Zbornik gozdarstva in lesarstva, 52, 1997, s. 279 - 322

GDK 181.45 : 181.351 : 161.4 : 160.201 : 160.203 : 174.7 *Picea abies* Karst : (497.12)

Prispelo / *Arrived*: 17. 2.1997
Sprejeto / *Accepted*: 5. 3.1997

MIKOBIOINDIKACIJA ONESNAŽENOSTI DVEH GOZDNIH RASTIŠČ

Hojka KRAIGHER*

Izvleček

Na dveh različno onesnaženih, rastiščno primerljivih gozdnih raziskovalnih ploskvah v imisijski coni termoelektrarne Šoštanj smo v vegetacijski sezoni 1993 v volumsko enakih vzorcih zemlje raziskovali pojavljanje tipov ektomikorize. Na kratko smo opisali dvajset morfotipov ektomikorize, dva tipa ektomikorize, *Lactarius lignyotus* x *Picea abies* in *Hydnum rufescens* x *Picea abies,* smo natančno opisali z anatomskimi in molekularnimi metodami. Pojavljanje trosnjakov ni ustrezalo pojavljanju tipov ektomikorize v vzorcih tal. Tip ektomikorize *Hydnum rufescens* x *Picea abies* bi lahko uporabili za kazalca neonesnaženih rastišč, *Paxillus involutus* x *Picea abies* pa za kazalca onesnaženih gozdnih rastišč v Sloveniji.

Ključne besede: tipi ektomikorize, *Picea abies,* anatomska karakterizacija, molekularne metode, mikobioindikacija

MYCOBIOINDICATION OF POLLUTION OF TWO FOREST SITES

Abstract

In two differently polluted forest research plots in the emission area of the Coal Power Plant in Šoštanj, which were comparable regarding their site characteristics, the types of ectomycorrhizae were studied during the vegetation season of 1993, in soil cores of equal volumes. Twenty morphotypes of ectomycorrhizae were briefly described, while two types, *Lactarius lignyotus* x *Picea abies* and *Hydnum rufescens* x *Picea abies,* were comprehensively characterised by anatomical and molecular methods. The occurrence of fungal fruitbodies did not correspond to the occurrence of ectomycorrhizal types. It might be proposed that type *Hydnum rufescens* x *Picea abies* be used as a bioindicatior of unpolluted sites, while *Paxillus involutus* x *Picea abies* as bioindicator of polluted forest sites in Slovenia.

Key words: types of ectomycorrhizae, *Picea abies,* anatomical characterisation, molecular methods, mycobioindication

* Doc. dr., dipl. biol., dipl. inž.gozd., Gozdarski inštitut Slovenije, Večna pot 2, 1000 Ljubljana, SLO

CONTENTS

1 INTRODUCTION

Forest ecosystems are, functionally and structurally, highly organized systems of biotic and abiotic components, linked into a sensitive dynamic equilibrium. Air pollution can influence all and each of the components of these systems at different levels. Directly it can influence the physiology of forest trees by damaging their photosynthetic apparatus, leaves and needles at the cellular level (SCHÜTT *et all.l.* 1984, REHFUESS 1988, BLANK / ROBERTS / SKEFFINGTON 1988, FINK 1988, WOLFENDEN / MANSFIELD 1991 etc.). Indirectly it can induce changes in mycorrhizosphere by influencing root systems, by changes in the living component in forest soil and by influences on the principal linking component in the forest floor, the mycelium of fungi, which live in a symbiosis with roots of higher plants (SCHLECHTE 1985, MC COOL 1988, COWLING 1989 etc.).

Different methods are in use for detection of forest site pollution: direct measurements of emissions are largely complemented by methods of bioindication, whereby bioindication organisms react to specific environmental conditions (or to a specific pollutant) by their specific physiological response. Biomonitoring of forest site pollution implies the analysis of damage and abundance of chosen "indicator" organisms in the ecosystem, the method which is known as passive biomonitoring; while active biomonitoring implies the introduction of "monitor" organisms into the site, for which a specific physiological reaction is known to occur under the influence of a specific pollutant (STEUBING / JAEGER 1981, ARNDT / NOBEL / SCHWEIZER 1987, KREEB 1990, BATIČ 1994).

1.1 MYCORRHIZAL SYMBIOSIS

Permanent associations of roots with hyphae of fungi were first named 'mycorrhiza' by FRANK in 1885. He distinguished two main forms of mycorrhiza, ectotrophic (now named ectomycorrhiza) and endotrophic mycorrhiza (now named endomycorrhiza). In symbiosis a bidirectional transport of nutrients occur: assimilates from the plant into fungus, and water and mineral nutrients from the fungus into the host plant (HARLEY / SMITH 1983). Each mycorrhizal form is associated with an ecosystem and soil environment with distinctive characteristics regarding the rate of decomposition, mineralization, availability of nutrients, and the dynamics of the ecosystem (READ 1991). A review on the role of ectomycorrhizae in forests has recently appeared (KRAIGHER 1996) in this journal, therefore only a short presentation of the role of ectomycorrhizae in forest ecosystems is shown below.

Forest trees with ectomycorrhiza dominate acid soils with surface litter accumulation and seasonally released organic and inorganic nutrients (key factors being N, P and C/N ratio) (READ 1991). In late-stage forest successions ectomycorrhizal roots are known to develop mainly in the upper organic soil horizons (MIKOLA / LAIHO 1962). There, mycorrhizae can short-circuit nutrient cycles by directly reacquiring nutrients in organic form from plant and fungal litter, and they may reallocate resources between different plant individuals, preventing the loss of resources from the entire ecosystem (PANKOW / BOLLER / WIEMKEN 1991). Hyphal connections between different plants of the same or different species which transferred water, minerals and also assimilates between roots of different plants were demonstrated (FRANCIS / READ 1984, MILLER ALLEN 1992, SIMARD 1996). In symbiosis functional compatibility of different fungal species and strains in combination with forest trees differs regarding their physiological effectiveness (GIANINAZZI-PEARSON 1984), while any anthropogenic impacts can influence this highly interactive forest environment (AMARANTHUS / PERRY 1994).

1. 2 CHANGES IN SPECIES DIVERSITY OF ECTOMYCORRHIZAL FUNGI

Since the early seventies an increasing number of reports have been concerned with a decrease in species diversity and the abundance of sporocarps of macromycetes in Europe (reviews and comments in papers by ARNOLDS 1991, JAENIKE 1991, PILTAVER 1990, GOGALA 1990, KRAIGHER / BATIČ / AGERER 1995, KRAIGHER 1996).

ARNOLDS (1988) has investigated the species diversity of different functional groups of macromycetes in the Netherlands by: i) comparing the lists of records of 15 forays made between 1912 and 1954 with those made between 1973 and 1982; ii) mapping of selected species in different periods; iii) repeated analysis of permanent plots with mycocoenological methods. All methods showed similar changes in the fruiting of the macromycete flora of the Netherlands: i) the group of terrestrial saprophytes in grass- and heathlands have declined; ii) lignicolous fungi as a whole appeared to have increased; iii) the most dramatic change was shown by ectomycorrhizal fungi, among which 77% (100 sps) of 130 investigated species, showed a decrease in numbers. The greatest established decrease was for fungi predominantly associated with coniferous trees (66%), mainly for hydnaceous fungi (Cantharellaceae, Hydnaceae, Gomphidiaceae and the *Cortinarius, Dermocybe, Tricholoma* and *Suillus*) genera.

Similar observations were done by GUILLITE 1987, HŐILAND 1986, , JAKUCS *et all.l.* 1986, P.J.JANSEN 1985, A.E.JANSEN and DE VRIES 1989, SCHAFFERS / TERMOSHUIZEN 1989, AGERER 1989, BLASCHKE 1986, GÖBL 1986, 1989, HOLOPAINEN 1989, KOWALSKI 1987, MEJSTŘIK / CUDLIN 1987, GULDEN *et all.* 1992 and others. Direct fumigation studies are also available, showing that the vitality of ectomycorrhizal root tips depends on the species of ectomycorrhizae (WOLLMER / KOTTKE 1990, QIAN *et all.* 1993), and that the occurence of fruitbodies does not necessarily correlate with the percentage of ectomycorrhizal root tips per type (SHAW *et all.* 1992).

Possible causes for the decline of ectomycorrhizal fungi were discussed by ARNOLDS (1991) with regard to natural successions, sporocarp removal, forest management, and air pollutants, which can act directly by influencing fungi, by forest soil acidification, nitrogen deposition, litter and herb layer removal or assimilate partitioning through influences on tree vitality. A widespread decline, predominantly in categories of macromycetes, associated with old broad-leaved and coniferous forests, especially in mycorrhiza-formers, has been found to account for more than half of total species listed (ING 1994).

1.3 MYCOBIOINDICATION OF FOREST SITE POLLUTION

FELLNER (1989) founded a myco-bioindication method for forest site pollution, based in part on evaluation of the ratio of mycorrhiza forming fungi to all macromycetes found at the locality studied, and in part on the degree of impoverishment of mycorrhizal mycocoenoses. He distinguished three phases of such impoverishment: 1) inhibition of sporocarp production; 2) loss of species diversity with the proportion of mycorrhizal species in the total count of macromycetes dropping to ca 35%; 3) partial to total destruction of the assemblages of ectomycorrhizal fungi which then contributed only 10-20% of the total number of macromycete species.

ARNOLDS (1991) has tested Fellner's phases in respect of decline in macromycetes in the Netherlands and suggested an adaptation in respect to different sensitivities of different ectomycorrhizal species. For instance, it was demonstrated that fruitbodies of hydnoid fungi ceased to be produced eventhough overall sporocarp productivity had not been significantly decreased. Therefore, he further elaborated Fellner's scheme for poor and acidic soils in the Netherlands, proposing the lists of most critical species - thise with a tendency to disappear - and of less critical species, which seem to replace the disappearing ones by increasing abundance in moderately polluted areas. Only in the last phase a total absence of ectomycorrhizal sporocarps can occur.

The long-lasting tolerant species may vary in different forest communities and regions. FELLNER (1989), from observations in Krkonoše, suggested that the fungus *Russula mustelina* might be used as a bioindication mycorrhizal species for the estimation of forest soil pollution by acid depositions. However, the occurrence of fruitbodies depends on a range of climatic factors in different years, while ectomycorrhizal types are supposed to be present in the soils at any time of the year and in any climatical conditions. Furthermore, a range of ectomycorrhizal fungi, such as Fungi Imperfecti, do not produce any sporocarps. As a result, a more detailed study of ectomycorrhizal potential of forest sites by ectomycorrhizal types determination was proposed at the 3rd International Mycological Congress in Regensburg in 1990, with a view to apply the results to bioindication.

1.4 OBJECTIVES OF OUR STUDY

The objectives of our studies were:

- determination and characterisation of ectomycorrhizal types on Norway spruce from two different forest sites by anatomical and molecular tools;
- possible application of these studies for the development of a new bioindication method, which would be based on the determination of naturally occuring types of ectomycorrhizae on Norway spruce as "indicators" of forest site pollution.

2 SITE PRESENTATION

Two forest research plots, which showed comparable site characteristics but were differently influenced by the emissions of the local Coal Power Plant (Šoštanj CPP) were chosen for our studies. In the two forest research plots, the predominant rock types are acidophilous silicates, the corresponding soil types are distric cambisols of different depths (URBANČIČ 1990). The predominant phytocenological association belongs to Querco - Luzulo Fagetum (MARINČEK 1987). The predominant tree species is Norway spruce (*Ibid.)*. The forests have been surveyed for damage classes (IGLG 1988, FERLIN 1990, KOLAR 1989), which result from the more or less direct influences of the Šoštanj CPP.

