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MYCORRHIZAL AND SAPROBIC MACROFUNGI OF TWO ZINC WASTES IN SOUTHERN POLAND

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Ectomycorrhizal and saprobic fungi of two industrial wastes in southern Poland (calamine spoil in Bolesław and zinc waste in Chrzanów) were studied. Pine (*Pinus sylvestris*) accompanied by birch (*Betula pendula*) were present in the investigated area. Fruitbodies of 68 species were recorded, but only 10 were common to both sites. Mycorrhizal species were the most common group on the zinc waste, whereas saprobic fungi prevailed on the calamine spoil. The differences in species composition between sites might be due to differences in plant cover, but also to the toxicity of the material at the sites. Among mycorrhizal species, members of Cortinariaceae and Tricholomataceae were most frequently recorded. Most ectomycorrhizal species had a broad host range, and only a few species known to be associated exclusively with pine or birch were found. Analysis of ectomycorrhizas by classical and molecular (PCR-RFLP) methods revealed that the fungi forming the most abundant fruitbodies were also present in the form of ectomycorrhizas. A few ascomycete and basidiomycete fungi not recorded as fruitbodies were present as pine symbionts.

Key words: Industrial waste, calamine spoil, *Pinus sylvestris*, ectomycorrhizal fungi, saprobic fungi, ectomycorrhizas.

INTRODUCTION

Fungi are important components of the soil microbial consortium. Their activity strongly influences the soil structure, water balance and nutrient availability (Walker et al., 1989; Tinker et al., 1992; Marschner and Dell, 1994). They may strongly affect soil formation processes, either directly or by interaction with other soil biota (Tisdall, 1994), and they influence the nutritional and health status of the plants occurring in a given habitat. Their activity is especially important in disturbed areas such as heaps and wastes created by industrial activity.

Revegetation of industrial wastes is of great interest in areas where mining activity has occurred. Even decades after mining has stopped, vegetation cover is slow to form, because of the soil's poor chemical and physical properties and the phytotoxicity of metals such as Pb, Cd, As and Tl (Adriano, 2001). The establishment of trees is especially important for phytostabilization of such places. Their survival may be strongly influenced by efficient strains of mycorrhizal fungi, and can be further enhanced by the development and activity of the mycelium of saprobic fungi. Numerous members of both groups of fungi form fruitbodies and/or mycorrhizas which allow the species to be identified and the strain to be isolated and characterized, including characterization of the mechanisms that allow the fungus to survive under harsh conditions. This might open the way for researchers to study the role of the fungus within the substratum. Understanding the role of individual fungal species and their interactions with other soil biota is crucial to selecting the most efficient fungi associating with the plants and designing optimal restoration practices.

The southern part of Poland has been a center of lead and zinc industry since the Middle Ages (Grodzińska et al., 2000). Several smelters and associated industrial plants and ore mines are still

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TABLE 1. Total and extractable [in 1 M NH_4NO_3 and 0.1M $Ca(NO_3)_2$] heavy metal content in zinc wastes localized in Bolesław and Chrzanów ($\mu g \cdot g^{-1}$). NR – non recultivated plot; R – recultivated plot

Locality	Cd			Pb			Zn		
	Total	Extr. in NH_4NO_3	Extr. in $Ca(NO_3)_2$	Total	Extr. in NH_4NO_3	Extr. in $Ca(NO_3)_2$	Total	Extr. in NH_4NO_3	Extr. in $Ca(NO_3)_2$
Bolesław, NR	265.4	5.3	2.9	14029.4	7.9	4.8	37743	236.9	9.8
Bolesław, R	205.4	6.5	2.3	14500.0	2.6	3.9	49705	377.4	6.9
Chrzanów	453.8	2.0	1.5	6878.3	0.9	4.2	25614	66.4	2.9

TABLE 2. Selected element and organic matter content and pH in substratum from Bolesław and Chrzanów wastes. NR – nonrecultivated plot; R – recultivated plot

Locality	P_2O_5 [mg $100g^{-1}$]	N [%]	Ca [%]	Organic matter [%]	pH in H_2O
Bolesław, NR	0.64	0.23	55000	1.41	7.55
Bolesław, R	0.70	0.60	55000	1.98	7.42
Chrzanów	0.50	0.50	2500	1.50	7.40

functioning. Their production resulted in the formation of waste areas where the material from ore beneficiation processes (e.g., flotation) has been stored. The wastes are subject to spontaneous succession, and to recultivation which the mining enterprises are obliged by law to implement. One of the main trees that appear spontaneously or are introduced is Scots pine (*Pinus sylvestris* L.).

The present research was carried out in two areas. The first is a century-old remnant of an ore mine, composed of limestone and metalliferous dolomite. The second is a 40-year-old zinc waste created as a deposit of post-flotation material. Both the ore mine and the zinc waste are rich in heavy metals. For decades they have been the object of intensive investigation of fauna and flora (Dobrzańska, 1955; Szafer and Zarzycki, 1972; Godzik, 1993; Grodzinska et al., 2000; Szarek-Łukaszewska and Niklińska, 2002; Wierzbicka and Rostański, 2002; Wierzbicka and Potocka, 2002; Dmowski, 2000). Relatively little work has been done on the microbial activity of the spoils, and the research has been focused mostly on endomycorrhizal fungi (Turnau, 1998; Jurkiewicz et al., 2001; Orłowska et al., 2002). A few papers concerned selected ectomycorrhizal species (Turnau et al., 1996, 2001, 2002).

This study examines the species composition of the macrofungal populations of these two industrial wastes by recording their fruitbodies, and analyzes the occurrence of ectomycorrhizal species by comparing their above- and belowground features (fruitbodies and ectomycorrhizas). The aim is to create a database for further work on the functioning of ectomycorrhiza and the mechanisms of heavy metal detoxification.

MATERIALS AND METHODS

STUDY SITES

The observations were carried out at two industrial wastes situated on the western border of the Krakowsko-Czestochowska Upland. Both are rich in heavy metals (Tab. 1), but differ in their origin and material properties. The site in Bolesław near Olkusz is a calamine spoil approximately 100 years old, where open-cast mining was stopped at the beginning of the 20th century (Szarek-Łukaszewska and Niklińska, 2002). The substratum is composed of Triassic oolitic limestone and metalliferous dolomite containing mostly cadmium, lead and zinc (Dobrzańska, 1955; Godzik, 1993), resembling initial soils classified as silt loam type. It was composed of 77% stones (> 1 mm); the mechanical composition of the < 1 mm fraction was as follows: 1–0.1 mm – 30%; 0.1–0.05 mm – 7%; 0.05-0.02 mm – 44%; 0.02–0.006 mm – 9%; 0.006–0.002 – 6%; 0.002 – 4%. The non-restored area is inhabited by plants such as *Armeria maritima* (Mill.) Willd. subsp. *halleri* (Wallr.) Á. Löve & D. Löve, the only species associated with soils rich in heavy metals (Szafer, 1959), *Biscutella laevigata* L., *Silene vulgaris* (Moench) Garcke, *Reseda lutea* L., *Gypsophila fastigiata* L., *Erysimum odoratum* Ehrh. and *Cerastium arvense* L. (Grodzińska et al., 2000). Scattered *Betula pendula* Roth and dwarf *Pinus sylvestris* L. are also present. The restored part, which was scarified and planted with *P. sylvestris* and *B. pendula* seedlings 30 years ago, is of sward type dominated by grasses, accompanied by *Plantago lanceolata* L.

and *B. laevigata* L., present mostly on the edges. Tables 1 and 2 summarize some of the properties of both the restored and non-restored parts of the waste. The area is impacted by industrial pollutants from the smelter in Bukowno and from the Upper Silesian Industrial Center (Godzik, 1993).

The zinc waste in Chrzanów was created as the result of deposition of material from flotation processes. The material resembled coarse sand composed of the following fractions: 1–0.1 mm – 84%; 0.1–0.05 mm – 4%; 0.05–0.02 mm – 4%; 0.02–0.006 mm – 2%; 0.006–0.002 – 2%; < 0.002 – 1%. It was characterized by basic pH, very low content of organic matter, nitrogen and phosphorus, and high concentration of calcium (Tab. 2). The waste was recultivated ~20 years ago by planting Scots pine. Birch (*Betula pendula*) appeared spontaneously on the waste and accompanies pine as an admixture.

