CZECH MYCOLOGY

VOLUME 52
OCTOBER 2000

3

CZECH SCIENTIFIC SOCIETY FOR MYCOLOGY PRAHA

ISSN 0009-0476

Vol. 52, No. 3, October 2000

CZECH MYCOLOGY

formerly Česká mykologie
published quarterly by the Czech Scientific Society for Mycology
http://www.natur.cuni.cz/cvsm/

Czech Mycology is an international scientific journal publishing papers in all aspects of mycology. Publication in the journal is open to members of the Czech Scientific Society for Mycology and non-members.

Contributions to: Czech Mycology, National Museum, Department of Mycology, Václavské nám. 68, 115 79 Praha 1, Czech Republic. Phone: 02/24497259 or 96151284

SUBSCRIPTION. Annual subscription is Kč 400,– (including postage). The annual subscription for abroad is US $ 86,– or DM 136,– (including postage). The annual membership fee of the Czech Scientific Society for Mycology (Kč 270,– or US $ 60,– for foreigners) includes the journal without any other additional payment. For subscriptions, address changes, payment and further information please contact **The Czech Scientific Society for Mycology, P.O.Box 106, 111 21 Praha 1, Czech Republic. http://www.natur.cuni.cz/cvsm/**

This journal is indexed or abstracted in:
Biological Abstracts, Abstracts of Mycology, Chemical Abstracts, Excerpta Medica, Bibliography of Systematic Mycology, Index of Fungi, Review of Plant Pathology, Veterinary Bulletin, CAB Abstracts, Rewiew of Medical and Veterinary Mycology.

No. 2 of the vol. 52 of Czech Mycology appeared in 20. 4. 2000

CZECH MYCOLOGY
Publication of the Czech Scientific Society for Mycology

Volume 52 October 2000 Number 3

Open Letter to the scientific community of mycologists. Inputs from referees requested.

REINHARD AGERER[1], JOE AMMIRATI[2], TIMOTHY J. BARONI[3], PAUL BLANZ[4], RÉGIS COURTECUISSE[5], DENNIS E. DESJARDIN[6], WALTER GAMS[7], NILS HALLENBERG[8], ROY HALLING[9], DAVID L. HAWKSWORTH[10], EGON HORAK[11], RICHARD P. KORF[12], GREG M. MUELLER[13], FRANZ OBERWINKLER[14], GERHARD RAMBOLD[15], RICHARD C. SUMMERBELL[7], DAGMAR TRIEBEL[16] and ROY WATLING[17]

[1] Institut für Systematische Botanik, Section Mykologie, Universität München, Menzinger Str. 67, D-80638 München, Germany.
[2] Department of Botany, University of Washington, Seattle, Washington 98195, USA.
[3] Department of Biological Sciences, POB 2000, SUNY-College at Cortland, Cortland, NY 13045, USA.
[4] Institut für Botanik, Universität Graz, Holteigasse 6, A-8010 Graz, Austria.
[5] Département de Botanique, Faculté des Sciences Pharmaceutiques et Biologiques, BP 83, F-59006 Lille Cedex, France.
[6] Department of Biology, San Francisco State University, 1600 Holloway Ave., San Francisco, CA 94132, USA.
[7] Centraalbureau v. Schimmelcultures, P. O. Box 273, NL-3740 AG Baarn, The Netherlands.
[8] Department of Plant Taxonomy, University of Göteborg, Carl Skottsbergs Gata 22, S-41319 Göteborg, Sweden.
[9] Institute of Systematic Botany, New York Botanical Garden, Bronx, New York 10458-5126, USA.
[10] MycoNova, 114 Finchley Lane, Hendon, London NW4 1DG, UK.
[11] Geobotanisches Institut ETH, Herbarium Z+ZT, Zollikerstr. 107, CH-8008 Zürich, Switzerland.
[12] Department of Plant Pathology, Cornell University, 401 Plant Science Bldg., Ithaca, NY 14853-4203, USA.
[13] Department of Botany, The Field Museum, 1400 S. Lake Shore Dr., Chicago IL, 60605-2496, USA.
[14] Institut für Biologie I, Lehrstuhl Spezielle Botanik und Mykologie, Auf der Morgenstelle 1, D-72076 Tübingen, Germany.
[15] Lehrstuhl für Pflanzensystematik, Universität Bayreuth, Universitätsstr. 30 NW I 101251, D-95447 Bayreuth, Germany.
[16] Botanische Staatssammlung München, Mycology Department, Menzinger Str. 67, D-80638 München, Germany.
[17] Royal Botanic Garden, Inverleith Row, Edinburgh EH3 5LR, UK.