The two forest research plots (at 800 - 900 m above sea level) were established for ecological studies in the eighties (LOVREC 1989, LEŠNJAK *et all.* 1989). The predominant rock types are acidophilous silicates, the corresponding soil types are distric cambisols of different depths (URBANČIČ 1990). The predominant phytocenological association belongs to Querco - Luzulo Fagetum (MARINČEK 1987). The predominant tree species is Norway spruce *(Ibid.)*. The forests have been surveyed for damage classes (IGLG 1988, FERLIN 1990, KOLAR 1989). The main difference between the two plots is the influence of the emissions from the Šoštanj CPP. Regarding the relief, climatic, previous forest decline and lichenological studies, the plot in Zavodnje is more influenced by emissions from the Šoštanj CPP, which lies approximately 6 km to the east from the plot. The plot in Mislinjski jarek, which is situated approximately 20 km north-east of the CPP, represents a relatively unpolluted site.

Air pollution in the two research plots was assessed by mapping of epiphytic lichens on Norway spruce (BATIČ 1991). Index of air purity (IAP) was determined by mapping of the frequency and cover of lichen thalli types as described in BATIČ (1990). IAP values can be between 0 (polluted) and 54 (clean air).
Zavodnje: IAP between 8 and 12 (representing very polluted air)
Mislinja: IAP between 25 and 40 (representing clean air and protected exposition)

The station for automated measurements of SO_2, NO_2 and O_3 near the plot in Zavodnje showed maximum one-hour concentrations of SO_2 in 1993 at 3612 μ g/m^3; maximum one-hour concentrations of NO_2 in 1993 at 129 μg/m^3; maximum one-hour concentrations of O_3 in 1993 at 204 μg/m^3 (CIGLAR *et all.* 1994).

3 METHODS

Soil cores were taken for the determination and estimation of the abundance of ectomycorrhizae in the vegetation season 1993 (6 times, in 2 to 5 replicates per plot, with soil cores of upper diameter 4.8 cm and lower diameter 4.0 cm, 18 cm deep, which equalled 274 ml soil volume). The samples were stored at 4 - 8^o C in plastic bags. For comprehensive characterisation and identification of ectomycorrhizae, soil cores from directly underneath fruitbodies of mycorrhizal fungi were cut with a sharp knife, wrapped in foil and stored at 4 - 8^o C until processing. For the identification of ectomycorrhizae the wrapped samples were soaked in water and mycelial connections were followed from fruitbodies to ectomycorrhizae under a dissecting microscope. Two types were comprehensively described after the method by AGERER (1987-1993; 1991a) and by molecular tools (after GARDES / BRUNS 1993) as presented in KRAIGHER (1994), KRAIGHER / AGERER / JAVORNIK (1995), AGERER / KRAIGHER / JAVORNIK (1996) and KRAIGHER (1996)).

For the determination of ectomycorrhizal types in soil cores the morphotypes were separated and all ectomycorrhizal root tips from each morphotype from samplings in 1993 were counted (modified after GRAND / HARVEY 1984). If possible, the morphotypes were determined after their anatomical characteristics in respect to all adequate literature: AGERER (1987-1993, 1986, 1991a, 1991b, 1992 etc), GRONBACH (1988), WEISS (1988), TREU (1990), UHL (1988), BRAND (1991), INGLEBY *et all.*, (1990), HAUG / PRITSCH (1992), PILLUKAT / RAIDL / AGERER (1992), WALLER *et all.* (1993) and others. The morphotypes, which could not be determined sufficiently for distinction after the above literature, were either comprehensively characterised, following the prescribed methodology, or a brief description was done and the distinctive morphotypes were given a reference number. The types which could not be described (they were either too old, too young or they were too rare to be analysed sufficciently for disctinction from other types), were categorized as 'unidentifiable'.

Representative ectomycorrhizal systems were fixed in FAA, marked and stored at the Institute's Herbarium LJU FU2 SLO collection. In the cases when identification of ectomycorrhizae through connections to fruitbodies was achieved, these were stored as exicates in Herbarium LJU FU2 collection. Fungal species were determined at the plot and checked for their identity at the Institute by Andrej PILTAVER, according to: MOSER 1978 (Polyporales, Boletales, Agaricales, Russulales), BON 1987, JÜLICH 1984 (Aphyllophorales, Heterobasidiomycetes, Gastromycetes), BREITENBACH / KRÄNZLIN, 1981-1991. At the time of sampling the occurrence of fruitbodies was noted. Their abundance was estimated as single observation (+), a few (++), frequent (+++), massive occurrence (++++).

4 RESULTS

4.1 TYPES OF ECTOMYCORRHIZAE OCCURING IN TWO FOREST RESEARCH PLOTS

Type 148

- white mycorrhiza with ochre tint, monopodial-pyramidal ramifications, straight to slightly bent unramified ends, length of ramified system up to 6.5 mm, of unramified ends 0.4 - 1.1 mm, diameter of unramified ends and axes 0.5 mm; mantle surface shiny, sometimes irregular patches of brown cortical cells visible, surrounded by a loose woolly cover of emanating hyphae, soil particles can remain on surface;

- mantle in surface view plectenchymatous, type A, hyphal cells with thick walls at ramifications, globular hyphal ends, slightly tortuous, no clamps, hyphal ramifications at right angles; typical inflations throughout the mantle, intrahyphal hyphae, repair mechanism;
- smooth, brownish rhizomorphs, in lactic acid reddish, 0.08 mm thick, undifferentiated; in rhizomorphs irregularly shaped emanating hyphae, with inflations, H-anastomoses, open or with septa, with thick walls, formed between two hyphae running alongside, simple septa, globule at the septum onesided;
- colour reactions negative for most chemicals, with $FeSO_4$ hyaline - decolored to bluish, guiaiacol on mantle bluish, on rhizomorphs red-brown, KOH on rhizomorphs ochre;
- new type, the suggested name - a comprehensive description will follow - is *Piceirhiza inflata;*
- Zavodnje SLO 00148 (07.10.1992).

Type 149

- whitish mycorrhiza with olive-ochre patches, monopodial-pyramidal ramifications, up to 20 mm long systems, slightly bent;
- with several white, smooth, thick rhizomorphs;
- mantle structure plectenchymatous, type A, anastomoses rare, septa with doliporus-like structures, no clamps, diameter of hyphae 5 to 7 mm, cell walls hyaline, non-thickened; rhizomorphs 10 to 80 mm thick, highly differentiated, with one single or a few central hyphae, type E or F, diameter of middle hyphae 8 to 16 mm;
- type determined as belonging to *Xerocomus badius* group;
- Zavodnje SLO 00149 (07.10.1994).

Type 150

- pyramidal to coralloid ramification, soil particles difficult to remove; mantle surface smooth to grainy, greyish;
- in surface view of mantle parallel agglutinated hyphae, plectenchymatous; no

clamps, occasionally found bottle-like cystidia;

- could belong to *Russula xerampelina* or *R. illota* group;
- Zavodnje SLO 00150 (15.10.1994).

Type 156

- dark grey to black mycorrhiza, older parts light grey, monopodial-pyramidal ramification, mantle surface smooth;
- mantle in surface view with very parallel oriented hyphae; septa thick, cross-walls can be seen together with longitudinal walls and depositions on with a different refraction index (bluish), the walls are collapsing, greyish-brown;
- possibly similar to *Piceirhiza parallela* (HAUG / PRITSCH 1992)
- Zavodnje SLO 00156 (15.10.1992).

Type 164

- long ochre-brown, scarcely ramified, monopodial-pyramidal ECM, mantle surface slightly grainy;
- mantle type P, pseudoparenchymatous - epidermoid with a net of thin laticifers on top; cells irregularly shaped to roundish, irregularly indented, 10 to 15 mm; laticifers with orange latex, which looks like irregularly shaped thick walled cells or incrustrations, but disappear in lactic acid; 5 to 7.5 mm in diameter, without septa;
- belonging to *Lactarius theiogalus* group (laticifers without septa, but thinner than described by GRONBACH 1988);
- Zavodnje, SLO 00164 (05.11.1992).

Type 163

- monopodial-pyramidal system, totally covered with soil particles (Figure 2);
- mantle surface with short emanating hyphae, appearance warty to wooly, colour violet-brownish;
- mantle plectenchymatous, in deeper layers well pronounced stellar pattern, cells colourless, surface densely covered with soil particles, hyphae thickwalled and tightly glued together; rhizomorphs not observed, emanating hyphae colourless to yellowish, clampless, smooth, if rough because of adhering soil particles; repair mechanism: new hyphae grow out of broken

ones;

- foreign hyphae also present; rather thinwalled hyphae filled with contents on the very tip;
- new type, the suggested name, after a comprehensive description will have been done, is *Piceirhiza terraphila;*
- Zavodnje SLO 00163 (05.11.1992).

Type 185

- black simple or monopodially ramified ectomycorrhizae with straight emanating hyphae, predominantly from its tips;
- stellate patterned mantle;
- belonging to group *Cenococcum geophilum*;
- Mislinja SLO 00214, Zavodnje SLO 00185.

Type 186

- irregularly monopodial-pyramidal, greyish to olive-grey mycorrhizae, covered with soil particles;
- mantle surface plectenchymatous; hyphae (1) 2 - 3 mm in diameter, 10 - 40 mm distance between septa, with clamps, Y-ramified or with simple anastomoses;
- resembling *Piceirhiza harenosa* (BERG 1989);
- Mislinja SLO 00186.

Type 196

- irregularly pinnate ramification, white mycorrhiza covered with white, flattened rhizomorphs;
- mantle surface plectenchymatous, septa with doliporus-like structures, bluish-white autofluorescence under UV-filter; emanating hyphae with clamps, 3 - 5 mm in diameter; rhizomorphs undifferentiated, anastomoses with contact-clamps;
- resembling *Cortinarius obtusus* group;
- Zavodnje SLO 00196, Mislinja SLO 00222.

Type 204

- irregularly monopodial-pyramidal ramifications, brownish colour with silvery patches, wooly emanating hyphae, covered with soil particles;
- mantle surface plectenchymatous, septa with central globular thickenings; inner mantle layers with hyaline gelatinous matrix inbetween hyphae; emanating hyphae 2 - 3.5 mm in diameter, without clamps, frequently ramified, forming anastomoses with septum; granulated, often with gelatinous substances on outer cell walls, with irregular crystalloid appositions;
- resembling *Elaphomyces granulatus (Piceirhiza glutinosa)* group;
- Zavodnje SLO 00204.

Type 207

- irregularly pinnate or unramified mycorrhizae, of bright orange colour, covered with emanating hyphae and flattened rhizomorphs;
- mantle surface plectenchymatous; emanating hyphae with clamps, 3.5 - 5 mm in diameter, smooth; rhizomorphs undifferentiated, hyphae loosely connected; anastomoses open, no contact clamps, very thick walled;
- resembling *Piceirhiza aurea* group;
- Zavodnje SLO 00207.

Type 213

- irregularly monopodial-pyramidal ramified mycorrhiza, brownish colour of root cortex cells visible, very tips whitish, rhizomorphs of no distinct colour, smooth;
- mantle surface plectenchymatous, with a dense net of oily droplets containing hyphae, inner surface pseudoparenchymatous; hyphae without clamps, 2.5 - 5 mm in diameter; rhizomorphs rare, undifferentiated;
- resembling *Piceirhiza oleiferans* (WALLER *et all.* 1993);
- Mislinja SLO 00213.

Type 241

- unramified or monopodially ramified mycorrhiza, of dark brown to black colour, emanating hyphae black; older parts carbonizing;
- mantle surface plectenchymatous; outer hyphal net formed of dark hyphae in simple three-directional stellate pattern; inner layers of uncoloured hyphae,

hyphae, forming the Hartig net, dark; emanating hyphae 2-3 mm in diameter, young hyphae hyaline, older brown to black coloured, warty; simple septa, ramifications rare, anastomoses simple;

- identified as belonging to the *Piceirhiza horti-atrata* group;
- Zavodnje SLO 00241.

Type 244

- monopodial-pyramidally ramified mycorrhiza of olive-green colour with smooth to slightly warty surface with adhering soil particles; without rhizomorphs and very few emanating hyphae;
- mantle surface pseudoparenchymatous, with epidermoid cells, occasionally dark coloured, interwoven by a loose net of thin laticifers of 4-5 mm in diameter; thick walled but rather thin emanating hyphae, without clamps;
- *Lactarius* sp., possibly belonging to the *Lactarius necator* group (AGERER, pers.comm.);
- Zavodnje SLO 00244.