FUNGAL FRUITBODY OBSERVATIONS

Fruitbodies of mycorrhizal fungi were recorded during 1994–2001 at the waste in Chrzanów, and in 1999–2001 at the spoil in Bolesław. Fungal fruitbodies were collected from the whole area of the wastes, including the recultivated part of the spoil in Bolesław. Only fruitbodies formed aboveground were considered.

The nomenclature follows Hansen and Knudsen (1992, 1997, 2000). Reference fruitbody specimens of the recorded fungi are stored in the mycological collection of the Institute of Botany Herbarium (KRAM) at the Jagiellonian University in Cracow.

ECTOMYCORRHIZAL INVESTIGATIONS

The material for investigation of pine ectomycorrhizas was collected in the form of soil blocks ~2.5 dm^3 in volume, taken to a depth of ~15 cm, approximately 20 soil blocks per site. Roots were rinsed in tap water and analyzed under a dissecting microscope. Ectomycorrhizas were grouped into morphotypes based on their morphological and anatomical features, and characterized according to methods described by Agerer and coworkers (1991, 1987–2003). The structural features were analyzed with a light microscope equipped with Nomarski interference contrast optics, a JVC color CCD camera and a computer image analysis system (IMAGE PRO PLUS 3.0 for Windows, Media Cybernetics, Silver Spring, U.S.A.). Ectomycorrhizas were identified by both molecular (PCR-RFLP) and classical (Agerer, 1991) methods.

Reference mycorrhizal material is stored as FAA-fixed voucher specimens in the Mycorrhizal Unit of the Institute of Botany at the Jagiellonian University.

MOLECULAR ANALYSIS

PCR-RFLP analysis was carried out according to Gardes et al. (1991) and Gardes and Bruns (1993, 1996), with the modifications of Agerer et al. (1996). Extracted DNA was not cleaned before the PCR reaction. The rDNA ITS region was amplified with two universal primers, ITS1 and ITS4 (White et al., 1990). Amplification products were digested with 4 enzymes (AluI, EcoRI, *HaeIII*, HinfI, TaqI). DNA was fractionated using 2% agarose gel in 0.5 × TAE buffer (Sambrook et al., 1989), and the profiles were compared after ethidium bromide staining using the KODAK 1 D Software System. Only fragments larger than 100 bp were considered for analysis. Up to 5% variation of the size of compared fragments was accepted (Kåren et al., 1997).

RESULTS AND DISCUSSION

FUNGAL FRUITBODIES

Fruitbodies of 68 species of macromycetes were found in the investigated area (Tab. 3). Only 10 species occurred at both wastes; 28 species were present only at Bolesław and 30 at Chrzanów. At both sites the most common were members of Cortinariaceae and Tricholomataceae (Basidiomycota). Mycorrhizal fungi were the most numerous group of species at the waste in Chrzanów, representing over 70% of the taxa. At Bolesław the predominant group of macromycetes were saprobic fungi with 50% of the species, while mycorrhizal fungi represented slightly more than 40% of the species.

Most mycorrhizal species probably have a broad host range. Species known to form symbiosis exclusively with pine included *Chroogomphus rutilus*, *Suillus luteus* and *Rhizopogon roseolus*, common to both sites. *Lactarius pubescens*, *L. torminosus*, *Leccinum scabrum*, Russula emetica and *R. depallens* were among the characteristic associates of birch. All species that occurred at both investigated sites were mycorrhizal fungi. Most of them formed abundant fruitbodies (e.g., *Rhizopogon roseolus*, *Suillus luteus*, *Thelephora terrestris*, *Tricholoma scalpturatum*). Species of *Cortinarius*, *Hebeloma* and *Inocybe* were common tree symbionts in Chrzanów and occurred only sporadically in Bolesław (with the excep-

TABLE 3. List of species recorded at study sites in Bolesław (B) and Chrzanów (Ch). NR – non-recultivated plot; R – recultivated plot; M – mycorrhizal species (status according to selected literature, e.g., Trappe 1962, Agerer 1987–2003, Agerer and Rambold 1997, descriptions published in Descriptions of Ectomycorrhizae). S – saprobic species; P – parasitic species; EG – ecological group; * – species recorded in Chrzanów and Jaworzno region by Wojewoda (1973, 1979, 1981); ? – mycorrhizal status not confirmed. Systematic arrangement after Kirk et al. (2001)

Taxon	EG	Family	B, NR	B, R	Ch
ASCOMYCOTA					
1. *Geoglossum cookeianum* Nannf.	S	Geoglossaceae	+		
2. *Helvella crispa* Scop.: Fr.	M(?)	Helvellaceae			+*
3. *Helvella lacunosa* Afzel: Fr.	M(?)	Helvellaceae	+		+
4. *Helvella elastica* Bull.: Fr.	S	Helvellaceae			+
5. *Pulvinula constellatio* (Berk. & Broome) Boud.	S	Pyronemataceae			+*
6. *Sphaerosporella brunnea* (Alb.& Schwein.: Fr.) Surček & Kubička	M	Pyronemataceae			+
7. *Pyronema domesticum* (Sowerby: Fr.) Sacc.	S	Pyronemataceae			+
BASIDIOMYCOTA					
1. A*uriscalpium vulgare* S.F. Gray	S	Auriscalpiaceae	+	+	
2. *Bovista plumbea* Pers.: Pers.	S	Lycoperdaceae	+		
3. *Chroogomphus rutilus* (Schaeff.: Fr.) O.K. Miller	M	Gomphidiaceae	+		+*
4. *Clitocybe clavipes* (Pers.: Fr.) Kumm.	S	Tricholomataceae			+*
5. *Clitocybe dealbata* (Sow.: Fr.) Kumm.	S	Tricholomataceae			+*
6. *Clitocybe inornata* (Sow.: Fr.) Gill.	S	Tricholomataceae	+	+	
7. *Clitocybe metachroa* (Fr.) Kumm.	S	Tricholomataceae			+
8. *Clitocybe* sp. 1	S	Tricholomataceae	+	+	
9. *Clitocybe* sp. 2	S	Tricholomataceae	+	+	
10. *Clitocybe* sp. 3	S	Tricholomataceae	+	+	
11. *Collybia tuberosa* (Bull.: Fr.) Kumm.	S	Tricholomataceae			+*
12. *Cortinarius* cfr. *decipiens* (Pers.: Fr.) Fr.	M	Cortinariaceae			+
13. *Cortinarius semivestitus* Mos.	M	Cortinariaceae			+
14. *Cortinarius* sp. 1	M	Cortinariaceae		+	
15. *Cortinarius* sp. 2	M	Cortinariaceae			+
16. *Crucibulum laeve* (Huds.) Kambly	S	Nidulariaceae		+	
17. *Cyathus olla* (Batsch: Pers.) Pers.	S	Nidulariaceae	+		
18. *Entoloma papillatum* (Bres.) Dennis	S	Entolomataceae	+	+	
19. *Galerina marginata* (Batsch) Khn.	S	Cortinariaceae		+	
20. *Geastrum minimum* Schwein.	M(?)	Geastraceae		+	
21. *Gloeophyllum sepiarium* (Wulfen: Fr.) P. Karsten	S	Gloeophyllaceae		+	
22. *Gymnopilus penetrans* (Fr.) Murr.	S	Cortinariaceae		+	
23. *Hebeloma* cfr. *anthracophilum* R. Maire	M	Bolbitiaceae			+
24. *Hebeloma crustuliniforme* (Bull.) Quél.	M	Bolbitiaceae			+*
25. *Hebeloma fastibile* (Pers.: Fr.) Kumm.	M	Bolbitiaceae			+
26. *Hebeloma longicaudum* (Pers.: Fr.) Kumm.	M	Bolbitiaceae			+
27. *Hebeloma mesophaeum* (Pers.) Quél.	M	Bolbitiaceae	+	+	+*
28. *Hebeloma sinapizans* (Paulet) Gill.	M	Bolbitiaceae	+	+	
29. *Hemimycena pseudogracilis* (Kühn.&Maire) Sing.	S	Tricholomataceae		+	
30. *Heterobasidion annosum* (Fr.) Bref.	S, P	Bondarzewiaceae		+	
31. *Hypholoma fasciculare* (Huds.: Fr.) Kumm.	S	Strophariaceae			+
32. *Inocybe* cfr. *rimosa* (Bull.: Fr.) Kumm.	M	Cortinariaceae			+
33. *Inocybe flocculosa* (Berk.) Sacc.	M	Cortinariaceae			+
34. *Inocybe lacera* (Fr.) Kumm. var. *regularis* Kuyper	M	Cortinariaceae			+*
35. *Inocybe rimosa* (Bull.: Fr.) Kumm.	M	Cortinariaceae			+
36. *Inocybe* sp.	M	Cortinariaceae	+		
37. *Lactarius pubescens* Fr.	M	Russulaceae	+		+*
38. *Lactarius torminosus* (Schaeff.: Fr.) Pers.	M	Russulaceae			+
39. *Leccinum scabrum* (Bull.: Fr.) S.F. Gray	M	Boletaceae			+*
40. *Lepiota cristata* (Bolt.: Fr.) Kumm.	S	Agaricaceae	+		

TABLE 3. (cont.)