To help minimise invalid publication of newly proposed scientific names of fungi, Korf (1995) provided advice on how to guarantee valid publication, and offered a few simple guidelines for authors, reviewers, and editors. He regretted that 'unfortunately many of the errors are committed by highly respected mycologists, and published in thoroughly respectable journals' and emphasised that 'although the ultimate responsibility for publishing correct names lies with authors, clearly reviewers and editors are shirking their duties to advise authors of such errors prior to publication'.

In order to be published validly, names must be introduced according to requirements of the International Code of Botanical Nomenclature (ICBN; Greuter et al. 1994, 2000). Since 1990 it has been compulsory to deposit the vouchers for new species and infraspecific taxa, the name-bearing types, in an herbarium or other collection. It is generally accepted that such voucher specimens should be deposited in publicly accessible reference collections such as herbaria.

However, voucher collections are invariably necessary not only when new fungi are described, but also in connection with any scientific study, whether by taxonomists, systematists, physiologists, chemists, molecular biologists, pathologists, ecologists, clinicians, etc., dealing with organisms. It is essential to preserve voucher specimens as dried material or, where possible, in addition as permanently preserved living cultures. When none of the investigated material is preserved, it is impossible to confirm the identity of the investigated taxa. If species concepts have changed, it is particularly crucial to be able to re-identify the organism at a later time. There are several examples of entities once thought to be species but now revealed as species complexes, where the species concept has been or will be changed, including *Pisolithus tinctorius* (Burgess et al. 1995) and *Paxillus involutus* (Fries 1985, Hahn and Agerer 1999). In such cases, re-identification of the original material is indispensable in order to know which organism was studied so that previous work will continue to be relevant. In recent years molecular biological studies have a tremendous impact on systematics, taxonomy, and ecology. DNA sequences are frequently obtained from fungal cultures. Too often there is no record either of an exact citation of the fungal material used, such as an unequivocal number referring to collection accession data and the voucher culture, or reference to the institution where the material has been deposited. Frequently, only personal or laboratory strain numbers are given, which make it hard to trace the origin of the fungal material. Only accession numbers allocated by permanent public or other open institutional collections can ensure the retrieval of voucher material over the long-term. It is not yet common practice to publish complete collection or isolation data, or to deposit vouchers, except in taxonomic articles.

Conservation of dried fruit-bodies from which cultures are made is also indispensable in order to allow checking of anatomical and morphological features that

cannot be reproduced in culture. The cultures also can be checked using molecular methods after prolonged preservation, in order to exclude the possibility of contamination. While it is rarely possible to culture fungi from dried specimens, the associated collection details are indispensable not only to clarify the geographical and ecological source, but also to facilitate the possibility of recollecting the fungus in the same site. This requires as detailed and exact a description of the sampling locality as possible, preferably including geographic co-ordinates something now facilitated by hand-held or wrist-band global positioning devices.

Voucher specimens are equally important for a wide range of other investigations. Dennis' (1960: xxii) remark that "records that cannot be verified are mere waste paper" applies to numerous aspects of our discipline. Studies of the species composition of any habitat depend on properly determined fungi, and so will require dried vouchers deposited in publically accessible collections. This applies, for example, not only to fruit-bodies, but indeed to any other form of fungal structure, such as sclerotia, or ectomycorrhizas (Agerer 1991) used in scientific work. Ecological, chemical, applied, and physiological studies quite often rely on ecotypes of species, which could later be considered, depending upon the species concepts applied, as separate species. In the seventies, Hawksworth (1974), Yocum and Simons (1977) and Ammirati (1979) were among the first to point out the importance of voucher material particularly in chemical, but also other physiological and ecological studies. In ecological studies on ectomycorrhizas, the increasing use made of RFLP patterns or DNA sequences for the detection of the symbionts requires comparison with those of identified fruit-bodies. In many studies, the identified ectomycorrhizas are completely consumed by the extraction and amplification methods. Instead, voucher specimens should be stored, when individual tips of a larger hyphal system have been used. Even more important is the citation and preservation of the fruit-body specimen from which DNA was extracted for comparison with that that obtained from ectomycorrhizae.