Type 247

- monopodial-pyramidal ectomycorrhiza with woolly emanating mycelium and yellowish rhizomorphs;
- mantle plectenchymatous, ca 10 mm thick, innermost layers showing palmetti-structures; emanating hyphae granulated, with clamps, 2.5 - 4 mm in diameter; rhizomorphs consisting of loosely interwoven hyphae, not differentiated, oval to roundish, 10 - 80 mm in diameter, anastomoses resembling H, or clamp-connections;
- similar to *Amphinema byssoides*;
- Mislinja SLO 00247.

Type 250

- monopodially-pinnate ramification, ochre-brown, surface smooth to slightly spiny, rhizomorphs ochre to reddish brown;
- mantle surface plectenchymatous, emanating hyphae and cystidia thick walled, 3.5 - 4.5 mm in diameter, with clamps; rhizomorphs slightly differentiated, type C, up to 150 mm in diameter;

- identified as belonging to the *Thelephora terrestris* group;
- Zavodnje SLO 00250, Mislinja SLO 00385.

Type 291

- monopodial-pyramidal systems of yellowish to yellow-olive colour with brighter yellow dots , most frequent close to ramifications;
- mantle structure pseudoparenchymatous with angular cells and with conical heaps of flat cells; with yellowish autofluorescence under UV-filter; emanating hyphae 2-3 mm in diameter, without clamps; rhizomorphs rare;
- identified as belonging to the *Russula ochroleuca* group;
- Zavodnje SLO 00291, Mislinja SLO 00315.

Type 310

- irregularly monopodial-pyramidal ramified mycorrhiza, rather thin, bright ochre, with silvery shiny patches, 2 - 15 mm long, nonramified ends 0.3-0.4 mm in diameter;
- rhizomorphs mostly roundish, ochre, with sclerotia; rhizomorphs with centrally arranged hyphae of conspicuously thicker diameter; hyphae with clamps; sclerotia smooth, round, shiny, 0.2-0.4mm in diameter;
- identified as belonging to the *Paxillus involutus* group;
- Zavodnje SLO 00310.

Type 316

- Monopodially ramified dark brown to black mycorrhiza, beaded, with occasionally emanating hyphae in bundles;
- mantle surface pseudoparenchymatous, formed of angular cells with heaps of roundish cells on outer surface; emanating hyphae 2.5 - 5.5 mm in diameter, with clamps, brownish coloured; cystidia rare, spherical to conical;
- identified as belonging to the *Piceirhiza nigra* group;
- Mislinja SLO 00316.

Type 349

- Monopodially-pyramidal ramifications, bright-brown to whitish coloured, young tips slightly violet tinted, woolly emanating hyphae;

- mantle surface densely plectenchymatous, inner layers forming palmetti structures; emanating hyphae 2 - 3.5 mm in diameter, with clamps, simple anastomoses, rhizomorphs undifferentiated, type A/B;
- identified as belonging to the *Laccaria bicolor* group;
- Zavodnje SLO 00349.

Characterization of *Lactarius lignyotus* Fr. x *Picea abies* (L.) Karst. (characterised comprehensively in KRAIGHER / AGERER / JAVORNIK 1995)

- The *Lactarius lignyotus x Picea abies* type is characterised basically by its irregular-monopodial-pyramidal mycorrhizal system of white colour, plectenchymatous mantle, laticifers, (4)5-7 mm in diameter, and cystidia-like protruding hyphal ends.
- Using molecular characterisation, this type could be distinguished from a closely related *Lactarius picinus x Picea abies* by the different restriction digest pattern of the amplified ITS region by the enzyme *Hinf 1*. The amplified ITS region for both fungi shows a distinct fragment at approximately 850 bp. Using *Alu1* restriction enzyme two bands are observed for both fungi at a position of 530 bp and 250 bp. Using *Hinf1* restriction enzyme, *Lactarius picinus* shows two bands at 360 bp and 250 bp region, while *Lactarius lignyotus* and its ectomycorrhiza only show one band at 360 bp region.

Characterization of *Hydnum rufescens* Fr. x *Picea abies* (L.)Karst. (characterised comprehensively in AGERER / KRAIGHER / JAVORNIK 1996)

- The *Hydnum rufescens x Picea abies* type is primarily characterised by its neat monopodial-pyramidal mycorrhizal systems of a peachy-white colour with frequent rhizomorphs, plectenchymatous mantle, emanating hyphae (1.5)2-4 mm in diameter, with clamps and trumpet-like inflations, rhizomorphs irregularly shaped, an intermediate type between A and D organization type (AGERER 1991a), with highly complicated ramifications, several hyphae growing out of a single inflated hyphal end, most hyphae contain oily droplets, outer hyphae warty.
- The amplified ITS region shows a distinct band at approximately 840 bp. This fragment can be digested into two bands using the *Alu1* enzyme at 480 and 160 bp, or into three bands with the enzyme *Hinf1* at 330, 290 and 130 bp position.

4.2 OCCURRENCE OF ECTOMYCORRHIZAE IN SOIL CORES

Number of root tips (nonmycorrhizal, unidentified ectomycorrhizae and ectomycorrhizal types) from six samplings with soil cores in 1993, each from 2 to 5 replicates per plot, are represented in Table 1 and in Figures 1,2,3, 4.

Table 1: Occurence of nonmycorrhizal and different ectomycorrhizal root tips in soil cores.

Preglednica 1: Pojavljanje nemikoriznih in različnih ektomikoriznih korenin v vzorcih zemlje.

Plot / Type	M 08.04.	M 07.05.	M 15.06.	M 19.08.	M 16.09.	M 21.10.	M Total	Z 08.04.	Z 07.05.	Z 15.06.	Z 19.08.	Z 16.09.	Z 21.10.	Z Total
Roots	105	36	74	130	69	99	513	136	66	204	50	74	39	569
SNT	3465	914	2235	5009	1338	4777	17738	2455	1143	1819	2170	1984	1682	11253
148	-	-	-	-	-	-	-	89	64	272	654	28	1364	2471
149	30	16	-	-	-	5	51	76	-	673	350	-	1009	2108
150	-	50	-	275	15	161	501	-	-	-	-	-	-	-
156	-	-	-	-	-	-	-	-	-	159	105	-	949	1213
163	-	-	-	-	-	-	-	-	-	1629	1343	29	-	3001
164	-	-	-	-	-	9	9	47	-	6	-	-	-	53
185	382	40	1157	407	425	524	2935	-	13	98	35	105	79	330
186	1075	572	-	-	-	-	1647	-	-	-	-	-	-	-
196	-	51	-	69	15	34	169	11	-	-	227	-	73	311
204	-	-	-	38	-	-	38	72	-	-	119	-	172	363
207	-	-	9	25	-	-	34	138	-	-	-	-	-	138
213	841	263	-	20	-	1301	2425	-	-	-	-	-	-	-
241	-	-	-	-	-	-	-	-	106	-	-	-	-	106
244	-	-	-	364	-	-	364	-	270	-	16	-	-	286
247	127	214	-	894	272	2122	3629	-	-	-	-	-	-	-
250	-	33	-	-	48	231	312	77	-	154	-	33	27	291
291	13	-	72	100	-	171	356	139	-	938	278	425	-	1780
310	862	-	-	47	51	-	960	78	25	307	222	-	2605	3237
316	-	-	-	60	63	80	203	-	-	-	-	-	76	76
349	-	-	-	269	-	-	269	5	-	-	413	289	-	707
404	73	701	535	512	1357	2068	5246	-	-	-	-	-	-	-
428	-	33	148	39	473	185	878	-	-	-	-	-	-	-
Other	-	-	-	37	3	185	225	-	38	-	56	-	56	150
No. t.	8	10	5	16	10	15	24	10	6	9	12	6	10	17
No.s.	3	3	2	5	3	5	21	3	3	2	5	3	5	21

M=Mislinja, Z=Zavodnje, SNT=old unidentifiable types; 148 = *Piceirhiza inflata*, 149 = *Xerocomus badius*, 150 = *Russula* sp., 156 = *Piceirhiza parallela*, 163 = *Piceirhiza terraphila*, 164 = *Lactarius theiogalus*, 185 = *Cenococcum geophilum*, 186 = *Piceirhiza harenosa*, 196 = *Cortinarius* sp., 204 = *Elaphomyces granulatus*, 207 = *Piceirhiza aurea*, 213 = *Piceirhiza oleiferans*, 241 = *Piceirhiza horti-atrata*, 244 = *Lactarius* sp., 247 = *Amphinema byssoides*, 250 = *Thelephora terrestris*, 291 = *Russula ochroleuca*, 310 = *Paxillus involutus*, 316 = *Piceirhiza nigra*, 349 = *Laccaria bicolor*, 404 = *Hydnum rufescens*, 428 = *Lactarius lignyotus*, Other: other undescribed types (can include more than one type); No.t.= number of types; No.s.=number of soil cores (274 ml volume each) analysed per sampling; all numbers as total no. of mycorrhizal (or nonmycorrhizal = Roots) root tips per sampling (dates indicated below plot sign); Total = total numbers for 6 samplings in 2 to 5 replicates per plot.

Throughout the year 1993 the most frequent types occurring in Zavodnje were *Paxillus involutus, Piceirhiza inflata, Xerocomus badius* and *Russula ochroleuca*. In Mislinja the most frequent types were *Hydnum rufescens*,which was also highly constant in all samplings, *Russula* sp., *Amphinema byssoides, Cenococcum geophilum;* a highly constant type in all samplings was also *Lactarius lignyotus*. The occurrence of the types of ectomycorrhizae and roots are shown in Figures 1,2,3, 4. The percentages are calculated from total countings of 38502 tips from Mislinja and 28443 from Zavodnje. The percentages of types are calculated from 20251 vital and identifiable ectomycorrhizal types from Mislinja and 16621 from Zavodnje.

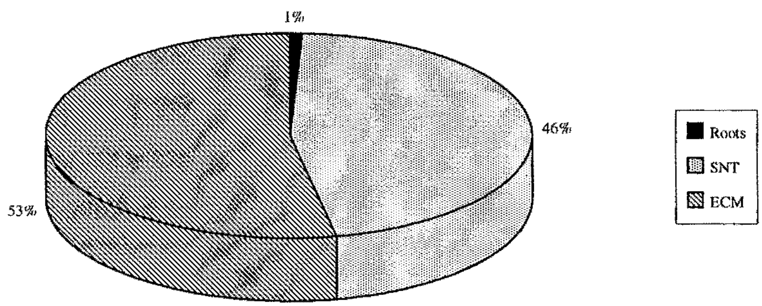

Figure 1: Percentage of nonmycorrhizal root tips (Roots), unidentifiable ectomycorrhizae (SNT) and viable ectomycorrhizal root tips (ECM) in soil cores from Mislinja.

Slika 1: Deleži nemikoriznih korenin (Roots), nedoločljivih tipov (SNT) in vitalnih tipov ektomikorize (ECM) v vzorcih tal iz Mislinje.

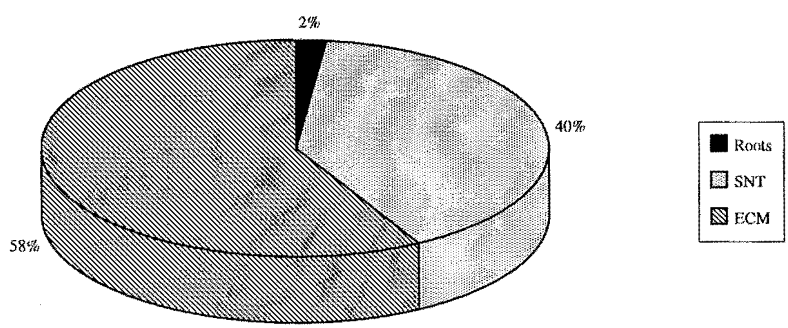

Figure 2: Percentage of nonmycorrhizal root tips (Roots), unidentifiable ectomycorrhizae (SNT) and viable ectomycorrhizal root tips (ECM) in soil cores from Zavodnje.