Taxon	EG	Family	B, NR	B, R	Ch
41. *Lycoperdon perlatum* Pers.: Pers.	S	Lycoperdaceae		+	
42. *Lycoperdon lividum* Pers.	S	Lycoperdaceae	+		
43. *Marasmius limosus* Quél.	S	Marasmiaceae			+
44. *Mycena pura* (Pers.: Fr.) Kumm. var. *pura*	S	Tricholomataceae		+	
45. *Paxillus involutus* (Batsch : Fr.) Fr.	M	Paxillaceae			+*
46. *Pholiota highlandensis* (Peck) Smith & Hesler [= *Ph. carbonaria* (Fr.: Fr.) Sing.]	S	Strophariaceae		+	
47. *Ramaria abietina* (Pers: Fr.) Quél.	M	Ramariaceae		+	
48. *Rhizopogon roseolus* (Corda) Th.M. Fries	M	Rhizopogonaceae	+	+	+*
49. *Russula* cfr. *consobrina* (Fr.: Fr.) Fr.	M	Russulaceae			+
50. *Russula emetica* (Schaeff.: Fr.) Pers. var. *emetica*	M	Russulaceae			+*
51. *Russula depallens* (Pers.: Fr.) Fr.	M	Russulaceae	+		+
52. *Schizophyllum commune* Fr.: Fr.	S	Schizophyllaceae		+	
53. *Scleroderma bovista* Fr.	M	Sclerodermataceae			+*
54. *Scleroderma citrinum* Pers.	M	Sclerodermataceae		+	+*
55. *Scleroderma verrucosum* (Bull.: Pers.) Pers. ss. Greville	M	Sclerodermataceae			+
56. *Strobilurus tenacellus* (Pers.: Fr.) Sing.	S	Marasmiaceae	+	+	
57. *Stropharia aeruginosa* (Curt.: Fr.) Quél.	S	Strophariaceae		+	
58. *Stropharia cyanea* (Bull.) Tuomikoski	S	Strophariaceae			+
59. *Suillus luteus* (L.: Fr.) Roussel	M	Suillaceae	+	+	+*
60. *Thelephora terrestris* Ehrh.: Fr. forma *terrestris*	M	Thelephoraceae	+	+	+*
61. *Tricholoma scalpturatum* (Fr.) Quél.	M	Tricholomataceae	+	+	+*

tion of *Hebeloma* spp.). The fruitbodies of these fungi were found mostly in places of scarce plant cover. The more dense vegetation of the Bolesław spoil could be one of the reasons for this difference, although it could also result from differences in the physical structure or chemical composition of the material (Tabs. 1, 2). The data on the bioavailability of heavy metals indicate higher toxicity at the Bolesław spoil than at the Chrzanów wastes. This could also account for the generally lower diversity of all mycorrhizal fungi at the Bolesław site. Despite the higher toxicity of this site, which is more than 100 years old, it had better developed vegetation than the Chrzanów waste; this might simply be a matter of the time needed for the development and adaptation of plants at a given site (Wierzbicka and Rostański, 2002; Wierzbicka and Potocka, 2002). The higher toxicity of the Bolesław waste might be why the site yielded no records of *Paxillus involutus* fruitbodies, which were present though rare at the Chrzanów waste. This species is ubiquitous, with a broad host range. It is known to be comparatively tolerant of heavy metals. In vitro studies of strains of several mycorrhizal fungi, including those isolated from industrial waste in Chrzanów, showed this fungus to be much less tolerant of heavy metals than, for example, *Suillus luteus* (Blaudez et al., 2000), which was one of the most abundant fruitbody producers at both sites. On the other hand, *Paxillus involutus* is one of the species that appear early in the succession of ectomycorrhizal fungi. The investigated wastes and trees differ in age, and the differences in successional stage cannot be excluded as an additional reason for the differences in species diversity, especially since there is no comprehensive information on successional stages of fungi in substratum containing such high levels of heavy metals.

The better development of the vegetation cover, leading to the deposition of more organic matter at the Bolesław mine spoil, could account for the higher diversity of saprobic fungi at that site. Saprobic fungi constituted ~25% and 50% of recorded fungal species in Chrzanów and Bolesław, respectively. Litter-inhabiting species were frequent (e.g., *Bovista plumbea, Clitocybe inornata, Entoloma papillatum* and *Lepiota cristata* in Bolesław; *Helvella elastica, Clitocybe clavipes, C. dealbata* and *C. metachroa* in Chrzanów). A few pyrophilic fungi associated with burned areas were noted at these sites (e.g., *Pyronema domesticum* and *Sphaerosporella brunnea* in Chrzanów; *Pholiota highlandensis* in Bolesław) (Breintenbach and Kränzlin, 1981; Turnau, 1984). *Sphaerosporella brunnea* is also an ectomycorrhizal species (Danielson, 1984).

