÜTTERMANN, A.; WARGO, P.M., 1991: Ontology and Physiology. In: SHAW, C.G. III; KILE, G.A. (eds) *Armillaria* Root Disease. Agricultural Handbook No. 691. Washington DC: USDA Forest Service. 21–47.

GÜNTHARDT-GOERG, M.S.; VOLLENWEIDER, P., 2003: Cellular injury, heavy metal uptake and growth of poplar, willow and spruce influenced by heavy metals and soil acidity. In: MENCH, M.; MOCQUOT, B. (eds) Risk assessment and sustainable land management using plants in trace element-contaminated soils. COST Action 837, 4th WG2 Workshop, Bordeaux 2002. Villenave d'Ornon, France, INRA Centre Bordeaux-Aquitaine. 165–171.

GUILLAUMIN, J.J.; MOHAMMED, C.; ANSELMI, N.; COURTECUISSE, E.; GREGORY, S.C.; HOLDENRIEDER, O.; INTINI, M.; LUNG, B.; MARXMÜLLER, H.; MORRISON, D.; RISHBETH, J.; TERMORSHUIZEN, A.J.; TIRRO, A.; VAN DAM, B., 1993: Geographical distribution and ecology of the *Armillaria* species in western Europe. Eur. J. For. Pathol. 23: 321–34.

HARRINGTON, T.C.; WINGFIELD, B.D., 1995: A PCR-based identification method for species of *Armillaria*. Mycologia 87: 280–288.

HERMLE, S.; GÜNTHARDT-GOERG, M.S.; SCHULIN, R., 2006: Effects of heavy metal contaminated soil on the performance of young trees growing in model-ecosystems under field conditions. Environ. Pollut. 144, 2: 703–714.

KABATA-PENDIAS, A.; PENDIAS, H. 2001: Trace elements in soils and plants, 3rd ed. Boca Raton, London, New York, Washington DC, CRC Press. 413 pp.

KILE, G.A.; MCDONALD, G. I.; BYLER, J.W., 1991: Ecology and disease in natural forests. In: SHAW, C.G. III; KILE, G.A. (eds) *Armillaria* Root Disease. Agricultural Handbook No. 691. Washington DC: USDA Forest Service. 102–121.

MENON, M.; HERMLE, S.; ABBASPOUR, K.; GÜNTHARDT-GOERG, M.S.; OSWALD, S.E.; SCHULIN, R., 2005: Water regime of metal-contaminated soil under juvenile forest vegetation. Plant Soil 271: 227–241.

MORRISON, D.J., 1974: Ion uptake by rhizomorphs of *Armillaria mellea*. Can. J. Bot. 53: 48–51.

PROSPERO, S.; HOLDENRIEDER, O.; RIGLING, D., 2003: Primary resource capture in two sympatric *Armillaria* species in managed Norway spruce forests. Mycol. Res. 107: 329–338.

PROSPERO, S.; HOLDENRIEDER, O.; RIGLING, D., 2004: Virulence of *Armillaria cepistipes* and *Armillaria ostoyae* on four Norway spruce provenances. For. Pathol. 34: 1–14.

PROSPERO, S.; HOLDENRIEDER, O.; RIGLING, D., 2005: Competitive ability of *Armillaria cepistipes* and *Armillaria ostoyae* in capturing stump resources: an experimental study. For. Pathol. 35: 1–11.

RISHBETH, J., 1982: Species of *Armillaria* in southern England. Plant Pathol. 31: 9–17.

RIZZO, D.M.; BLANCHETTE, R.A.; PALMER, M.A., 1992: Biosorption of metal ions by *Armillaria* rhizomorphs. Can. J. Bot. 70: 1515–1520.

SHAW, C.G. III; KILE, G. A. (eds) 1991: *Armillaria* Root Disease. Agricultural Handbook No. 691. Washington, DC: USDA Forest Service. 223 pp.

SMITH, M.L.; BRUHN, J.N.; ANDERSON, J.B., 1992: The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature 356: 428–431.

VELLEMAN, P.F., 1997: DataDesk Version 6. Handbooks 1–3. Ithaca, NY., Data Description Inc.

WARGO, P.M.; CAREY, A.C., 2001: Effects of metals and pH on in vitro growth of *Armillaria ostoyae* and other root and butt rot fungi of red spruce. For. Pathol. 41: 5–24.

YETIS, U.; OZCENGIZ, G.; DILEK, F.B.; ERGEN, N.; DOLEK, A., 1998: Heavy metal biosorption by white-rot fungi. Water Sci. Technol. 38: 323–330.

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