Voucher cultures are urgently needed when clinically relevant fungi are investigated and their etiologic data and their impact on human beings have to be evaluated (de Hoog and Guého 1985). Further, where cultural or chemical features are crucial for the evaluation of newly described fungi such as yeasts, the non-availability of cultures can make interpretation impossible and frustrate other researchers (Banno *et al.* 1993, Hawksworth 1984). Sufficient information on clinical direct microscopy or histopathology results to determine whether an isolate was medically significant or a biomedical contaminant is essential for later evaluations. In cases of apparently exotic fungi, a brief notation of relevant patient travel history is strongly recommended.

Additional documentation requirements apply to strains deposited in the major service collections of fungal cultures, such as ATCC (American Type Culture Collection, Manassas, Virginia, USA), CBS (Centraalbureau voor Schimmelcultures,

Baarn/Utrecht, The Netherlands), or IMI (CABI Bioscience (UK Centre), Egham, Surrey, UK); these and other culture collections often provide forms for depositors to simplify the documentation process. In such major culture collections, the cultures are safely stored with cryo-preservation methods, and may be revived at any time. For sporulating fungi, the citation of the allocated accession number is generally enough to meet the goal of reproducibility of scientific results, i. e. to confirm the identity of the species studied. But a comparison with naturally grown material is only possible when the original collection or isolation details have been cited. A completely different situation arises in cultures which are sterile and thus cannot be identified by normal methods. For such cultures, preservation of vouchers is particularly important together with exact collection data of the fruit-bodies and the herbarium or other collection where they have been deposited. Misidentifications can then be detected, new species concepts applied to the material, and recollection of new living material from the site of the original fruit-body might still be possible.

The addresses of public and open institutional dried reference collections and herbaria can be found in *Index Herbariorum* (Holmgren, Holmgren and Barnett 1990), and of microbial culture collections in the *World Directory* (Sugawara et al. 1993); these works both contain generally applied acronyms, which are convenient and informative enough for citation. Public and institutional collections ensure that the material in their care is well-curated and preserved in a proper way for centuries, and they usually loan dried material free of charge, subject to certain requirements. Whilst the long-term maintenance of private herbaria is often uncertain and the mailing expenses exceed a private budget, nearly all of the international herbaria and other institutions that house fungi will warmly accept properly dried and documented fungal material. Living cultures are normally supplied for a charge to cover the post of preparation and carriage, again subject to particular regulations that may apply; details vary and are available from the collections' catalogues and web sites.

Particularly in recent years, the behaviour of the scientific community has set tongues wagging, especially in relation to falsified data in publications concerning human cancer. It is a fundamental principle of science that research work must be reproducible. Reproducibility requires that studies can be made using the same dried material or cultures as the original study used. As a consequence, publications lacking unambiguous reference to the locations where the critical study material can be accessed by later researchers should not be accepted for publication. They are of no or limited scientific value in that they cannot be reproduced. Editors and referees in all aspects of mycology are often confronted with such situations and it is therefore necessary to include advice for the deposition of voucher material in instructions for authors (e. g. Hawksworth 2000) and to regard this as a prerequisite for publication.

All scientists are responsible for their results. This responsibility lies not only in relation to the scientific community, but also in relation to those who support their research - the taxpayer, charities or other funding agencies, and ultimately society at large. The general public expects integrity from the scientific community. It is the responsibility of individual scientists, referees, and editors to rigorously apply the highest standards and make every effort to ensure that published research will be reproducible. Reproducibility in mycology is irrevocably and inextricably connected to the unequivocal citation of voucher specimens and cultures.

REFERENCES

AGERER, R. (1991): Characterization of ectomycorrhiza. – In: Techniques for the Study of Mycorrhiza (J. R. Norris, D. A. Read & A. K. Varma, eds): 25-73. [Methods in Microbiology Vol. 23.] Academic Press, San Diego.

AMMIRATI, J. (1979): Chemical studies of mushrooms: the need for voucher collections. – Mycologia 71: 437-441.