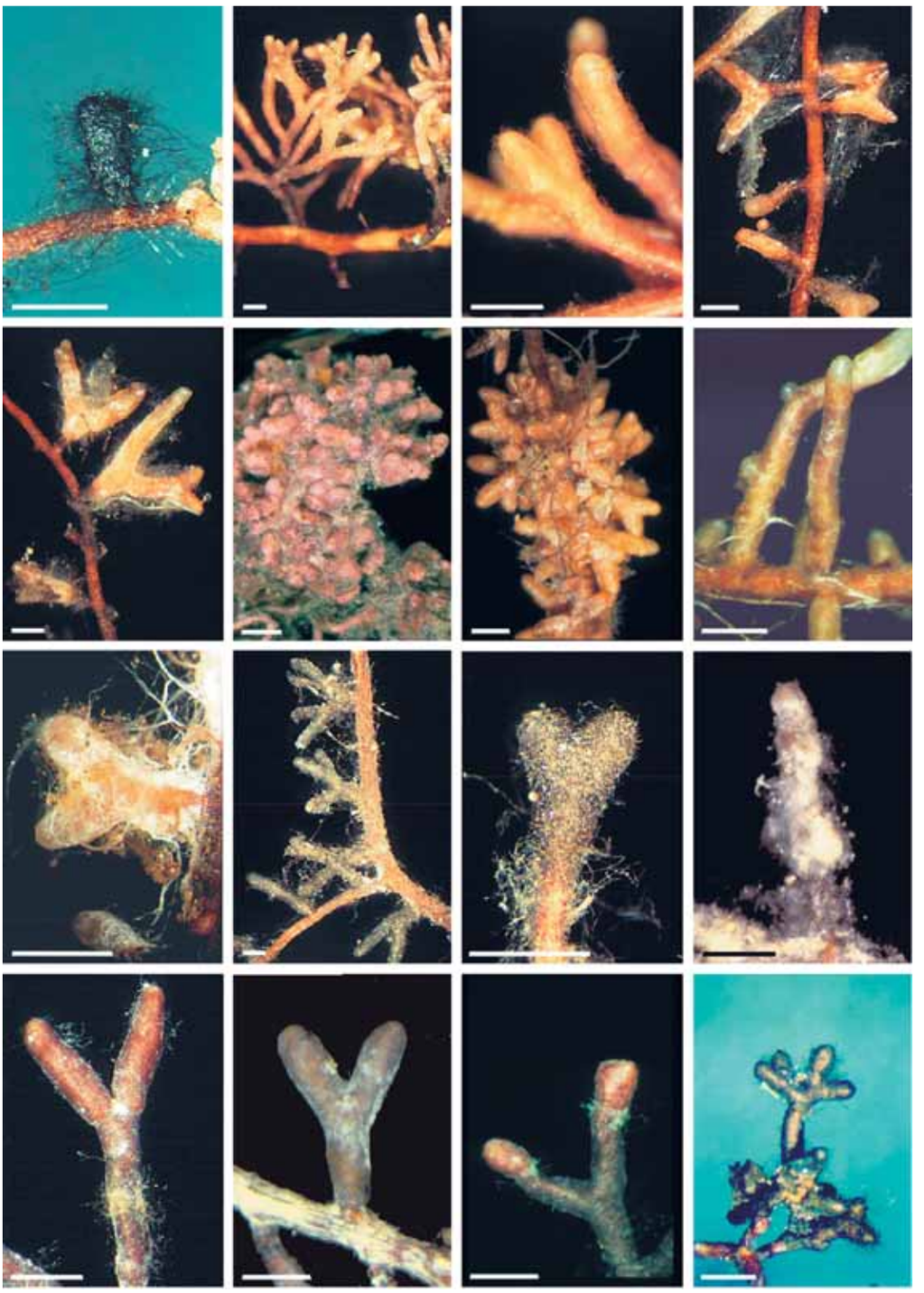


Fig. 1. Morphological features of selected ectomycorrhizas. (**a**) *Cenococcum geophilum*, (**b**) *Chroogomphus rutilus*, (**c**) *Chroogomphus rutilus*, ectomycorrhiza surrounded by curled cystidia, (**d**) *Hebeloma mesophaeum*, (**e**) *Hebeloma sinapizans*, (**f**) *Rhizopogon roseolus*, older, coralloid ectomycorrhizal system, (**g**) *Suillus luteus*, (**h**) *Thelephora terrestris*, (**i**) *Tricholoma scalpturatum*, (**j**) '*Pinirhiza acuto-nigra*,' (**k**) '*Pinirhiza acuto-nigra*,' surface covered with cystidia (**l**) '*Pinirhiza arenosa*,' (**m**) '*Pinirhiza brunnea*,' (**n**) '*Pinirhiza confusa*,' (**o**) '*Pinirhiza nuda*,' (**p**) '*Pinirhiza ovino-atra*'. Bars = 1 mm.

Among fungi inhabiting plant debris (including wood decomposers), *Marasmius limosus* (on reed leaves) and *Hypholoma fasciculare* were the only species recorded in Chrzanów, whereas in Bolesław this group was represented by several species.

Species preferring a grassy environment, such as *Bovista plumbea, Entoloma papillatum, Geoglossum cookeianum* and *Lepiota cristata* (Michael et al., 1968-1985; Cetto, 1978-1984; Breitenbach and Kränzlin, 1981; Rudnicka-Jezierska, 1991; Hansen and Knudsen, 1992), were present in Bolesław but almost absent in Chrzanów. This was again most probably related to the much better developed vegetation cover at the spoil in Bolesław.

The substratum of both sites was characterized by high calcium concentration and neutral or basic pH. Calciphilic species included both saprobic and mycorrhizal fungi such as *Clitocybe inornata*, *Geastrum minimum*, *Chroogomphus rutilus* and also most probably *Tricholoma scalpturatum* and *Lycoperdon lividum* (Gumińska, 1972, 2000; Moser, 1983; Breitenbach and Kränzlin, 1986, 1991; Rudnicka-Jezierska, 1991; Hansen and Knudsen, 1992, 1997; Bas et al., 1995). *Clitocybe clavipes* and *Scleroderma citrinum* usually prefer acidic pH (Breitenbach and Kränzlin, 1991; Rudnicka-Jezierska, 1991; Michael et al., 1968-1985). The occurrence of these species might be attributed to the formation of small ecological niches with low pH due to the acidifying properties of decomposing pine needles; however, adaptation by the particular strains of these fungi to higher substratum pH cannot be ruled out.

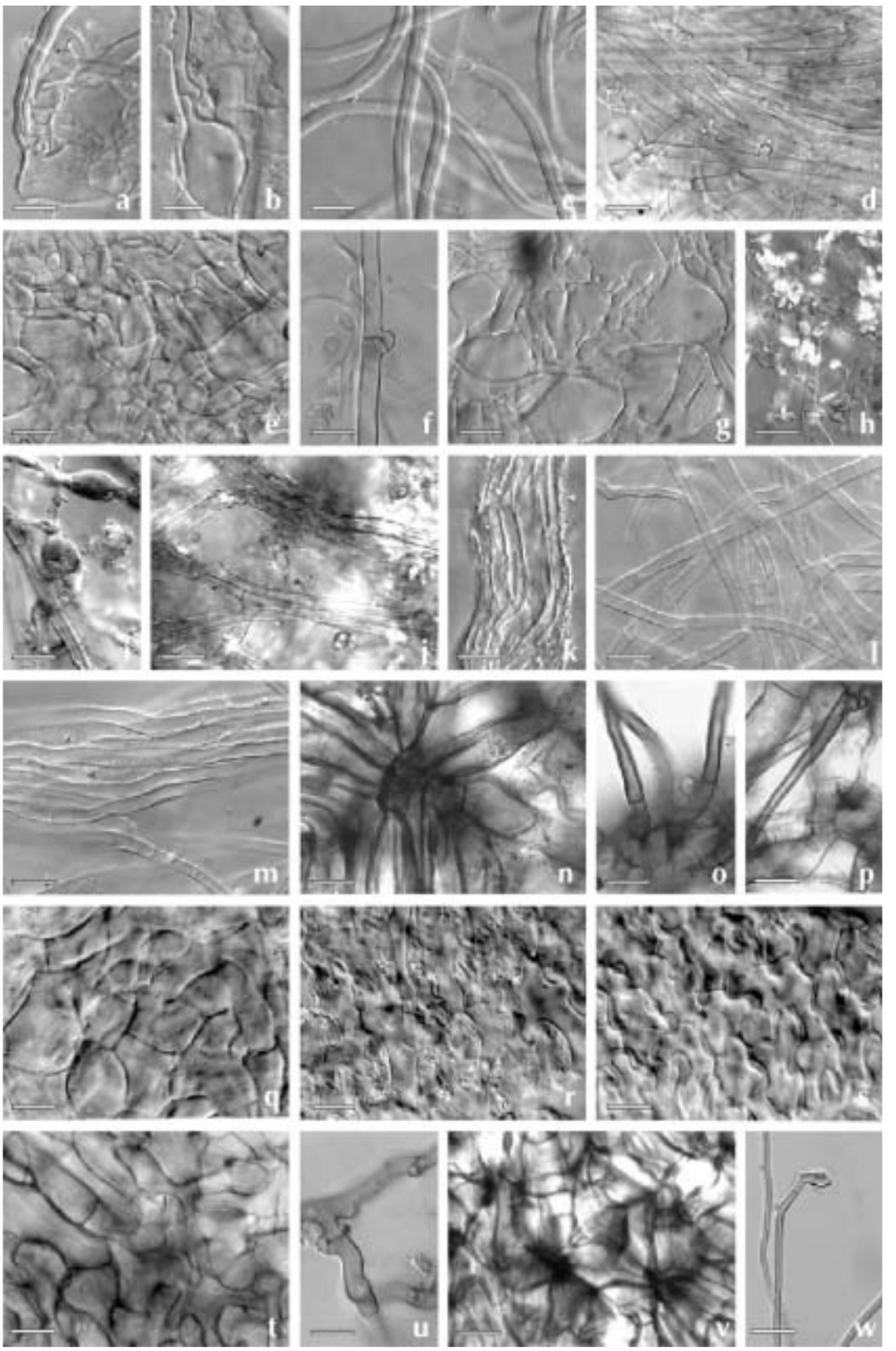
Most of the fungal species recorded from both wastes were observed in surrounding areas previously (Wojewoda, 1973, 1979, 1981). Among the fungi noted on the investigated sites, 3 species have been placed on the RED LIST OF FUNGI THREATENED IN POLAND (Wojewoda and Ławrynowicz, 1992): *Marasmius limosus* (in Chrzanów), *Entoloma papillatum* (in Bolesław) (both endangered but not fully evaluated, category 'I') and *Geastrum minimum* (in Bolesław) (vulnerable species, category 'V'). The last species is treated as endangered in Germany also (Benkert et al., 1996). It is a thermophilic and calciphilic fungus, recorded in Poland from less than 20 locations (Rudnicka-Jezierska, 1991). Malloch and Thorn (1985) regarded *Geastrum minimum* as a mycorrhizal symbiont of *Cistus monspeliensis*. Ectomycorrhizas of another species of *Geastrum* (*G. fimbriatum*) were described by Agerer and Beenken (1998). Other fungi observed at the sites in Bolesław and Chrzanów (*Chroogomphus rutilus, Clitocybe inornata, Collybia tuberosa, Geoglossum cookeianum*) have been placed on some European red data lists (Arnolds, 1989; Benkert et al., 1996).