Slika 2: Deleži nemikoriznih korenin (Roots), nedoločljivih tipov (SNT) in vitalnih tipov ektomikorize (ECM) v vzorcih tal iz Zavodenj.

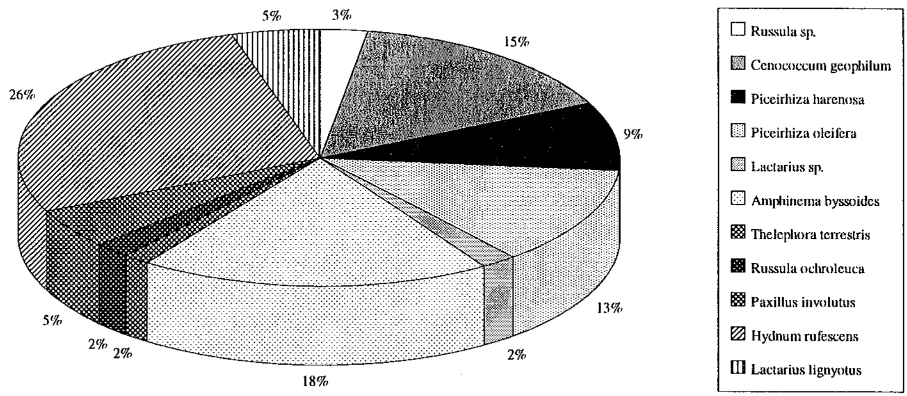

Figure 3: Ectomycorrhizal types, which occurred in more than 1 % of all viable types in soil cores from Mislinja.

Slika 3: Tipi ektomikorize, ki so predstavljali več kot 1% vseh vitalnih tipov ektomikorize v vzorcih tal iz Mislinje.

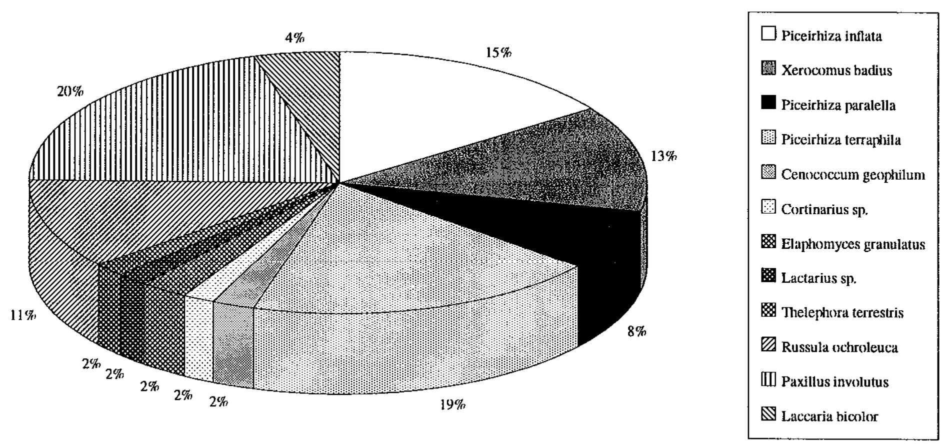

Figure 4: Ectomycorrhizal types, which occurred in more than 1 % of all viable types in soil cores from Zavodnje.

Slika 4: Tipi ektomikorize, ki so predstavljali več kot 1 % vseh vitalnih tipov ektomikorize v vzorcih tal iz Zavodenj.

4.3 COMPARISON OF THE OCCURENCE OF FRUITBODIES AND THE TYPES OF ECTOMYCORRHIZAE IN TWO FOREST RESEARCH PLOTS

The occurrence of fruitbodies was taken down and their abundance was estimated by ranking them into rare-single specimen (+), few (++), frequent (+++) and massive (++++) occurrence. In Table 2 a comparison of the occurrence of fruitbodies and the types of ectomycorrhizae in the two forest research plots on one sampling day is shown.

Table 2: Occurrence of the types of ectomycorrhizae (Mycorrhiza) in soil samples (as % of all types, but only the most frequent types are shown) and fungal fruitbodies (Fruitbody by ranks: -/none, +/occasional, ++/frequent, +++/massive occurence; only the most frequent species are listed) in two forest research plots on 21/10/1993. The total number of roots in soil cores from the same day from Mislinja was 11.972 , from which 99 were nonmycorrhizal, 4.777 were unidentifiable, and in soil cores from Zavodnje 8.146, from which 39 were nonmycorrhizal ans 1.682 were unidentifiable types.

Preglednica 2: Pojavljanje tipov ektomikorize (Mycorrhiza) v vzorcih zemlje (kot delež vseh tipov ektomikorize v %, navedeni samo pogostejši tipi) in trosnjakov gliv (Fruitbody, rangi: -/ni, +/posamezni, ++/pogosti, +++/masovno pojavljanje; navedeni samo trosnjaki pogostejših gliv) na dveh raziskovalnih ploskvah ob vzorčenju dne 21.10.1993. Skupno število analiziranih korenin vzorčenj tega dne je bilo v vzorcih iz Mislinje 11972, od tega 99 nemikoriznih korenin in 4777 starih nevitalnih tipov ektomikorize in v vzorcih iz Zavodenj 8146, od tega 39 nemikoriznih korenin in 1682 starih nevitalnih tipov ektomikorize.

Fungus	Zavodnje		Mislinja	
	Fruitbody (rank)	Mycorrhiza (%)	Fruitbody (rank)	Mycorrhiza (%)
Paxillus involutus	+++	41	++	0
Amanita gemmata	+++	0	-	0
Thelephora terrestris	+++	1	-	1
Xerocomus badius	++	16	++	1
Tricholoma saponaceum	++	0	-	0
Piceirhiza inflata	-	21	-	0
Piceirhiza parallela	-	15	-	0
Lactarius mitissimus	++	0	+++	0
Laccaria amethystina	++	0	+++	0
Lactarius lignyotus	-	0	+++	4
Russula ochroleuca	-	0	+++	1
Cortinarius (3 spp)	-	1	++	1
Hydnum rufescens	-	0	+	29
Amphinema byssoides	-	0	-	30
Piceirhiza oleiferans	-	0	-	18
Cenococcum geophilum	-	1	-	7

For each of the plots the same fungal species were observed in different sampling years. However, the most abundant fruitbody occurrence was in autumn 1993 (the occurrence from one sampling day are shown above).

5 DISCUSSION

In our project the identification and characterisation of ectomycorrhizal types on Norway spruce from two different forest sites by anatomical and molecular tools were used for the studies of their abundance on the two differently polluted forest research plots throughout the vegetation period. A possible application of the basic studies for the development of a bioindication method, based on identification of naturally occurring types of ectomycorrhizae on Norway spruce as "indicators", was suggested.

5.1 CHARACTERISATION AND IDENTIFICATION OF ECTOMYCORRHIZAL TYPES IN TWO FOREST RESEARCH PLOTS

By 1993, 82 types of ectomycorrhizae on Norway spruce were characterised using the anatomical method (by AGERER 1991a) and included into the Colour Atlas of Ectomycorrhizae (AGERER 1987 - 1993). The method is based on morphological and anatomical characteristics of ectomycorrhizae, whereby the range of features have to be recognized for each type and compared to all other known ectomycorrhizae. Therefore the identifications can not only be based on the key (as described in the Atlas), but have to be confirmed by comparisons with original publications, containing comprehensive descriptions of each type as well. Furthermore, from ecological studies of fungal fruitbodies (TRAPPE 1962, AGERER 1985, DEACON / FLEMING 1992) 300 to 500 fungal species are expected to form ectomycorrhizal associations with Norway spruce. Some authors expect even 2100 or more species of fungi which may be mycorrhiza - formers with North American trees (SMITH 1971). The numbers can be especially high in pronaturally managed forests with high natural diversity, as is the case in Slovenian forests. Therefore only 1/3 to 1/10 of all ectomycorrhizal types on spruce could be described so far, and in each project, concerning the types of ectomycorrhizae, new characterizations can be expected.

The comprehensive morphological and anatomical characterization of each type of ectomycorrhizae is a project which might last for several growth seasons. A number of characteristics can only be described on fresh material. These comprise all morphological characteristics regarding the shape and dimensions of ectomycorrhizae, surface views, colour, occurrence of rhizomorphs and emanating hyphae, most chemical tests and autofluorescence. In addition, the observation of several anatomical features of plan views of mantle, such as laticifers, colour of hyphal contents and their surface, and the same for rhizomorphs and emanating hyphae have to be done on fresh material (AGERER 1987-1993). Positive identifications of the fungal partner can also imply several years to trace mycelial connections from ectomycorrhizae to fruitbodies, which may not be produced every year. Furthermore, these connections are in several cases difficult to obtain, which especially holds for fungal species, which do not or can only rarely form rhizomorphs (such as the *Russula* and *Lactarius* genera).

Therefore in ecological studies (HAUG / PRITSCH 1992, ZHAO 1989, SCHERFOSE 1990, QIAN *et all.* 1993) brief descriptions of ectomycorrhizae are a prerequisite for further comparisons regarding site or pollution effects. These morphological and anatomical descriptions can lately be supplemented by molecular methods (GARDES *et all.* 1991).

In our approach we have joined brief descriptions of a fairly large number of ectomycorrhizal morphotypes to two comprehensive descriptions of new types of ectomycorrhizae, which have proved to be of interrest regarding their growth potential in unpolluted sites only. These two identifications of the fungal symbiont were confirmed by molecular method based on PCR - amplification, using basidiomycete specific primers, and the restriction digests of the amplified products (described in KRAIGHER / AGERER / JAVORNIK 1995, as modified from GARDES / BRUNS 1993).

Brief descriptions of morphotypes in soil cores from the two forest research plots in the emission zone of the CPP Šoštanj, which could be differentiated from one another, were based on the descriptions of most distinguishable characteristics of ectomycorrhizae. Whenever possible these types were compared to an already described type or a 'group' type. The introduction of this term was necessary to emphasise the possibility that the type considered could show all the characteristics of one single fungal species, but due to the number of related fungal species the characteristics described might also belong to another fungus. For example *Lactarius theiogalus x Picea abies*, as described already by GRONBACH 1988, showed the same characteristics as the morphotype described in our studies, but there is still a whole range of *Lactarius* spp. from the same *Tabidi* section (BON 1987), whose ectomycorrhizae have not been described on Norway spruce so far, therefore any of these fungi could also account for the relevant features from GRONBACH 1988.

In two cases (regarding the brief descriptions of morphotypes) no similarities could be found with any characterised type of ectomycorrhizae. Considering them as new types, longer descriptions were needed and new names for them, regarding their most obvious characteristics, were suggested: *Piceirrhiza inflata* for the type with reference number SLO 00148 and *Piceirhiza terraphila* for the type with reference number SLO 00163.

Piceirrhiza inflata is predominantly characterised by its white colour with ochre tint and brownish rhizomorphs, hyphae without clamps but with well distinguishable inflations, which occur throughout the mantle and in rhizomorphs. From the presence of the globular thickenings (here onesided) at the septum it is possible to conclude that the fungal partner belongs to Basidiomycetes. *Piceirrhiza terraphila* is characterized first by being totally covered with soil particles, adhering on a violet-brownish warty to wooly mantle, consisting of clampless hyphae which are tightly glued together and smooth emanating hyphae with a pronounced repair mechanism: a new hypha can grow out of a broken one. Any relationships could not be established so far.

A few more types were dubious regarding their identity - those which might belong to several *Russula* or to several *Lactarius* sps. In one case (*Amphinema* *byssoides*) the hyphae of this fungus were found also to invade other (minor) types which were then (for ease) included among descriptions under the name of *Amphinema byssoides*. If the hyphae of this fungus were not too numerous and the primary type was recognized, then the type would be included under the original type's name. Similar 'pirate' hyphae were also observed for *Cenococcum geophilum* and a few unidentified dark brown hyphae, which are also known to grow on the surface of other types (pers. comm. with colleagues from Munich, Tübingen and Scotland).