BANNO, I., BARNETT, J. A., DÉAK, T., GAMS, K. W., GOLUBEV, W. I., GUÉHO, E., HAWKSWORTH, D. L., HENNEBERT, G. L., HOFFMANN, P., JONG, S.-C., KURTZMAN, C. P., LACHANCE, M.-A., MARTINI, A., NAKASE, T., PITT, J. I., ROBERTS, I. N., SLÁVIKOVÁ, E., SPOENCER-MARTINS, I., SUIHKO, M.-L., URUBURU, F. and YARROW, D. (1993): Unavailable new species. – FEMS Microbiology Letters 108: i.

BURGESS, T., MALAJCZUK, N. and DELL B. (1995): Variation in Pisolithus based on basidiome and basidiospore morphology, culture characteristics and analysis of polypeptides using 1D SDS-PAGE. – Mycological Research 99: 1-13.

DENNIS, R. W. G. (1960): British Cup Fungi. Ray Society, London.

FRIES, N. (1985): Intersterility groups in Paxillus involutus. – Mycotaxon 24: 403-409.

GREUTER, W., BARRIE, F. R., BURDET, H.-M., CHALONER, W. G., DEMOULIN, V., HAWKSWORTH, D. L., JØRGENSEN, P. M., NICOLSON, D. H., SILVA, P. C., TREHANE, P. and MCNEILL, J., eds. (1994) International Code of Botanical Nomenclature (Tokyo Code).[Regnum Vegetabile No. 131.] Koeltz Scientific Books, Königstein.

GREUTER, W., BARRIE, F. R., BURDET, H.-M., DEMOULIN, V., FILGUERIAS, T. S., MCNEILL, J., NICOLSON, D. H., SILVA, P. C., SKOG, J. E., TREHANE, P., TURLAND, N. J. and HAWKSWORTH, D. L., eds. (2000) International Code of Botanical Nomenclature (St. Louis Code). [Regnum Vegetabile, No. 138] Koeltz Scientific Books, Königstein.

HAHN, C. and AGERER, R. (1999): Studien zum Paxillus involutus Formenkreis. – Nova Hedwigia 69: 241-310.

HAWKSWORTH, D. L. (1974): Mycologist's Handbook. Commonwealth Mycological Institute, Kew, Surrey.

HAWKSWORTH, D. L. (1984): Fungi in culture. – Nature 310: 18.

HAWKSWORTH, D. L. (2000): Mycological Research: Instructions and guidelines for authors. – Mycological Research 104: 119-127.

HOLMGREN, P. K., HOLMGREN, N. H. and BARNETT, L. C. (1990): Index Herbariorum. Part I. Herbaria of the World. 8th edn. [Regnum Vegetabile No.120.] New York Botanical Garden, New York. (http://www.nybg.org/bsci/ih/)

DE HOOG, G. S. and GUÉHO, E. (1985): A plea for the preservation of opportunistic fungal isolates. Diagnostic Microbiology and Infectious Disease 3: 369-372.

KORF, R. P. (1995): Authors, reviewers, and editors of articles proposing new names: A few guidelines. – Mycotaxon 54: 413-419.

SUGAWARA, H., MA, J., MIYAZAKI, S., SHIMURA, J. and TAKISHIMA, Y. (1993): World Directory of Collections of Cultures of Microorganisms. 4^{th} edn. World Federation of Culture Collections World Data Center on Microorganisms, RIKEN, Wako, Japan.

YOCUM, R. R. and SIMONS, D. M. (1977): Amatoxins and phallotoxins in Amanita species of northeastern United States. – Lloydia 40: 178-190.

CZECH MYCOL. 52 (3), 2000

Fruitbody quality and enzyme production of strains of Hericium erinaceus, an edible mushroom of medicinal relevance

A. PH. GRYGANSKI[1], B. KIRCHHOFF[2], and H. P. MOLITORIS[3] *)

[1]M. G. Kholodny Institute of Botany,
National Academy of Sciences of Ukraine,
Tereshchenkivska St. 2, 252601, Kyiv, Ukraine
[2]WESER-CHAMPIGNON, Neue Heerstr. 35,
31840 Hessisch-Oldendorf, Germany
[3]Botanical Institute, University of Regensburg,
Universitätstr. 31, 93040 Regensburg, Germany

Gryganski A. Ph. B., Kirchhoff B. and Molitoris H. P. (2000): Fruitbody quality and enzyme production of strains of Hericium erinaceus, an edible mushroom of medicinal relevance. – Czech Mycol. 52: 195–207