MACROMYCETES ON RECULTIVATED AND NON-RECULTIVATED PLOTS OF THE BOLESŁAW SPOIL

Among the 38 species of fungi recorded on the spoil in Bolesław, 23 were noted on non-recultivated and 28 on recultivated plots; 13 species were common to both plots (Tab. 3). Some of the species were clearly associated with the non-recultivated plot, although usually also present on the recultivated area. This group was represented mostly by mycorrhizal species: *Chroogomphus rutilus* and *Inocybe* sp. (not observed at the recultivated plot), *Thelephora terrestris, Rhizopogon roseolus* and *Suillus luteus*. The above-mentioned species are regarded mostly as 'early stage fungi' or at least are found in all successional stages, and they often prefer open habitats devoid of a thick litter layer (Last et al., 1987; Thermorshuizen, 1991; Shaw and Lankey, 1994; Gáper and Lizoň, 1995). On the other hand, species characteristic of places rich in organic matter, such as *Clitocybe inornata* and *Ramaria abietina* (Breitenbach and Kränzlin, 1991), were recorded from the recultivated plot in Bolesław.

Some mycorrhizal species such as those belonging to the genera *Ramaria, Scleroderma* and *Cortinarius* were restricted to the recultivated plot. Their frequency and abundance were generally low. Most are regarded as typical 'late-stage fungi' (Last et al., 1987; Hintikka, 1988). Blasius and Oberwinkler (1989) and Baar (1996) consider litter accumulation a major factor limiting the occurrence of fruitbodies of some ectomycorrhizal fungi, which, however, may still be present in the form of ectomycorrhizas (Baar, 1996). Among the mycorrhizal fungi, there were no differences between recultivated and non-recultivated plots in the frequency and abundance of fruitbodies of *Tricholoma scalpturatum*, which was common in Bolesław.

Differences between the two plots were evident in the case of saprobic species. The fruitbodies of fungi usually reported from grasslands, such as *Bovista plumbea* (Kreisel, 1962; Runge, 1971), *Geoglossum cookeianum* and *Lepiota cristata* (Cetto, 1977–1984; Breitenbach and Kränzlin, 1981), appeared mostly on the non-recultivated plot. The fungi common to both plots represented species associated with pine cones or needles, such as *Auriscalpium vulgare* and *Strobilurus tenacellus*, or species belonging to litter-inhabiting fungi such as *Entoloma* and certain *Clitocybe*.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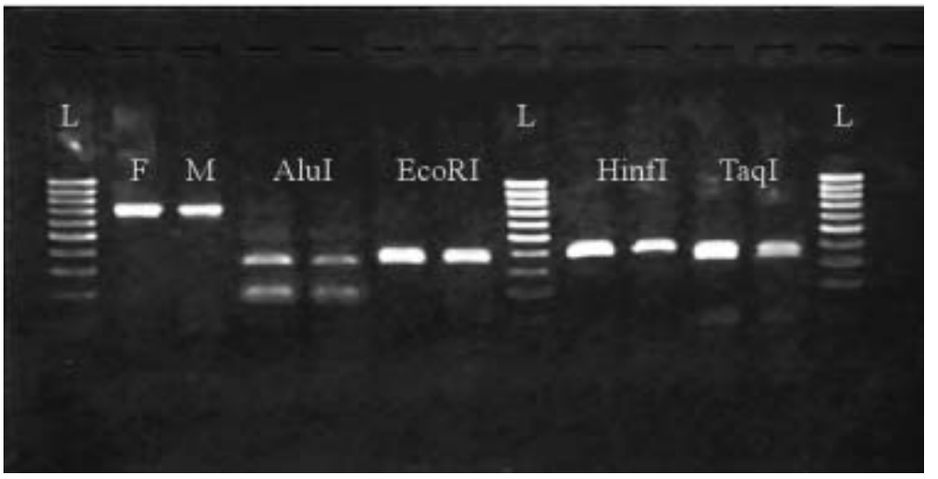

Fig. 3. Electrophoretic gel with fractionated, PCR-amplified rDNA of *Hebeloma mesophaeum* fruitbody (F) and mycorrhiza (M), after digestion with restriction enzymes (Alu I, EcoR I, Hinf I, Taq I). L = ladder.

ASSESSMENT OF ECTOMYCORRHIZAS

Samples collected from sites at Bolesław (the non-recultivated plot) and Chrzanów with similar plant cover were analyzed for mycorrhizal morphotypes. The most characteristic and numerous ectomycorrhizal morphotypes were selected, and their morphological and anatomical features were studied (Tab. 4). The morphotypes were also analyzed by PCR technique, and in a few cases the data were matched to those obtained for fruitbodies to confirm the identification of the mycorrhiza (Tab. 5). As no DNA cleaning procedure was used, the PCR method was especially useful for mycorrhizas devoid of brown or black pigment (a few basidomycete and all ascomycete mycorrhizas); the pigment can inhibit DNA amplification.

The fungi that formed the most abundant fruitbodies at both sites, such as *Rhizopogon roseolus*, *Suillus luteus*, *Tricholoma scalpturatum*, *Thelephora terrestris*, were also found among the ectomycorrhizas. Mycorrhizas of a few species not recorded in the form of aboveground fruitbodies were found at both sites, including ascomycetous mycorrhiza formed by species of Humariaceae, ascomycetous mycorrhiza with an affinity for tuberoids, and basidiomycetous mycorrhiza formed most probably by a *Tomentella* species. In addition, mycorrhizas of *Cenococcum geophilum*, an ascomycete possibly with an affinity for Loculoascomycetidae (LoBuglio et al., 1996) were observed in Bolesław. These findings indicate that assessment of mycorrhizal fungi on the basis of fruitbody observations alone is inaccurate; mycorrhizas should be considered in evaluating the diversity of ectomycorrhizal fungi. On the other hand, many species, especially those that occurred at the Chrzanów waste, were not recorded in the form of ectomycorrhizas (e.g., species of *Inocybe*, *Cortinarius* and some *Hebeloma*). This might be due to uneven distribution of mycorrhizas within the substratum, low frequency and abundance, a preference for deeper soil layers, and possibly also insufficient sampling. As the soil and root material from beneath the fruitbodies was not collected, it is likely that mycorrhizas of sparsely represented species were not detected.

Earlier investigations of the ectomycorrhiza population at the waste in Chrzanów revealed differences in the frequencies and abundance of ecto-

Fig. 2. Microscopic structures of selected ectomycorrhizas. **(a–c)** *Chroogomphus rutilus*, (**a**) Basal part of cystidium with clamp connection, (**b**) Swollen segment of hyphal net, (**c**) Bent, thick-walled cystidia; **(d–f)** *Hebeloma mesophaeum*, (**d**) Loosely organized outer mantle layer, (**e**) Middle mantle with enlarged hyphae, (**f**) Clamp connection on emanating hyphae; **(g)** *Hebeloma sinapizans*, middle mantle with some inflated hyphal cells; **(h–i)** *Rhizopogon roseolus*, (**h**) Suilloid crystals on mantle surface, (**i**) Pigment drops on emanating hyphae; **(j–k)** *Suillus luteus*, (**j**) Outer mantle layer, (**k**) Rhizomorph with enlarged central hypha; **(l–m)** *Tricholoma scalpturatum*, (**l**) Loosely organized mantle surface, (**m**) Rhizomorph fragment; **(n–p)** '*Pinirhiza acuto-nigra*,' (**n**) Middle mantle layer, star-like hyphal pattern, (**o**) Basal parts of cystidia, (**p**) Outer mantle surface with cystidium base; **(q)** '*Pinirhiza arenosa*,' middle mantle layer, hyphae considerably enlarged and swollen; **(r–s)** '*Pinirhiza confusa*,' (**r**) Hyphal net on mantle surface, (**s**) Middle, epidermoid mantle layer; **(t–u)** '*Pinirhiza brunnea*,' (**t**) Outer mantle layer with hyphae irregular in shape, (**u**) Emanating hyphae with clamp connection; **(v–w)** '*Pinirhiza ovino-atra*,' (**v**) Outer mantle layer, nest-like arrangement of hyphae, (**w**) Apical, swollen part of cystidium. Bars = 10 μm.