From the discussions on *Lactarius lignyotus* x *Picea abies* (KRAIGHER / AGERER / JAVORNIK 1995) and on *Hydnum rufescens* x *Picea abies* (AGERER / KRAIGHER / JAVORNIK 1996) we would hereby only like to stress the easy and fast application of the molecular methods, and the necessity of the combination of the classical anatomical methods of characterisation and determination of types of ectomycorrhizae with molecular markers. Based on these studies a conclusion was made that molecular biology approaches can not replace classical taxonomical procedures but they may simplify them and add to the objectivity, due to the possibility to compare restriction patterns of r-DNA of fruitbodies and of mycorrhizae which grew within the area of a single fungal population. The molecular methods can be employed as helpful tool to implement the anatomical characterisation and identification of ectomycorrhizae; the latter could be adapted to a simplified version if complemented by molecular methods; thus temporal and to a certain extent also spatial studies of ectomycorrhizal communities can be made friendlier.

5.2 OCCURRENCE OF TYPES OF ECTOMYCORRHIZAE IN TWO FOREST RESEARCH PLOTS

The two forest research plots were analysed for abundance of ectomycorrhizal types by regular sampling during the vegetation period 1993. Preparatory work on fungal types and methods of sampling was carried out in the years 1990 to 1992. As mycorrhizal fungi spread predominantly in upper soil horizons (MIKOLA / LAIHO 1962) and such distribution was also established in the Norway spruce forests on the Pohorje mountain range in north Slovenia (KRAIGHER 1991), the sampling was limited to organic soil horizons.

To allow volumetric or percental comparisons of the occurrence of nonmycorrhizal and mycorrhizal root tips as well as the abundance of different ectomycorrhizal types, it was decided to compare identical volumes of soil at each sampling in the two plots. Due to high heterogeneity of soil biotic and abiotic structure, several replicates were needed per each sampling period. It was planned to analyse 5 replicates at different locations per plot per sampling in 1993 in order to minimize the variability in each sampling period. The actual number of replicates per sampling varied from 2 to 5 due to the occurrence of new types, which needed brief descriptions, and to additional samplings of soil cubes from underneath fruitbodies in the autumn months with a view to generate comprehensive descriptions of ectomycorrhizae.

Between the percentages of nonmycorrhizal root tips, unidentifiable ectomycorrhizae and viable ectomycorrhizal root tips from Mislinja (Figures 1,3) and Zavodnje (Figures 2,4), only minor differences were observed. In total (Table 1), the same volumes of soil showed greater numbers of root and mycorrhizal root tips in samplings from Mislinja (a total of 38502) in comparison to Zavodnje (a total of 28443). Taking these different totals into account, no differences were established in the proportion of nonmycorrhizal or mycorrhizal root tips from both plots. Similar results were also reported by TERMORSHUIZEN / SCHAFFERS 1989a, 1989b, who found no significant differences in total counts of mycorrhizae under the influences of NH_3 or SO_2 pollution. However, organic soil horizons in Mislinja (see above) seem to be better overgrown with fine roots of Norway spruce in comparison to Zavodnje. This might be explained by previous work of WEISS / AGERER 1986 in which it was shown that either the lack of Ca and Mg fertilization or, separately, ozone treatment resulted in a reduction of fine root biomass. Similar data were reported by KOCOUREK / BYSTŘIČAN 1989 for heavily polluted Krušné hory in comparison to unpolluted Šumava mountains.

Regarding the occurrence of different types of ectomycorrhiza (Table 1) the differences between the two plots were clear. In respect of the number of types, a greater number was found to occur in Mislinja (24) in comparison to Zavodnje

(17). In the plot in Zavodnje the following types, as calculated from pooled samplings, were dominating throughout the year: *Paxillus involutus* (19.5%), *Piceirhiza terraphila* (18.1%), *Piceirhiza inflata* (14.9%), *Xerocomus badius* (12.7%), *Russula ochroleuca* (10.7%) and *Piceirhiza parallela* (7.3%). The three identified types from this list have been shown previously to occur on polluted sites: QIAN *et all.* 1993 have shown that the viable mycorrhizae from the polluted plots in the Tübingen area belonged predominantly to *Xerocomus badius* type; TURNAU / KOTTKE / OBERWINKLER 1993 have stressed their studies of heavy metal tolerance predominantly on *Paxillus involutus*, as this fungus was found previously to persist on heavily polluted plots, where it was accompanied also by *Russula ochroleuca*; this last fungus was found to grow well independently of pollution or liming also in Höglwald (TAYLOR / BRAND 1991). However, also opposite results were shown regarding *Paxillus involutus* by TERMORSHUIZEN / van der EERDEN / DUECK 1989 who have reported on decrease of mycorrhiza formation by this fungus under the influences of SO_2 treatment. SHAW *et all.* 1992, who have found high numbers of fruitbodies of the same fungus on fumigated plots, have discussed their results in connection to the above Dutch paper as an artefact, due to the presence of this fungus on the plots already before the fumigation started. In the view of our data, *Paxillus involutus* and the other above mentioned ectomycorrhizae do seem to share the ability to persist on heavily polluted sites, whereby a range of other fungi tend to disappear. Also *Laccaria bicolor* (occuring at 4.3% on this plot) was reported previously to persist on heavily polluted plots (TERMOSHUIZEN / van der EERDEN / DUECK 1989). However, as the period of ontogenetical development of this type is relatively short (WEISS 1988) it might be replaced by other fungi on a single mycorrhizal system, or in our samplings it might have been included among the unidentifiable types (as too old to be identified).

In the plot in Mislinja the following types as calculated from pooled samplings were either dominating or occurring regularly throughout the year: *Hydnum rufescens* (25.9%), *Russula* sp. (25%), *Amphinema byssoides* (17.9%), *Cenococcum geophilum* (14.5%), *Piceirhiza oleifera* (12%), *Piceirhiza harenosa* (8.1%) and *Lactarius lignyotus* (4.3%). Among these types we would like especially to stress on the constant and high frequency of *Hydnum rufescens*, which is known to be among the first fungal species, disappearing under the influences of pollution (ARNOLDS 1991). From this point of view this ectomycorrhiza can be applied as a bioindicating type for unpolluted sites and the plot in Mislinja can be considered as an unpolluted plot. This fungus did not appear on the plot in Zavodnje at all. The other type, occuring constantly in all samplings from Mislinja, although in lower frequency, was *Lactarius lignyotus*. This fungus or its ectomycorrhiza was not studied previously, as it is relatively rare in central Europe (AGERER pers.comm.). We suggest that it might also be used as a bioindicator of unpolluted sites.

The other ectomycorrhizae, occurring in the plot in Mislinja in relatively high frequencies, can not be considered as possible bioindication types from the following reasons: *Russula* sp. was not identified to species or group level and it could as well represent two different species. *Amphinema byssoides* was found to occur frequently in the plot in Mislinja, but its hyphae can also be found on other types. In our own studies we have found this ectomycorrhizae to occur as the predominant ectomycorrhizal type also in the Institute's nursery in Ljubljana, which might be considered as a relatively polluted site (unpublished). *Cenococcum geophilum* in our case seems to be more frequent in the plot in Mislinja, which might contradict the previous reports on this fungus as being well adapted to stress conditions (MEYER 1987). However, this ectomycorrhizae, probably formed by an asexual stadium of Deuteromycotina (Fungi Imperfecti), has been considered as a complex type, formed by several different fungal symbionts (AGERER pers.comm.). Therefore it might be possible that several of these fungi, showing similar characteristics in ectomycorrhizae, can be differently suited to different environmental conditions or can even show different competitiveness in natural forest ecosystems.

Paxillus involutus was also found in several samplings in Mislinja, representing 4.7% of all ectomycorrhizal types from this plot. This might suggest that this ectomycorrhiza has a broad range of distribution, but due to higher competitiveness of other fungal partners in nonpolluted sites, can not dominate in Norway spruce ectomycorrhizae in such plots where natural diversity is high. It merely takes over the position of the disappearing types due to pollution in heavily polluted plots.

5.3 COMPARISON OF FRUITBODY ABUNDANCE AND ECTOMYCORRHIZAE

The autumn of 1993 was very productive regarding fungal fruitbody occurrence in the whole of Slovenia. Therefore also the comparison on fruitbody abundance and ectomycorrhizae could best be done on the last sampling date in October 1993. The predominance in fruitbodies did not linearily follow the abundance in ectomycorrhizal types, i.e. in mycelium which is present in the soils. However, as the fruitbodes were not counted, but their presence was estimated by four ranks, no statistical correlations could be done.

In the plot in Zavodnje, the great number of fruitbodies of *Paxillus involutus* corresponded well to the percentage (41%) of the ectomycorrhizal types sampled at this date. *Xerocomus badius* fruitbodies were also fairly frequent at the plot, as well as the ectomycorrhizae of this type (16%). However, *Piceirhiza inflata* and *Piceirhiza parallela* could not be connected with any of the fungal fruitbodies occurring on the plot at this samplig date. Therefore the presence or absence of fruitbodies could not be correlated with the occurrence of different types of ectomycorrhizae. Such results were also reported by other authors (i.e. SHAW *et all.* 1992, MEHMANN *et all.* 1995, ICOM 1996). Furthermore, the presence of several ectomycorrhizae could be established throughout the vegetation season, while fruitbodies only appeared for a few weeks in the autumn.

The comparisons of fruitbody occurrence with ectomycorrhizae in the plot in Mislinja showed even fewer correlations. The predominant type of ectomycorrhiza was shown to be *Hydnum rufescens*, while its fruitbody was only noticed once on the plot. A few more have occurred in the vicinity of the plot. Neither *Amphinema byssoides* nor any other corticiaceous fungal species was noticed at all on the slopes of Mislinja and yet its 'pirate' hyphae and the type itself were rather frequent formers of ectomycorrhiza. A few fruitbodies of *Paxillus involutus* were found in the October sampling in Mislinja. However, at this sampling date, no ectomycorrhizae of this type were noticed. Therefore for this plot, it was even more apparent that no clear correlations were possible between fungal fruitbody occurrence and ectomycorrhizal types occurrence.

5.4 APPLICATION OF ECTOMYCORRHIZAL STUDIES FOR BIOINDICATION

From the occurrence of ectomycorrhizae in the two forest research plots and comparison with fungal fruitbody occurrence, we suggested the following (see also KRAIGHER 1996, KRAIGHER / BATIČ / AGERER 1996):

1. pollution can influence the distribution of mycorrhizal fungi, whereby several species can disappear while the others can profit from the new less competitive stage in the pollution-stressed forest ecosystem;
2. mycobioindication through mycocoenoses is a possible method to apply in bioindication of forest sites (as suggested by FELLNER 1989 and ARNOLDS 1988, 1991);
3. in Slovenia, mycobioindication through selective sensitive, in comparison to unsensitive, fungal species, i.e. the presence and abundance of the representatives of each of these two groups, seems to be the main choice for the application of this method, whereby the sensitive species in our case would be *Hydnum rufescens* and the nonsensitive one would be *Paxillus involutus*;
4. from the comparisons of fruitbody occurrence and types of ectomycorrhizae, the latter seems to be superior for the establishing of the presence of a certain fungus in a forest site, since: i) ectomycorrhizae can be present in forest soils throughout the year and in different vegetation seasons, while the presence of fruitbodies is restricted to a certain period in the year, and their presence can vary between different years due to general climatic conditions; ii) a great number of ectomycorrhizal fungi do not form sporocarps (Deuteromycotina), or belong to corticiaceous fungi, whose sporocarps are difficult to observe, or belong to hypogeus fungi, whose sporocarps need special sampling procedures, as they are nicely hidden bellow ground; iii) in several cases in our plots the identity of the fungal partner in ectomycorrhiza was not known, but ectomycorrhizae seemed to be restricted either to the polluted plot (such as *Piceirhiza inflata* and *Piceirhiza terraphila*) or to the unpolluted plot (such as *Piceirhiza oleifera* and *Piceirhiza harenosa*), which means that these fungi might also be suspected as differentially sensitive to pollution.