Cultivation experiments of 14 heterokaryotic strains of the edible and medicinally relevant mushroom *Hericium erinaceus* have shown a large variation in yield, quality and colour of the fruitbodies (Kirchhoff 1996). To determine the reasons for different fruitbody colours, phenoloxydases in the vegetative mycelium on agar and liquid media were investigated. It was shown that the colour of the fruitbodies correlates with the presence and activity of the phenoloxidase laccase. There is no correlation between fruitbody colour and presence of the phenoloxidase tyrosinase, responsible for browning of white button mushroom fruitbodies, *Agaricus bisporus*. The data obtained are important for selection or breeding of new *H. erinaceus* strains with good fruitbody qualities.

Key words: Fruitbody colour, fruit body quality, *Hericium erinaceum*, laccase, tyrosinase.

Gryganski A. Ph. B., Kirchhoff B. a Molitoris H. P. (2000): Kvalita plodnic a enzymatická produkce kmenů korálovce ježatého (Hericium erinaceus), jedlé a lékařsky významné houby. – Czech Mycol. 52: 195–207

Experimenty s kultivací 14 hetrokaryotických kmenů jedlé a lékařsky významné houby korálovce ježatého (*Hericium erinaceus*) ukázaly širokou variabilitu ve výnosu, kvalitě a barvě plodnic (Kirchhoff 1996). Ke stanovení příčin různého zbarvení plodnic, byly zkoumány fenoloxidázy ve vegatativním myceliu na agarových půdách a v tekutých mediích. Zjistilo se, že rozdíly v barvách plodnic souvisí s přítomností a aktivitou enzymu lakkázy. Nebyla zjištěna korelace mezi barvou plodnic a přítomností fenoloxidázy tyrozinázy, která je zodpovědná za hnědnutí pěstovaného žampionu *Agaricus bisporus*. Získaná data jsou významná z hlediska šlechtění a výběru nových kmenů *Hericium erinaceus* s dobrou kvalitou plodnic.

INTRODUCTION

The introduction of new species of edible mushrooms for the market is difficult because of the well developed market structure and high quality standards of

*) Corresponding author Prof. Dr. H. P. Molitoris,
E-mail: Hans-Peter. Molitoris@Biologie. Uni-Regensburg. De

commercial mushrooms. Consumers are used to the high quality of the white button mushroom, *Agaricus bisporus* (J. Lge) Imbach, and its established quality standards (Peters 1997). Colour is considered to be one of the most important factors in consumer perception of fresh mushroom quality. Mushrooms with discolouration are judged to be of lower quality and hence of lower commercial value (Burton 1988). Whiteness, compactness, fruitbody form, density and texture, and length and thickness of spines are also of importance to make *Hericium erinaceus* (Bull.: Fr.) Pers. more attractive for consumers. The colour of *H. erinaceus* fruitbodies, like that of other edible mushrooms, has two important aspects, namely colour of fruitbodies and its changes during fruitbody development, maturation and shelf life. For *H. erinaceus* it is important to produce clear white or snow-white fruitbodies, that do not change colour after mechanical damage, during storage, transport and sale (Figs. 1, 2). The main factor considered to be responsible for the browning by *A. bisporus* and, possibly, of other mushrooms, is tyrosinase. The browning of mushroom tissue is caused by the oxidation of phenols by tyrosinase to produce melanins (Rama et al. 1995). In addition to tyrosinase, laccase, another phenoloxidase, has also been studied, and may also be responsible for browning of harvested fruitbodies of *H. erinaceus*.. This paper deals with colour and the correlation of this feature with the presence and activity of the enzymes laccase and tyrosinase – important attributes of quality of fresh fruitbodies of *H. erinaceus*.

MATERIALS AND METHODS

Biological material

Fourteen strains of *Hericium erinaceus* (Bull.: Fr.) Pers. (He = heterokaryon: He1-He2, He4-He15) of different origin were obtained from culture collections and companies (Kirchhoff 1996). Six homokaryons (h = homokaryon: h1-h6) were obtained from the rarely occurring 1-nucleated chlamydospores of vegetative mycelium of strain He1. These cultures were maintained in the strain collection of WESER-CHAMPIGNON on malt peptone agar (MPA [malt extract 30 g, peptone 5 g, agar 16 g/l H_2O, pH 6]).