TABLE 4. Structural features of most important ectomycorrhizal morphotypes from Chrzanów (Ch) and Bolesław (B). Types of rhizomorphs, mantle and cystidia according to Agerer (1991, 1995, 1987–2003). Mycorrhizae arranged in alphabetical order

Color	Rhizomorph type	Mantle type	Special features	Hyphal junctions	Hyphal surface	Emanating hyphae
Cenococcum geophilum (Ascomycota), B (Fig. 1a)						
black	not observed	type G, plectenchyma, hyphae black, thick-walled, 3–6 µm in diam., forming a star-like pattern; inner mantle plectenchymatous, hyphae less pigmented	cystidia: thick-walled, black hyphae (here regarded as cystidia); lactifers: lacking; clamps: lacking	open bridges	mantle hyphae smooth, emanating hyphae smooth or rough, black	scarce
Chroogomphus rutilus (Gomphidiaceae, Basidiomycota), Ch. B (Figs. 1b,c, 2a–c)						
brownish, reddish-brown	not observed	type D, plectenchyma, without pattern, hyphal net with some swollen hyphal segments is present on mantle surface	cystidia: type A, up to 55 µm long, thick-walled, curved, septa simple, with clamp at the base; lactifers: lacking; clamps: present, less than semicircle, without hole	short open bridges	smooth, walls colorless	frequent, thin-walled
Hebeloma mesophaeum (Bolbitiaceae, Basidiomycota), Ch. B (Figs. 1d, 2d–f)						
yellowish, yellow, silvery in places	type B, very loosely organised, undifferentiated	type B, plectenchyma, thin, outer layer very loose, hyphae ~1.5 µm in diam., inner layers more compact, hyphae branched, swollen in places, ~1.5–4(6) µm in diam.	cystidia: lacking; laticifers: lacking; clamps: present, semicircle or more than semicircle in side view, with hole	contact clamps	smooth, walls colorless, rarely yellowish	frequent, thin-walled, septa usually thicker than walls
Hebeloma sinapizans (Bolbitiaceae, Basidiomycota), B (Figs. 1e, 2g)						
yellowish, yellow, silvery in places	type B, loosely organised, undifferentiated, sometimes lacking	type B, thin, plectenchyma, outer layer loose, hypahe 1–1.5 µm in diam., inner layers compact, hyphae branched and often swollen or irregular in shape, up to 4 µm in diam.	cystidia: lacking; laticifers: lacking; clamps: present, semicircle or more than semicircle in side view, with hole	short open bridges and contact clamps	smooth, walls colorless	frequent, septa usually as thin as walls
Rhizopogon roseolus (Rhizopogonaceae, Basidiomycota), Ch. B (Figs. 1f, 2h–i)						
silvery, white when young, later becoming pinkish, reddish and (the oldest) brownish	type F, highly differentiated, branched, central hyphae vessel-like, considerably enlarged, septa partially or totally dissolved, peripheral hyphae smaller, with complete septa	type B/C, thick, plectenchyma, hyphae 2–5 µm in diam.; inner mantle layers more compact, hyphae immersed in non-gelatinous material	cystidia: lacking laticifers: lacking clamps: lacking	short bridges, open or closed by septum	covered by crystals, crystalloids or drops of pigment, rarely smooth	usually frequent
Suillus luteus (Suillaceae, Basidiomycota), Ch. B (Figs. 1g, 2j–k)						
tip yellowish, below orange-brownish, or brownish, old brown, silvery in places	type F, highly differentiated, central part with enlarged vessel-like hyphae up to 30 µm in diam., with septa often partially or totally dissolved, some with crystalloids inside	type B, plectenchyma, in outer part loose, in inner layers compact, without pattern; hyphae branched, with dense septa	cystidia: lacking laticifers: lacking clamps: lacking	short bridges, open or closed by septum	smooth or covered by drops of pigment and crystalloids	scarce to abundant (especially beneath tip and in basal parts of system)

TABLE 4. (cont.)

Color	Rhizomorph type	Mantle type	Special features	Hyphal junctions	Hyphal surface	Emanating hyphae
Thelephora terrestris (Thelephoraceae, Basidiomycota), Ch. B (Fig. 1h)						
yellowish at top, yellow-brownish to brownish beneath, silvery in places	type B/C, undifferentiated or slightly differentiated, some thicker central hyphae up to 10 µm in diam., with complete septa present, margin with some emanating hyphae and occasionally with setae	type D, plectenchyma without pattern; surface a hyphal net, hyphae branched, 2–5 µm in diam.; middle mantle compact, septa dense, hyphal segments irregular in shape	cystidia: type A, rigid setae narrowing at tip, 1.5–2 µm in diam., up to 150 µm long, walls thickened, with 1–4 simple septa, clamp at base; lactifers: lacking; clamps: present, less than semicircle	short open bridges	smooth, walls colorless	infrequent, thin-walled
Tricholoma scalpturatum (Tricholomataceae, Basidiomycota), Ch. B (Figs. 1i, 2l–m)						
silvery, white, yellowish-white, yellowish-orange to reddish or reddish-brown in older parts	type B, non-differentiated or slightly differentiated, surface shredded, rarely smooth, with many side branches, hyphae of equal diam., in thicker rhizomorphs central hyphae slightly thickened; hyphae often with oily content	type B, without pattern, outer layer loosely organized, hyphae 2–5 µm in diam., anastomoses and ramifications frequent, branches often cross-like (symmetrical), inner layers more compact	cystidia: lacking laticifers: lacking clamps: lacking	long or short bridges, open or closed by septum	smooth, walls not thickened, colorless or yellowish	frequent, weakly ramified, with few junctions
'*Pinirhiza acuto-nigra*' (cf. *Tomentella* sp.; Basidiomycota), B (Figs. 1j-k, 2n–p)						
dark brown to black	not observed	type G/L, pseudoparenchyma, surface with cystidia and enlarged hyphal cells up to 17 µm in diam.; beneath surface hyphae form star-like pattern, hyphal cells elongated, rectangular, in star centers smaller	cystidia: type B, bottle-shaped, basal part swollen, with clamp, upper neck narrow, ~ 1.5 µm in diam., up to 7 µm long, with 0–2(3) septa, walls thickened; lactifers: lacking; clamps: present in emanating hyphae and at cystidium base	long or short bridges, open or closed by septum (simple or clamp)	smooth, walls brownish, thickened in mantle hyphae and cystidia	frequent, ramified and anastomizing
'*Pinirhiza arenosa*' (Basidiomycota), Ch (Figs. 1l, 2q)						
yellowish, brownish-yellow in older parts	not observed	type H, transitional type between plectenchyma and pseudoparenchyma, hyphal segments irregular in shape	cystidia: lacking; laticifers: lacking; clamps: present, semicircle or more than semic. in side view, with hole; globular structures often associated with septa	short open bridges and contact clamps	smooth, but coarsely covered with sand particles, walls yellowish	bent and curved, walls slightly thickened, septa as thin as walls
'*Pinirhiza brunnea*' (Basidiomycota), Ch. B (Figs. 1m, 2t–u)						
yellowish-beige in apical part, light brown beneath, brown to dark brown in older parts	not observed	type L/K, with gelatinous substance, surface with round or irregular cells with thickened walls, hyphal cells beneath irregular or epidermoid, walls thickened; middle and inner layer hyphae with thinner and paler walls	cystidia: lacking lactifers: lacking clamps: present on emanating hyphae, in mantle lacking	not observed	smooth, walls yellow-brownish to brownish	infrequent, with clamps (prevailing) and simple septa

TABLE 4. (cont.)