6 CONCLUSIONS

In our studies a range of ectomycorrhizal types was briefly described for the assessment of distribution of ectomycorrhizae in two research plots. Based thereupon, two comprehensive characterisations were done for *Lactarius lignyotus* x *Picea abies* and for *Hydnum rufescens* x *Picea abies*, which had not been characterised previously. The fungal partner was additionally characterized and identified by the electrophoretogram of the PCR amplified DNA fragment and the sizes of its restriction digest fragments. This characterization proved to be fast and objective. We suggest that PCR methods be used and further developed with every anatomical description of ectomycorrhizae in the future.

Regarding the occurrence of ectomycorrhizal types a difference was shown in respect of their occurrence and abundance in the two forest research plots. It has been shown that pollution can influence the distribution of mycorrhizal fungi, therefore mycobioindication through mycocoenoses is a possible method to be applied in the bioindication of forest site pollution. In Slovenia mycobioindication through selectively sensitive fungal species - in comparison to insensitive ones (two 'indicator' organisms) - seems to be the main choice for the application of this method, whereby the sensitive species in our case would be *Hydnum rufescens* and the nonsensitive one *Paxillus involutus*. From comparisons of fruitbody occurrence and types of ectomycorrhizae the latter seem to be superior for the establishing of the presence of a certain fungus in a forest site. With the application of molecular methods a large scale screening could also be possible in differently polluted forest sites.

7 SUMMARY

In our studies several taxonomical and ecological aspects of ectomycorrhizae of Norway spruce were studied with respect to their potential for use in the bioindication of forest site pollution in Slovenia. Therefore two comparable forest research plots of the Forestry Institute of Slovenia were chosen in the emission zone of the Coal Power Plant in Šoštanj, differing in the pollution impacts, the plot in Zavodnje representing the polluted site and the plot in Mislinja the relatively unpollutedone.

For taxonomical studies of ectomycorrhizae the classical method based on morphological and anatomical characteristics of the symbiotic organs was applied. It was supplemented with a molecular method based on the PCR amplification of the ITS region of ribosomal repeats using basidiomycete-specific primers and two restriction enzymes for the analysis of the amplified product.

A range of ectomycorrhizal morphotypes were briefly described for the assesment of ectomycorrhizal distribution in two forest research plots. Therefrom two comprehensive descriptions were done for *Lactarius lignyotus* x *Picea abies* and for *Hydnum rufescens* x *Picea abies*, which had not been characterised previously. The fungal partner was additionally characterized and identified by the electrophoretogram of the PCR amplified DNA fragment and the sizes of its restriction digest fragments.

Besides these two types 20 other types were found to occur on the two plots in the vegetation season 1993. At least two of them will still need comprehensive descriptions in the future as they have not been described so far.

In regular samplings with soil cores of ca 270 ml volume in 1993, a total of 21 soil cores were analysed from each plot. Total countings of all nonmycorrhizal and mycorrhizal root tips per plot showed 38502 tips in samples from Mislinja and 28443 tips in samples from Zavodnje, which showed a reduction in fine root biomass in the polluted plot. Regarding the occurrence of ectomycorrhizal types, a difference was clear as to the number of types, their occurrence and abundance in the two forest research plots. Based on our data it has been established that pollution can influence the distribution of mycorrhizal fungi, mycobioindication through mycocoenoses is a possible method to be applied in the bioindication of forest sites, mycobioindication through selectively sensitive - in comparison to insensitive - fungal species, i.e. the presence and abundance of the representatives of each of these two groups, seems to be the main application choice of this method in Slovenia; from comparisons of fruitbody occurrence and types of ectomycorrhizae it can be concluded that the latter seem to be superior for the establishing of the presence of a certain fungus in a forest site.

8 POVZETEK

Gozdni ekosistemi so funkcionalno in strukturno visoko organizirani sistemi biotskih in abiotskih komponent, povezanih v občutljivo dinamično ravnovesje. Onesnaževanje vpliva na vse in na posamezne komponente teh sistemov na različnih ravneh in se odraža v spremembah fiziologije gozdnih drevesnih vrst ter na spremembah vrstne sestave in abundanci vrst v gozdni flori in mikoflori.

V prispevku s pomočjo taksonomskih in ekoloških raziskav tipov ektomikorize pri smreki prikazujemo enega od pristopov k študiju vplivov onesnaževanja na gozdne ekosisteme. Postopke in rezultate raziskav smo poskušali uporabiti za bioindikacijo onesnaženosti gozdnih rastišč.

Terenski del raziskav smo izvajali na dveh različno onesnaženih, rastiščno primerljivih ploskvah na imisijskem območju termoelektrarne Šoštanj. Gozdna raziskovalna ploskev Gozdarskega inštituta Slovenije v Zavodnjah je predstavljala ploskev, ki je pod neposrednimi vplivi emisij, gozdna raziskovalna ploskev v Mislinji pa je predstavljala razmeroma neonesnaženo ploskev. Obe ploskvi ležita v pretežno smrekovih sestojih starosti 80 - 100 let, na nadmorski višini 850 m, talna podlaga je distrični kambisol, rastlinska združba je *Querco-Luzulo-Fagetum* oziroma *Luzulo-Fagetum illyricum*. Indeks čistosti zraka z analizami lišajev (IAP) je pokazal, da je ploskev v Zavodnjah zelo onesnažena, ploskev v Mislinji pa relativno neonesnažena (BATIČ 1991).

Taksonomske raziskave tipov ektomikorize pri smreki smo izvajali po klasični anatomsko-morfološki metodi (AGERER 1991a in 1987-1993) s svetlobnim mikroskopom z dodatki za nomarski interferenčni kontrast, fazni kontrast in epifluorescenco in z lupo s standardiziranimi (predpisanimi) postopki za fotografijo in meritve. Dva popolna opisa tipov ektomikorize smo dopolnili z molekularno metodo analize prednostno amplificirane regije ITS ribosomalnih genov (po GARDES / BRUNS 1993) (ITS = notranji transkripcijski presledek) ter encimsko cepljenih fragmentov produkta amplifikacije. Z uporabo bazidiomicetno specifičnih komplementarnih oligonukleotidov (začetnikov, angl. 'primer') je bilo mogoče prednostno pomnožiti ITS regijo ribosomalnih genov glivnega partnerja v simbiozi (opisano v KRAIGHER / AGERER / JAVORNIK 1995, AGERER / KRAIGHER / JAVORNIK 1996, metode v KRAIGHER 1996).

Na ploskvah smo vzorčili med leti 1990 in 1993. Prvotne študije smo omejili na spoznavanje tipov ektomikorize pri smreki na obeh ploskvah. Pri tem bi poudarili, da je bilo dotlej pri smreki opisanih 82 tipov ektomikorize (AGERER 1987-1993), glede na število gliv, ki se pojavljajo v smrekovih gozdovih severnega zmernega in borealnega pasu, pa pričakujejo, da bi bilo število tipov ektomikorize lahko od nekaj sto do tisoč (AGERER 1985, DEACON / FLEMING 1992). Zato je potrebno v vsaki ekološko usmerjeni študiji tipov ektomikorize le-te najprej na kratko ali popolno opisati in spoznati.

V vegetacijski sezoni 1993 smo na obeh ploskvah izvedli 6 vzorčenj v 2 - 5 ponovitvah (zaradi velike biotske in abiotske heterogenosti zgornjih horizontov tal) s sondo volumna 270 ml (velikost vzorca je bila omejena z možnostjo časovne izvedbe analiz med vzorčenji). V vsakem vzorcu smo prešteli vse nemikorizne in mikorizne kratke korenine. Med mikoriznimi smo izločili stare, nedoločljive tipe in tiste, ki so bili premalo razviti ali jih je bilo premalo za opis. Vse določljive tipe smo na kratko opisali in prešteli. Dva tipa ektomikorize s ploskve v Mislinji smo popolno opisali in identificirali glivnega partnerja s sledenjem povezavi med ektomikorizo in trosnjakom glive in z molekularno metodo.

Na ploskvi v Mislinji smo v skupno 21 sondah prešteli 38 502 kratkih korenin, na ploskvi v Zavodnjah pa 20251 kratkih mikoriznih in nemikoriznih korenin smreke (preglednica 1). Sklepali smo lahko na zmanjšanje biomase kratkih korenin na bolj onesnaženi ploskvi. O podobnih rezultatih so poročali WEISS / AGERER 1986 ter KOCOUREK / BYSTRIČAN 1989 za ploskve na Bavarskem in Češkem.

Glede na število in pojavljanje tipov ektomikorize smo ugotovili razlike med obema ploskvama (preglednici 1, 2, slike 1, 2, 3, 4). Na ploskvi v Mislinji smo določili skupno 24 tipov ektomikorize, na ploskvi v Zavodnjah pa 17. Tam smo skozi vso vegetacijsko dobo popisovali naslednje tipe ektomikorize: *Paxillus involutus* (20 %), *Piceirhiza terraphila* (18 %), *Piceirhiza inflata* (15 %), *Xerocomus badius* (13 %), *Russula ochroleuca* (11 %) in *Piceirhiza parallela* (7 %). Nekatere od teh tipov so tudi drugi avtorji opisovali kot pogoste na onesnaženih ploskvah: QIAN in sod. 1993 so poročali, da je najpogostejši vitalni tip na onesnaženih ploskvah v okolici Tübingena *Xerocomus badius*, TURNAU in sod. 1993 pa so svoje raziskave omejevali na vrsti *Paxillus involutus* in *Russula ochroleuca*, ker sta uspevali na močno onesnaženih področjih v okolici Krakova.

Na ploskvi v Mislinji smo skozi vse leto opisovali naslednje tipe ektomikorize: *Hydnum rufescens* (26 %), *Russula* sp. (25 %), *Amphinema byssoides* (18 %), *Cenococcum geophilum* (15 %), *Piceirhiza oleifera* (12 %), *Piceirhiza harenosa* (8 %) in *Lactarius lignyotus* (4 %). Med temi bi želeli poudariti predvsem pogosto pojavljanje in veliko številčnost tipa *Hadnum rufescens*. Te glive na ploskvi v Zavodnjah sploh nibilo. Za vse hidnoidne vrste je namreč značilno, da so med prvimi, ki izginejo z onesnaženih rastišč (ARNOLDS 1991). Zato bi lahko to glivo uporabili kot kazalec neonesnaženih rastišč in ploskev v Mislinji označili za neonesnaženo ploskev.

Iz primerjave med popisi tipov ektomikorize in popisi trosnjakov gliv smo ugotovili, da ni mogoče neposredno primerjati številčnosti posameznih vrst gliv v obeh razvojnih oblikah. Trosnjaki gliv so se pojavljali predvsem v jesenskem obdobju, medtem ko so tipi ektomikorize prisotni na gozdnih rastiščih skozi vse leto. Trosnjaki posameznih vrst gliv, npr. *Hydnum rufescens*, so bili prisotni na ploskvi v Mislinji le posamič, medtem ko je med tipi ektomikorize ta tip prevladoval. Pri nekaterih tipih ektomikorize, ki so se značilno pojavljali le na onesnaženi ali le na neonesnaženi ploskvi, pa glivnega partnerja še nismo identificirali.