1. Qualitative laccase- and tyrosinase-tests on the surface of mycelial colonies on agar media (Molitoris and Schaumann 1986)

Mycelium of the strains was cultivated for 2 weeks on agar media: MPA for tyrosinase-tests (application of p-cresol/glycine solution onto the colony surface), or MPA with guajacol (0.05 g/l) or with α-naphtol (0.05 g/l), for laccase-tests. The presence of laccase was determined by the appearance of brown-red (guajacol)

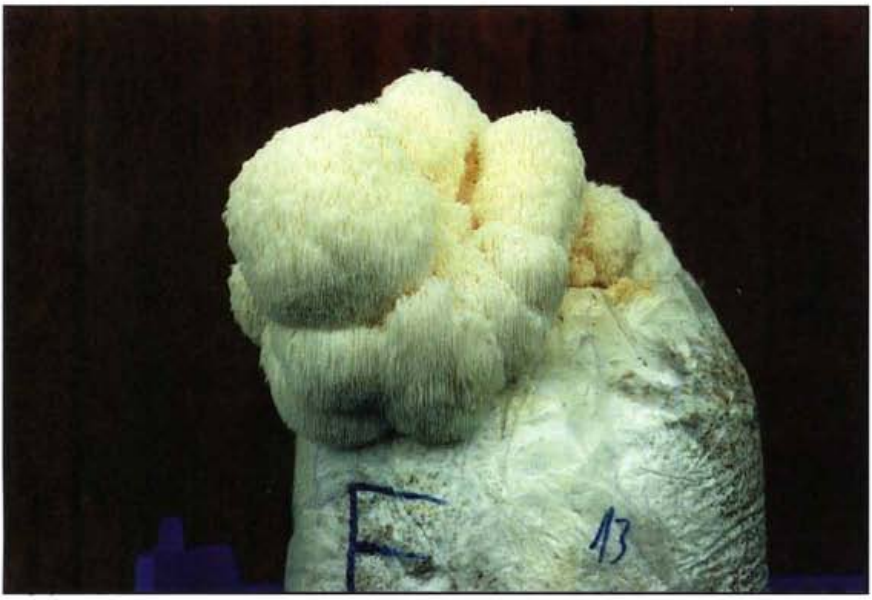


Fig. 1. White (good quality) fruitbodies of *Hericium erinaceus*.

Fig. 2. Coloured and distorted (bad quality) fruitbodies of *H. erinaceus*.

or blue (α-naphtol) on the plates 1, 3, 5, 7 and 10 days after inoculation. Presence of tyrosinase (reddish-brown colour) was tested with the heterokaryons 10 and 20 days after inoculation and with the homokaryons after 20 and 60 days because of their slow growth rate.

2. Qualitative laccase- and tyrosinase tests of culture filtrate and mycelial extract from liquid (surface) cultures

Mycelium was cultivated in liquid MP medium. 100 ml Erlenmeyer flasks with 50 ml sterile nutrient solution were inoculated with 5 × 5 mm mycelial pieces from MPA cultures. After one week of growth mycelial biomass and culture filtrate were separated by filtration. The pH of the culture filtrate was around 6 for the heterokaryons and around 5 for the homokaryons. For growth determinations the vegetative mycelium was washed with distilled water and weighed (wet weight).

For preparation of the mycelial extract 1 g mycelium (wet weight) was initially disrupted using mortar and pestle with 2 g washed sea sand for 3 min. and after addition of 6 ml 0.05M/pH 6 phosphate buffer for another 5 min. The disrupted material was centrifuged in an Eppendorf centrifuge 5417R for 20 min. at 8000 xg and 5 °C. The supernatant was used both for qualitative and quantitative enzyme determinations. For homokaryotic strains enzyme determinations could be performed only with the culture filtrates owing to lack of mycelium.

In order to prove the presence of tyrosinase in the presence of laccase, culture filtrate and mycelial extract were treated at 60 °C for 10 min. whereby laccase should be destroyed and tyrosinase should be activated. For the qualitative determination of laccase or tyrosinase, 0.5 ml of culture filtrate or mycelial extract was mixed in test tubes with 1 ml of water (control), 2.6-dimethoxyphenol (DMOP), guajacol or p-cresol for laccase determination or with 1 ml of tyrosine for tyrosinase determination. Heat-treated material was used in the same way. Colour change of the solutions was observed at 19 °C after 1 and 12 hours following mixing.