Color	Rhizomorph type	Mantle type	Special features	Hyphal junctions	Hyphal surface	Emanating hyphae
'*Pinirhiza confusa*' (tuberoid affinity; Ascomycota), Ch (Figs. 1n, 2r–s)						
yellow-brown, light brown to brown in older parts	not observed	type Q, thick, pseudoparenchyma, surface a hyphal net with irregular segments, outer mantle epidermoid, hyphae lobed, walls thickened, septa thin; middle mantle similar but walls thinner; inner mantle plectenchymatous	cystidia: not observed laticifers: lacking clamps: lacking	not observed	smooth, walls yellowish to yellow	rare
'*Pinirhiza nuda*' (cf. Humariaceae, Ascomycota), Ch (Fig. 1o)						
brownish, brown, mantle smooth, glistening	not observed	typeH, usually thin, hyphae brownish, up to 5(7) µm thick, ramified, often with short segments	cystidia: lacking laticifers: lacking clamps: lacking	rare, long and short bridges, open or closed by septum	on mantle hyphae smooth, on emanating hyphae rough, walls yellowish	rare, thick-walled, with globular structures at septa
'*Pinirhiza ovino-atra*' (unknown affinity), B (Figs. 1p, 2v–w)						
black, brown-black	type B, infrequent, undifferentiated, smooth and compact, hyphae 1.5–2.5 µm in diam.	type L, outer mantle a pseudoparenchyma formed by rectangular hyphal cells 4–7(10) µm in diam., inner mantle plectenchymatous	cystidia: type L, long, bent and curved, 1-1.5 µm in diam., walls thickened, in distal parts thin, tips often swollen, break easily, septa rare, simple; lactifers: lacking; clamps: lacking	not observed	smooth, walls brown or brownish	scarce, thicker than cystidia, walls thin, septa frequent

mycorrhizas and fruitbodies of the recorded species (Turnau et al., 2002). *Suillus luteus* and *Rhizopogon roseolus* represented up to 7% and 3% of all ectomycorrhizas, respectively, whereas their fruitbodies were very abundant. The most frequent of the analyzed mycorrhizas was an unidentified *Pinirhiza arenosa* (~70%), followed by less abundant *Tricholoma scalpturatum*, *Hebeloma mesophaeum*, and black and dark-brown mycorrhizas, members of Thelephoraceae. The observations of ectomycorrhizas at the spoil in Bolesław generally agree with those data, except that *Hebeloma* species were much less abundant.

Differences in the ability to accumulate heavy metals within the fungal mantle of ectomycorrhizas isolated from the Chrzanów waste were determined for the first time by means of electron energy loss spectroscopy (EELS) accompanied by TEM (Turnau et al., 1996), and subsequently by energy dispersion spectroscopy (EDS) connected with a scanning electron microscope (Turnau et al., 2002). Using particle-induced X-ray emissions (PIXE), Turnau et al. (2001) confirmed the filtering effect in mycorrhizas such as *Rhizopogon roseolus* and *Suillus luteus*. Although these mycorrhizas were not dominant, these species would be useful for introduction in polluted areas. Our results will provide a basis for further attempts to isolate the most effective fungal symbionts of the pine trees used in revegetation of polluted areas.

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TABLE 5. Results of amplification of ITS region, and RFLP analysis using four restriction enzymes (Alu I, EcoR I, Hinf I, Taq I). * – indicates double band

Mycorrhiza	ITS	Alu I	EcoR I	Hinf I	Taq I
Hebeloma mesophaeum	710	300, 210	360*	360*	365*
Hebeloma sinapizans	720	370, 215	365*	370*	365*
'*Pinirhiza arenosa*'	625	439	349, 293	325, 293	325, 245
Rhizopogon roseolus	745	400, 295	–	260*, 185	315*
Suillus luteus	710	705	710	255*, 155	–
Thelephora terrestris	680	255, 250	330, 305	330, 205	305*
Tricholoma scalpturatum	705	400, 295	–	260*, 185	315*

REFERENCES

ADRIANO D. 2001. *Trace elements in terrestrial environment*. Springer-Verlag, New York, Berlin, Heidelberg.

AGERER R. [ed.]. 1987–2003. *Colour atlas of ectomycorrhizae*. Einhorn Verlag, Schwäbisch Gmünd, Germany.

AGERER R. 1991. Characterization of ectomycorrhiza. In: Norris JR, Read DJ, Varma K [eds.], *Techniques for the study of mycorrhiza*, 25–73. *Methods in Microbiology* 23. Academic Press.

AGERER R, MÜLLER WR, and BAHNWEG G. 1996. Ectomycorrhizae of *Rhizopogon subcaerulascens* on *Tsuga heterophylla*. *Nova Hedwigia* 63: 397–415.

AGERER R, and BEENKEN L. 1998. *Geastrum fimbriatum* Fr. + *Fagus sylvatica* L. *Descriptions of Ectomycorrhizae* 3: 13–18.

AGERER R, and RAMBOLD G. 1997. *DEEMY. A system for characterization and determination of ectomycorrhizae.* CD-ROM. Institute for Systematic Botany, Section Mycology, Munich, Germany.

ARNOLDS E. 1989. A preliminary red data list of macrofungi in the Netherlands. *Persoonia* 14: 77–125.

BAAR J. 1996. The ectomycorrhizal flora of primary and secondary stands of *Pinus sylvestris* in relation to soil conditions and ectomycorrhizal succession. *Journal of Vegetation Sciences* 7: 497–504.

BAS C, KUYPER THW, NOORDELOOS ME, and VELLINGA EC. 1995. *Flora Agaricina Neerlandica*, vol. 3. AA. Balkema, Rotterdam, Brookfield.

BENKERT D, DÖRFELT H, HARDTKE HJ, HIRSCH G, KREISEL H, KRIEGLSTEINER GJ, LÜDERITZ M, RUNGE A, SCHMID H, SCHMITT JA, WINTERHOFF W, WÖLDECKE K, and ZEHFUß HD. 1996. Rote Liste der Großpilze Deutschlands. In: *Rote Liste gefährdeter Pflanzen Deutschlands. Schriftenreihe für Vegetationskunde* 28: 377–426.

BLASIUS D, and OBERWINKLER F. 1989. Succession of mycorrhizae: a matter of tree age or stand age? *Annales Scientie Forestaliae* 46: 758–761.

BLAUDEZ D, JACOB C, TURNAU K, COLPAERT JV, AHONEN-JONARTH U, and FINLAY R. 2000. Differential responses of ectomycorrhizal fungi to heavy metals *in vitro*. *Mycological Research* 104: 1366–1371.

BREITENBACH J, and KRÄNZLIN F. 1981. *Pilze der Schweiz*, Band 1, *Ascomyceten*. Verlag Mycologia, Luzern.

BREITENBACH J, and KRÄNZLIN F. 1986. *Pilze der Schweiz*, Band 2. *Nichtblätterpilze: Heterobasidiomycetes, Aphyllophorales, Gasteromycetes*. Verlag Mykologia, Luzern.

BREITENBACH J, and KRÄNZLIN F. 1991. Pilze der Schweiz, Band 3. *Röhrling und Blätterpilze* 1 Teil, *Strobilomytcetaceae* und *Boletaceae, Paxillaceae, Gomphidiaceae, Hygrophoraceae, Tricholomataceae, Polyporaceae*. Verlag Mycologia, Luzern.

CETTO B. 1977–1984. *Der grosse Pilzführer*, Band 1–4. BVL Verlagsgesellschaft, München.

DANIELSON RM. 1984. Ektomycorrhizae formation by the operculate Discomycete *Sphaerosporella brunnea* (*Pezizales*) . *Mycologia* 76: 454–461.

DMOWSKI K. 2000. Environmental monitoring of heavy metals with magpie (*Pica pica*) feathers – an example of Polish polluted and control areas. In: Market B, Friese P [eds.], *Trace elements in the environment*, 455–477. Elsevier Science, Amsterdam.

DOBRZAŃSKA J. 1955. Flora and ecological studies on calamine flora in the district of Bolesław and Olkusz. *Acta Societatis Botanicorum Poloniae* 24: 357–417.

GÁPER J, and LIZOŇ P. 1995. Sporocarp succession of mycorrhizal fungi in the Norway spruce plantation in formerly agricultural land. In: Baluška F et al. [eds.], *Structure and function of roots*. Kluwer Academic Publishers, Netherlands.

GARDES M, and BRUNS T. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.

GARDES M, and BRUNS T. 1996. Community structure of mycorrhizal fungi in a *Pinus muricata* forest: above- and below ground views. *Canadian Journal of Botany* 74: 1572–1583.

GARDES M, WHITE TJ, FORTIN JA, BRUNS TD, and TAYLOR JW. 1991. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. *Canadian Journal of Botany* 69: 180–190.

GODZIK B. 1993. Heavy metal content in plants from zinc dumps and reference areas. *Polish Botanical Studies* 5: 113–132.

GRODZIŃSKA K, KORZENIAK U, SZAREK-ŁUKASZEWSKA G, and GODZIK B. 2000. Colonization of zinc mine spoils in southern Poland – preliminary studies on vegetation, seed rain and seed bank. *Fragmenta Floristica et Geobotanica* 45: 123–145.

GUMIŃSKA B. 1972. Mikoflora Pieninskiego Parku Narodowego (część III). *Zeszyty Naukowe UJ, Prace Botaniczne* 4: 128–141.

GUMIŃSKA B. 2000. Grzyby wielkoowocnikowe (macromycetes). *Flora i Fauna Pienin – Monografie Pienińskie* 1: 47–53.