Glede na rezultate naših raziskav smo sklepali naslednje:

1. onesnaževanje lahko vpliva na distribucijo mikoriznih gliv, med katerimi lahko nekatere izginejo, medtem ko druge lahko izkoristijo novonastali manj kompetitivni stadij v zaradi onesnaženja osiromašenem gozdnem ekosistemu;
2. mikobioindikacijo z analizo mikocenoz je mogoče uporabiti v ugotavljanju onesnaženosti gozdnih rastišč (kot sta predlagala FELLNER 1989 in ARNOLDS 1988 in 1991);
3. v Sloveniji bi predlagali metodo mikobioindikacije z analizo prisotnosti občutljivih v primerjavi z na onesnaženje neobčutljivimi vrstami gliv, to je z raziskavo prisotnosti in abundance predstavnikov vsake od teh skupin; v naših raziskovalnih razmerah se je pokazala kot občutljiva vrsta *Hydnum*

rufescens, kot neobčutljiva pa *Paxillus involutus;*

4. iz primerjave med pojavljanjem trosnjakov in tipi ektomikorize sklepamo, da so slednji primernejši za ugotavljanje prisotnosti določene vrste gliv na gozdnem rastišču, iz naslednjih vzrokov:

- ektomikoriza je v gozdnih tleh prisotna skozi vse leto in v različnih vegetacijskih sezonah, medtem ko je pojavljanje trosnjakov omejeno na določeno obdobje v letu, odvisno pa je od klimatskih dejavnikov v celem letu;
- veliko število ektomikoriznih gliv ne tvori trosnjakov (Deuteromycotina) ali se uvršča med skorjaste glive, katerih trosnjake je težko opaziti, ali sodi med podzemne glive, katerih trosnjaki so skriti pod zemljo;
- v nekaj primerih smo zabeležili povezanost določenih tipov ektomikorize bodisi z onesnaženo ali le z neonesnaženo ploskvijo, vendar pri njih glivnega partnerja še nismo identificirali; kljub temu bi te tipe lahko uvrstili med razlikovalno občutljive na onesnaženje.

Pri tovrstnih raziskavah bi si lahko v veliki meri pomagali z uporabo molekularnih metod analiz regije ITS ribosomalnih genov. Metoda se je izkazala kot natančna, objektivna in hitra. Predlagali smo, da se značilni vzorci pomnožene DNA uvrstijo v ključ za določanje tipov ektomikorize kot eden od določevalnih znakov za identifikacijo glivnega partnerja do ravni vrste.

9 REFERENCES

AGERER, R., 1985. Zur Ökologie der Mykorrhizapilze.- Bibliotheca Mycologica 97, 160 p.

AGERER, R., 1986. Studies on Ectomycorrhizae III. Mycorrhizae Formed by Four Fungi in the Genera *Lactarius* and *Russula* on Spruce.- Mycotaxon 27, pp. 1-59.

AGERER, R., 1987-1993. Colour Atlas of Ectomycorrhizae.- Einhorn Verlag, Műnchen.

AGERER, R., 1989. Impacts of artificial acid rain and liming on fruitbody production of ectomycorrhizal fungi. Agric., Ecos. & Environment 28, pp. 3-8.

AGERER, R., 1991a. Characterization of ectomycorrhiza.- In: Techniques for the study of mycorrhiza (Ed: Norris JR, Read DJ, Varma AK). Methods in microbiology 23. Academic Press, London, pp. 25-73.

AGERER, R., 1991b. Streuzersetzende Grospilze im Höglwald-Projekt: Reaktionen im vierten Jahr der Behandlung.- Forstwiss. Cbl. 39, pp. 99-103.

AGERER, R. / WEISS, M., 1989. Studies on ectomycorrhizae. XX. Mycorrhizae formed by *Thelephora terrestris* on Norway spruce.- Mycologia 81, pp. 444-453.

AGERER, R. / KRAIGHER, H. / JAVORNIK, B., 1996. Identification of ectomycorrhizae of *Hydnum rufescens* on Norway spruce and the variability of the ITS region of *H. rufescens* and *H. repandum* (Basidiomycetes).- Nova Hedwigia 63, 1-2, pp. 183-194.

AMARANTHUS, M.P. / PERRY, D.A., 1994. The functioning of ectomycorrhizal fungi in the field: linkages in space and time.- Plant and Soil 159, pp. 133-140.

ARNDT, U. / NOBEL, W. / SCHWEIZER, B., 1987. Bioindikatoren - Möglichkeiten, Grenzen und neue Erkentnisse. Ulmer, Stuttgart.

ARNOLDS, E., 1988. The changing macromycete flora of the Netherlands.- Trans.Br.Myc.Soc. 90, pp. 391-406.

ARNOLDS, E., 1991. Decline of ectomycorrhizal fungi in Europe.- Agric., Ecosyst., Envir. 35, pp. 209-244.

BATIČ, F., 1990. Lichen Mapping in Jugoslavia, especially in Slovenia.- Stuttgarter Beitr. Naturk. Nr. 456, pp. 121-124.

BATIČ, F., 1991. Bioindikacija onesnaženosti zraka z epifitskimi lišaji.- Gozd.vestn. 49, s. 248-254.

BATIČ, F., 1994. Bioindikacija onesnaženosti zraka in njen pomen pri vzpostavitvi integralnega monitoringa.- V: Varstvo zraka. Stanje in ukrepi za izboljšanje stanja v Sloveniji. Bled, 28.-30.3.1994. Zavod za tehnično izobraževanje, Ljubljana, s. 12/01 - 12/10.

BERG, B., 1989. Charakterisierung und Vergleich von Ektomykorrhizen gekalkter Fichtenbestände.- Diss. Univ. München.

BLANK, L.W. / ROBERTS, T.M. / SKEFFINGTON, R.A., 1988. New perspectives on forest decline.- Nature 336, pp. 27-30.

BLASCHKE, H., 1986. Mycorrhiza formation and function in Norway spruce stands affected by forest decline in Bavaria.- Universität München Lehrstuhl für Forstbotanik, pp. 731-733.

BON, M., 1987. Mushrooms and Toadstools of Britain and North-western Europe.- Hodder & Stoughton, London.

BRAND, F., 1991. Ektomykorrhizen an *Fagus sylvatica* Charakterisierung und Identifizierung, ökologische Kennzeichnung und unsterile Kultivierung.- Libri Botanici 2, 229 p.

BREITENBACH, J. / KRAENZLIN, F., 1981 - 1991. Pilze der Schweiz. Band 1 Ascomyceten.- Mykologische Gesellschaft Luzern, Verlag Mykologia, 313 pp.

CIGLAR, R. *et all.,* 1994. Varstvo zraka in ukrepi za izboljšanje stanja v Sloveniji.- V: Varstvo zraka. Stanje in ukrepi za izboljšanje stanja v Sloveniji. Bled, 28.-30.3.1994. Zavod za tehnično izobraževanje, Ljubljana, s. 4/01 - 4/18.

COWLING, E., 1989. Comments of Repporters for the International Congress on Forest Decline Research, Friedrichshafen, FRG.- In: ICFDR (Ed: Ulrich, B.), Vol.2, Karlsruhe, FRG, pp. 935-944.

DEACON, J.W. / FLEMING, L.V., 1992. Interactions of Ectomycorrhizal Fungi.- In: Mycorrhizal Functioning: an Integrative Plant-Fungal Process (Ed.: Allen, M.F.). Routledge, Chapman & Hall, pp. 249-300.

FELLNER, R., 1989. Mycorrhiza-forming fungi as bioindicators of air pollution.- Agr., Ecos., Envir. 28, pp. 115-120.

FERLIN, F., 1990. Vpliv onesnaževanja ozračja na rastno obnašanje in rastno zmogljivost odraslih smrekovih sestojev.- Mag. delo, VTOZD za gozd., BF, Univ. v Lj., 142 p.

FINK, S., 1988. Histological and cytological changes caused by air pollutants and other abiotic factors.- In: Air Pollution and Plant Metabolism. Proc. 2nd Int. Symp. on Air Pollution and Plant Metabolism. Munich, Elsevier Appl. Sci., London & NewYork, pp. 36 - 54.

FRANCIS, R. / READ, D.J., 1984. Direct transfer of carbon between plants connected by vesicular-arbuscular mycorrhizal mycelium.- Nature 307, pp. 53-56.

FRANK, A.B., 1885. Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze.- Ber.Deutsch.Bot.Ges. 3, pp. 128-145.

GARDES, M. *et all.,* 1991. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA.- Can.J.Bot. 69, pp. 180-190.

GARDES, M. / BRUNS, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts.- Molecular Ecology 2, pp. 113-118.

GIANINAZZI-PEARSON, V., 1984. Host-Fungus Specificity, Recognition and Compatibility in Mycorrhizae.- In: Genes involved in Plant-Microbe Interactions (Ed: Verma, Hohn), pp. 225-254.

GÖBL, F., 1986. Wirkung simulierter saurer Niederschlage auf Boden und Fichtenjungpflanzen in Gefassversuch. III. Mycorrhiza-untersuchungen.- Cbl. Ges. Forstwesen 103, pp. 89-107.

GÖBL, F., 1989. Mycorrhiza- und Feinwurzeluntersuchung in Waldschadensgebiet Gleingraben / Steiermark.- Österr. Forstz. 6, pp. 16-18.

GOGALA, N., 1990. Vzroki propadanja mikoriznih gliv.- V: Zbornik seminarja: Izkoriščanje in varstvo gozdne mikoflore. Univerza v Ljubljani, BF, VTOZD za gozd., s. 48-46.

GRAND, L.F. / HARVEY, A. E., 1984. Quantitative measurement of ectomycorrhizae on plant roots.- In: Methods and Principles of Mycorrhizal Research (Ed: N.C.Schenk), pp. 157-164.

GRONBACH, E., 1988. Charakterisierung und Identifizierung von Ektomykorrhizen in einem Fichtenbestand mit Untersuchungen zur Merkmalsvariabilitat in sauer beregneten Flächen.- Bibl. Mycologica 125, 216 p.

GUILLITTE, O., 1987. Observations mycologiques et "Pluies acides" en Belgique.- Mem. Soc. Roy. Bot. Belg. 9, pp. 67-78.

GULDEN G. *et all.,* 1992. Macromycetes and air pollution. Mycocoenological studies in three oligotrophic spruce forests in Europe.- Bibl. Mycologica 144, J. Cramer, Berlin, Stuttgart, 81 p.

HARLEY, J.L. / SMITH, S.E., 1983. Mycorrhizal Symbiosis.- Academic Press, London & New York.

HAUG, I. / PRITSCH, K., 1992. Ectomycorrhizal Types of Spruce (*Picea abies* (L.) Karst.) in the Black Forest, A Microscopical Atlas.- Kernforschungszentrum Karlsruhe, 89 p.

HÖILAND, K., 1986. Is the mycorrhizal symbiosis being damaged by pollution?- Acid Magazine 4, pp. 30-31.

HOLOPAINEN, T., 1989. Ecological and untrastructural responses of Scots pine mycorrhizas to industrial pollution.- Agric., Ecos., Env. 28, pp. 185-189.

ICOM 1., 1996. Program and Abstracts.- First International Conference on Mycorrhizae. August 4-9, 1996, University of California Berkeley, USA.

IGLG (Inštitut za gozdno in lesno gospodarstvo), 1988. Črna knjiga o propadanju gozdov v Sloveniji.- IGLG, Ljubljana.

ING, B., 1994. Red Data Lists and decline in fruiting of macromycetes in relation to pollution.- In: Fungi and environmental change, Silsoe College, Cranfield University, March 28-31 1994, British Mycological Society 11th General Meeting, Abstacts, 1 p.

INGLEBY, K. *et all.,* 1990. Identification of ectomycorrhizas. ITE research publication no.5.- Institute of Terrestrial Ecology, 112 p.

JAENIKE, J., 1991. Mass Extinction of European Fungi.- Tree 6, pp. 174-175.

JAKUCS, P. *et all.,* 1986. Acidification of soil and decay of sessile oak in the "Sikfokut project" area (N-Hungary).- Acta Botanica Hungarica 32, pp. 303-322.

JANSEN, A.E. / DE VRIES, F.W., 1989. Mycorrhizas on Douglas fir in the Netherlands.- Agric., Ecos., Envir. 28, pp. 197-200.

JANSEN, P.J., 1985. Zure regen en paddestoelen, een verkenning in de peel.- Coolia 28, pp.13-16.

JÜLICH, W., 1984. Die Nichtblätterpilze, Gallertpilze und Bauchpilze, Aphyllophorales, Heterobasidiomycetes, Gastromycetes. Band IIB/1 - Basidiomyceten - 1. Teil.- Gustav Fisher Verlag, Stuttgart , New York, 626 p.

KOCOUREK, R. / BYSTRIČAN A., 1989. Fine root and mycorrhizal biomass in Norway spruce (*Picea abies* /L./ Karsten) forest stands stands under different pollution stress.- Agric., Ecos., Envir. 28, pp. 235-242.