HANSEN L, and KNUDSEN H. [eds.]. 1992. *Nordic Macromycetes*, vol. 2. *Polyporales, Boletales, Agaricales, Russulales*. Nordsvamp, Copenhagen.

HANSEN L, and KNUDSEN H. [eds.]. 1997. *Nordic Macromycetes*, vol. 3. *Heterobasidioid, aphyllophoroid and gastromycetoid basidiomycetes*. Nordsvamp, Copenhagen.

HANSEN L, and KNUDSEN H. [eds.]. 2000. *Nordic Macromycetes*, vol. 1. *Ascomycetes*. Nordsvamp, Copenhagen.

HINTIKKA V. 1988. On the macromycete flora in oligotrophic pine forest of different ages in South Finland. *Acta Botanica Fennica* 136: 89–94.

JURKIEWICZ A, TURNAU K, MESJASZ-PRZYBYLOWICZ J, PRZYBYLOWICZ W, and GODZIK B. 2001. Heavy metal localization in mycorrhizas of *Epipactis atropurpureum* (Orchidaceae) from zinc wastes in Poland. *Protoplasma* 218: 117–124.

KÅREN O, HÖGBERG N, DAHLBERG A, JONSSON L, and NYLUND J-E. 1997. Inter- and intraspecific variation in the ITS region of the rDNA of the ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. *New Phytologist* 136: 313–325.

KIRK PM, CANNON PF, DAVID JC, and STALPERS JA [eds.]. 2001. *Ainsworth and Bisby's dictionary of the fungi*. 9th Edition. CAB International, Wallingford, UK.

KREISEL H. 1962. Taksonomisch-pflanzengeographische Monographiae der Gattung *Bovista*. *Beihefte zur Nova Hedwigia* 25: 1–244.

LAST FT, DIGHTON J, and MASON PA. 1987. Succession of sheathing mycorrhizal fungi. *TREE* 2: 157–161.

LOBUGLIO KF, BERBEE ML, and TAYLOR JW. 1996. Phylogenetic origins of the asexual mycorrhizal symbiont *Cenococcum geophilum* Fr. and other mycorrhizal fungi among the Ascomycetes. *Molecular Phylogenetics and Evolution* 6: 287–294.

MALLOCH D, and THORN RG. 1985. The occurrence of ectomycorrhizae in some species of *Cistaceae* in North America. *Canadian Journal of Botany* 63: 872–875.

MARSCHNER H, and DELL B. 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* 159: 89–102.

MICHAEL E, HENNIG B, and KREISEL H. 1968–1985. *Handbuch für Pilzfreunde*, I–VI. Veb. G. Fischer Verlag, Jena.

MOSER M. 1983. *Keys to Agarics and Boleti* (*Polyporales, Boletales, Agaricales, Russulales*). Roger Philips, London.

ORŁOWSKA E, ZUBEK SZ, JURKIEWICZ, A, SZAREK-ŁUKASZEWSKA G, and TURNAU K. 2002. Influence of restoration on arbuscular mycorrhiza of *Biscutella laevigata* L. (Brassicaceae) and *Plantago lanceolata* L. (Plantaginaceae) from calamine spoil mounds. *Mycorrhiza* 12: 153–159.

RUDNICKA-JEZIERSKA W. 1991. Podstawczaki (*Basidiomycetes*), Purchawkowe (*Lycoperdales*), Tęgoskórowe (*Sclerodematales*), Pałeczkowe (*Tulostomales*), Gniazdnicowe (*Nidulariales*), Sromotnikowe (*Phallales*), Osiakowe (*Podaxales*). *Flora Polska, Grzyby (Mycota)* 23. Instytut Botaniki PAN, Kraków.

RUNGE A. 1971. Stäbling (Lycoperdaceen) Funde unter besonderer Bercksichtigung. *Westfalens Zeitschrift für Pilzkunde* 37: 149–159.

SAMBROOK J, FRITSCH EF, and MANIATIS T. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, New York.

SHAW PJA, and LANKEY K. 1994. Studies on the scots pine mycorrhizal fruitbody succession. *Mycologist* 8: 172–174.

SZAFER W. 1959. *The vegetation in Poland*. Państwowe Wydawnictwo Naukowe, Warszawa.

SZAFER W, and ZARZYCKI K. [eds.]. 1972. *Szata roślinna Polski*. Państwowe Wydawnictwo Naukowe, Warszawa.

SZAREK-ŁUKASZEWSKA G, and NIKLIŃSKA M. 2002. Concentration of alkaline and heavy metals in *Biscutella laevigata* L. and *Plantago lanceolata* L. growing on calamine spoils (S. Poland). *Acta Biologica Cracoviensia Series Botanica* 44: 29–38.

THERMORSHUIZEN AJ. 1991. Succession of mycorrhizal fungi in stands of *Pinus sylvestris* in The Netherlands. *Journal of Vegetation Science* 2: 555–564.

TINKER PB, JONES MD, and DURALL DM. 1992. A functional comparison of ecto- and endomycorrhizas. In: Read DJ, Lewis DH, Fitter AH, and Alexander IJ [eds.], *Mycorrhizas in ecosystems*, 303–310. CAB International, Wallingford, UK.

TISDALL JM. 1994. Possible role of soil microorganisms in aggregation of soil. *Plant and Soil* 159: 115–121.

TURNAU K. 1984. Post-fire cup-fungi of Turbacz and Stare Wierchy mountains in the Gorce Range (Polish Western Carpathians). *Zeszyty Naukowe UJ, Prace Botaniczne* 12: 145–170.

TURNAU K. 1998. Heavy metal uptake and arbuscular mycorrhiza development of *Euphorbia cyparissias* on zinc wastes in South Poland. *Acta Societatis Botanicorum Poloniae* 67: 105–113.

TURNAU K, KOTTKE I, and DEXHEIMER J. 1996. Toxic element filtering in *Rhizopogon roseolus/Pinus sylvestris* mycorrhizas collected from calamine dumps. *Mycological Research* 100: 6–22.

TURNAU K, PRZYBYLOWICZ W, and MESJASZ-PRZYBYLOWICZ J. 2001. Heavy metal distribution in *Suillus luteus* mycorrhizas – as revealed by proton microscopy and PIXE. *Journal of Nuclear Instruments* 181: 649–658.

TURNAU K, MLECZKO P, BLAUDEZ D, CHALOT M, and BOTTON B. 2002. Heavy metal binding properties of *Pinus sylvestris* mycorrhizas from industrial wastes. *Acta Societatis Botanicorum Poloniae* 71: 253–261.

WALKER RF, WEST DC, MCLAUGHLIN SB, and AMUNDSEN CC. 1989. Growth, xylem pressure potential, and nutrient absorption of loblolly pine on a reclaimed surface mine as affected by an induced *Pisolithus tinctorius* infection. *Forest Sciences* 35: 569–581.

WHITE TJ, BRUNS T, LEE S, and TAYLOR J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, and White TJ [eds.], *PCR Protocols: a guide to methods and applications*, 315–322. Academic Press, New York.

WIERZBICKA M, and POTOCKA A. 2002. Lead tolerance in plants growing on dry and moist soils. *Acta Biologica Cracoviensia Series Botanica* 44: 21–28.

WIERZBICKA M, and ROSTAŃSKI A. 2002. Microevolutionary changes in ecotypes of calamine waste heap vegetation near Olkusz, Poland: A review. *Acta Biologica Cracoviensia Series Botanica* 44: 7–19.

WOJEWODA W. 1973. Grzyby wielkoowocnikowe ('*macromycetes*') Ziemi Chrzanowskiej i Jaworzna, część I. *Studia Ośrodka Dokumetacji Fizjograficznej* 2: 57–86.

WOJEWODA W. 1979. Grzyby wielkoowocnikowe ('*macromycetes*') Ziemi Chrzanowskiej i Jaworza. Część II. *Studia Ośrodka Dokumentacji Fizjograficznej* 7: 67–108.

WOJEWODA W. 1981. Grzyby wielkoowocnikowe ('*macromycetes*') Ziemi Chrzanowskiej i Jaworzna, część III. *Studia Ośrodka Dokumentacji Fizjograficznej* 8: 187–201.

WOJEWODA W, and ŁAWRYNOWICZ M. 1992. Czerwona lista grzybów wielkoowocnikowych zagrożonych w Polsce. In: Zarzycki K, Wojewoda W, Heinrich Z [eds.], *Lista roślin zagrożonych w Polsce*, 27–56. Instytut Botaniki PAN, Kraków.