KOLAR, I., 1989. Umiranje smreke v gozdovih Šaleške doline.- Zb. gozd. in les. 34, s. 121-198.

KOWALSKI, S., 1987. Mycotrophy of Trees in Converted Stands Remaining Under Strong Pressure of Industrial Pollution.- Angew. Botanik 61, pp. 65-83.

KRAIGHER, H., 1991. Mineralna prehrana mikoriznih smrek na Pohorju.- Magistrska naloga, Biol.odd. BF, Ljubljana, 126 p.

KRAIGHER, H., 1994. Citokinini in tipi ektomikorize pri sadikah smreke (*Picea* *abies* (L.) Karst.) kot kazalci onesnaženosti gozdnih rastišč.- Doktorska disertacija, Oddelek za agronomijo, Biotehniška Fakulteta, Univerza v Ljubljani, 156 p.

KRAIGHER, H. / AGERER, R. / JAVORNIK, B., 1995. Ectomycorrhizae of *Lactarius lignyotus* on Norway spruce, characterized by anatomical and molecular tools.- Mycorrhiza 5, pp.175-180.

KRAIGHER, H. / BATIČ, F. / AGERER, R., 1995. Mycobioindication of forest site pollution.- In: Proceedings of the BIOFOSP, 22.-31.08.1995, GIS in Odd. za agronomijo BF, Ljubljana, pp. 95-100.

KRAIGHER, H. / BATIČ, F. / AGERER, R., 1996. Types of ectomycorrhizae and mycobioindication of forest site pollution.- Phyton (Horn, Austria) 36, pp. 115 - 120.

KRAIGHER, H., 1996. Tipi ektomikorize - taksonomija, pomen in aplikacije.- Zb. gozd. in les. 49, s. 33 - 66.

KREEB, K. H., 1990. Methoden zur Pflanzenoekologie und Bioindikation.- Gustav Fischer Verlag, 317 p.

LEŠNJAK, M. *et all.,* 1989. Air pollution and damage on vegetation near TE - Šoštanj Thermal Power Plant in Slovenia, Yugoslavia.- In: Man and his Ecosystem (Ed:Brasser, Mulder), Proc. of the 8th world clean air congress, Vol. 2, Elsevier, Amsterdam, The Netherlands, pp. 125-130.

LOVREC, M., 1989. Primarna produkcija in razdelitev biomase pri sadikah smreke (*Picea abies* (L.) Karsten).- Diplomsko delo, BF VTOZD za biologijo, Ljubljana, 94 p.

MARINČEK, L., 1987. Bukovi gozdovi na Slovenskem.- Delavska enotnost, Ljubljana, 153 p.

MC COOL, P.M., 1988. Effect of air pollutants on mycorrhizae.- In: Air Pollution and Plant Metabolism, Proc. of the 2nd Int.Symp.on Air Pollution and Plant Metabolism, Munich, FRG, 6-9-4-1987, Elsevier Appl. Sci. London, New York, pp. 356-365.

MEHMANN, B. *et all.,* 1995. Coincidence between molecularly or morphologically classified ectomycorrhizal morphotypes and fruitbodies in a spruce forest.- In: Biotechnology of Ectomycorrhizae (Eds. Stocchi, V. *et all.*), Plenum Press, New York, pp. 41-52.

MEJSTRIK, V. / CUDLIN, P., 1987. Experiences with Mycorrhizal Seedlings

Inoculation Used for Reforestation in Immission Stress Areas.- Angew. Botanik 61, pp. 47-52.

MEYER, F.H., 1987. Extreme Standorte und Ektomykorrhiza.- Angew. Botanik 61, pp. 39-46.

MIKOLA, P. / LAIHO, O., 1962. Mycorrhizal relations in the raw humus layer of northern spruce forests.- Comm. Inst. Forest. Fenniae 55.18, Helsinki, pp. 1-13.

MILLER, S.L. / ALLEN E.B., 1992. Mycorrhizae, Nutrient Translocation, and Interaction Between Plants.- In: Mycorrhizal Functioning: An Integrative Plant-Fungal Process. (Ed.: Allen, M. F.), Routledge, Chapman & Hall, pp. 301-331.

MOSER, M., 1978. Die Röhrlinge und Bätterpilze (Polyporales, Boletales, Agaricales, Russulales).Band IIb/2 - Basidiomyceten - 2.Teil.- Gustav Fischer Verlag, Stuttgart, New York, 532 p.

PANKOW, W. / BOLLER, T./ WIEMKEN, A., 1991. Structure, function and ecology of the mycorrhizal symbiosis.- Experientia 47, pp. 311-400.

PILLUKAT, A. / AGERER, R., 1992. Studien an Ektomykorrhizen XL*. Vergleichende Untersuchungen zur baumbezogenen Variabilität der Ektomykorrhizen von *Russula ochroleuca*.- Zeitschrief für Mykologie 58, pp. 211-242.

PILTAVER, A., 1990. Vzroki spreminjanja in propadanja mikoflore v Evropi.- In: Zbornik republiškega seminarja: Izkoriščanje in varstvo gozdne mikoflore, Univerza v Lj., BF, VTOZD za gozdarstvo, s. 34-48.

QIAN, X.M. *et all.,* 1993. Vergleichende untersuchungen an mykorrhizen und mikropilzen der rhizosphäre nach saurer beregnung und kalkung im fichtenbestand 'Höglwald'.- Abschlussbericht über das Projekt 0339175E.- Univ. Tübingen, Botanisches Institut, Spezielle Botanik / Mykologie und Botanischer Garten, Auf der Morgenstelle 1, 72076, Tübingen, 83 p.

RAIDL, S. / AGERER, R., 1992. Studien an Ektomykorrhizen XLII. Ontogenie der Rhizomorphen von *Laccaria amethystina, Hydnum rufescens* und *Sarcodon imbricatus*.- Nova Hedwigia 55, pp. 279-307.

READ, D.J., 1991. Mycorrhizas in Ecosystems.- Experientia 47, pp. 376-390.

REHFUESS, K.E., 1988. Acidic deposition -extent and impact on forest soils, nutrition, growth and disease phenomena in Central Europe: a review.-

Water, Air, Soil Pollution 48, pp. 1-20.

SCHAFFERS, A.P. / TERMORSHUIZEN, A.J., 1989. A field survey on the relations between air pollution stand vitality and the occurrence of fruitbodies of mycorrhizal fungi in plots *of Pinus silvestris*.- Agr., Ecos., Envir. 28, pp. 449-454.

SCHERFOSE, V., 1990. Feinwurzelverteilung und Mykorrhizatypen von *Pinus sylvestris* in verschiedenen Bodentypen.- Diss. Dokt., Fachb. Landespflege der Universität Hannover, 166 p.

SCHLECHTE, G., 1985. Zur Mykorrhizapilzflora in geschädigten Forstbeständen.- Institut für Forstbotanik der Universität Göttingen, pp. 225-232.

SCHÜTT, P. *et all.,* 1984. Der Wald stirbt an Stress.- Bertelsmann Verlag, München, S 32, 128 p.

SHAW, P.J.A. *et all.,* 1992. The effects of sulphur dioxide and ozone on the mycorrhizas of Scots pine and Norway spruce in a field fumigation system.- Mycol. Res. 96, pp. 785-791.

SIMARD, S.W., 1996. Interspecific carbon transfer in ectomycorrhhizal tree species mixtures.- Doct. Thesis, Oregon State University, 210 p.

SMITH, A., 1971. Taxonomy of Ectomycorrhiza-Forming Fungi.- In: Mycorrhizae, Proc. of 1st NACOM, U.S. Department of Agriculture, Forest service, pp. 1-8.

STEUBING, L. / JAEGER, H.J., 1981. Monitoring of air pollutants by plants. Methods and problems.- Dr.W.Junk Publishers, The Hague, London, 161 p.

TAYLOR, A.F.S. / BRAND, F., 1991. Reaction of the Natural Norway Spruce Mycorrhizal Flora to Liming and Acid Irrigation.- In: Mycorrhizas in Ecosystems (Ed: Read, D.J., Lewis, D.H., Fitter, A.H., Alexander, I.J.), CAB International, Cambridge, 404 p.

TERMORSHUIZEN, A.J. / SCHAFFERS, A.P., 1989a. Succession of mycorrhizal funghi in stands Pinus sylvestris in the Netherlands.- Agr., Ecos., Environment 28, pp. 503-507.

TERMORSHUIZEN, A.J./ SCHAFFERS, A.P., 1989b. The relation in the field between fruitbodies of mycorrhizal fungi and their mycorrhizas.- Agr., Ecos., Environment 28, pp. 509-512.

TERMORSHUIZEN, A.J. / VAN DER EERDEN, L. / DUECK, T.A., 1989. The effect of SO2 pollution on mycorrhizal and non-mycorrhizal seedlings of *Pinus sylvestris*.- Agr., Ecos., Environment 28, pp. 513-518.

TRAPPE, J.M., 1962. Fungus associates of ectotrophic mycorrhizae.- Bot.Rev. 28, pp. 538-606.

TREU, R., 1990. Charakterisierung und Identifizierung von Ektomykorrhizen aus den Nationalpark Berchtesgaden.- Bibl. Mycologica 134, 196 p.

TURNAU, K. / KOTTKE, I. / OBERWINKLER, F., 1992. *Paxillus involutus-Pinus sylvestris* Mycorrhizae from Heavily Polluted Forest.- Bot. Acta 106, pp. 193-276.

UHL, M., 1988. Identifizierung und charakterisierung von ektomykorrhizen an *Pinus silvestris* und ektomykorrhizen aus der gattung *Tricholoma*.- Diss. Dokt., Fachb. Biologie, Ludwig- Maximilians-Universitat München, 254 p.

URBANČIČ, M., 1990. Talne razmere na objektih za ekofiziološke raziskave.- Inštitut za gozdno in lesno gospodarstvo, Ekspertiza, Ljubljana, 55 p.

WALLER, K. *et.all.*, 1993. *Piceirhiza oleiferans*, eine neue Ektomykorrhizen-Art an *Picea abies*.- Sendtnera 1, pp. 11-22.

WEISS, M., 1988. Ektomykorrhizen von *Picea abies* Syntheze, Ontogenie und Reaktion auf Umweltschadstoffe.- Diss. Dokt., Fachb. Biologie, Ludwig-Maximilians-Universität München, 141 p.

WEISS, M. / AGERER, R., 1986. Reaktionen des Wurzelsystems von *Picea abies* (L.) Karst. auf Mineralstoffernährung und auf Belastung des Sprosses mit Ozon und saurem Nebel.- Forstw.Cbl. 105, pp. 230-233.

WOLFENDEN, J. / MANSFIELD, T.A., 1991. Physiological disturbances in plants caused by air pollutants.- Proc.of the Royal Society of Edinburg 97B, pp. 117-138

WOLLMER, H. / KOTTKE, L., 1990. Fine root studies in situ and in the laboratory.- Env. Pollut. 68, pp. 383-407.

ZHAO, Z., 1989. Untersuchungen über Zusamenhänge zwischen chemischen Eigenschaften von Waldböden und Buchenmykorrhizen im Wienerwald.- Dissertationen der BOKU 35, Wien, 98 p.

10 ACKNOWLEDGEMENTS

This study is part of a Doctoral Dissertation, financed by the Ministry of Science and Technology of the Republic of Slovenia (MST) (Young Researchers Programme and J4-0261-0404/1992-1995), the Ministry for Agriculture and Forestry (MAF) and the TEMPUS M_JEP 4667, done under the supervision of Prof. Dr. Franc Batič, Ljubljana, Prof. Dr. David E. Hanke, Cambridge, and Prof. Dr. Reinhard Agerer, Munich. Determination of fungal fruitbodies were done by Andrej Piltaver. Molecular methods were applied by the author in the laboratory of the Center for Plant Breeding and Biotechnology, Ljubljana, as part of a joint project L4-6287-0404/94 (MST & MAF). The publication was prepared as part of the current projects L4-7402 (MST & MAF) and 1487/8 (MAF). Special thanks are due also to Mrs. Jana Janša for her help in sample preparation and countings.