3. Quantitative determination of laccase activity with DMOP in liquid culture

Culture filtrate or mycelial extract (0.4 ml) was mixed with 2.0 ml substrate (DMOP) and 0.3 ml Sörensen's buffer pH 5 in a 1 cm cuvette. DMOP solution: 100 mg 2.6-Dimethoxy-phenol was dissolved in 1 litre NaOH-citrate buffer 0.1 M, pH 5. Sörensen's buffer: A) 21.008 g citric acid + 200 ml 1N NaOH made up to 1 litre with distilled water; B) 0.1N NaOH; C) 1 litre of solution A was mixed with 41.7 ml solution B. The enzyme reaction was started by addition

of the enzyme solution. Readings were taken in an Eppendorf Spektrallinien Photometer with a 1101 M Cd-Lampe at 468 nm after 30 sec. reaction time at room temperature (19 °C).

RESULTS

1. Qualitative enzyme tests on agar media

Laccase (guajacol)

The colour reaction (brown-red colouring of the inoculum) of the heterokaryotic strains commenced usually 1 hour after inoculation, in some strains after 30 minutes. The enzyme reaction (brown-red zone in agar around inoculum or mycelial colony) (Fig. 3) appeared usually on the third day. After this time, colour intensity and size of the reaction zone did not change except for strain He10. The colour of the heterokaryotic fruitbodies correlated with the results of the guajacol laccase tests. The majority of strains with a white fruitbody (Table 1, colour intensity 1–3) had no positive enzyme reaction. All strains with yellow to brown fruitbodies (Table 1, colour intensity 4–6) had a strong positive reaction in the guajacol laccase test.

Only one out of six homokaryotic strains tested showed a positive reaction in the guajacol laccase test.

Laccase (α-naphtol)

The colour reaction of the heterokaryotic strains started usually 1 hour after inoculation (blue colour of the inoculum). The enzyme reaction (blue zone around colony, fig. 4) appeared usually on the fifth day. Colour intensity and extent of the reaction zone did not change after day 5. However, after 10 days the mycelium overgrew the coloured zone, prohibiting further observation. There was no correlation between the laccase test (both presence and intensity of reaction) and fruitbody colour.

Only one out of six homokaryotic strains tested showed a positive reaction of laccase with α-naphtol. The reaction was finished after 3 days of growth and did not change thereafter.

Tyrosinase (p-cresol and glycine)

Tyrosinase activity was observed on the 10th day after inoculation for strains He2 and He14 and after 20 days for strains He4, He6, and He12-He15. Possibly, all *H. erinaceus* strains possess intracellular tyrosinase which would not allow observation of the reaction on the colony surface. Only old or destroyed cells may

Table 1. Correlation between colour of fruitbodies and qualitative tests for laccase (4 days, 25 °C) for the heterokaryotic strains (He) on agarised media.

Strain	Colour of fruitbodies*	Laccase (guajacol)**
He6	1	–
He2	2	+
He13	2	–
He1	3	–
He10	3	–
He9	3	++
He8	3	+++
He12	4	+
He14	4	+
He7	4	++
He4	4	+++
He11	5	+
He5	5	+++
He15	6	++

* Colour of fruitbody: 1 = white; 2–3 = white to yellow; 4–5 = yellow to brown; 6 = brown.
** Laccase intensity: – = no reaction; + = present; ++ = strong; +++ = very strong.

Table 2. Correlation between colour of fruitbodies and the results of qualitative laccase tests in of culture filtrate (CF) and mycelial extract (ME) from liquid culture (4 days, 25 °C) of heterokaryotic *Hericium* strains.

		Laccase (CF)** guajacol	Laccase (ME)** guajacol	Laccase (CF)** DMOP
Strain	Colour of fruitbodies*	after 12 hours	after 12 hours	after 1 hour
He6	1	–	–	–
He2	2	–	+	–
He13	2	+	–	+
He1	3	–	–	–
He10	3	++	–	++
He8	3	+++	+	+
He9	3	+++	+	++
He7	4	+	+	+
He4	4	+	+++	++
He12	4	+++	++	++
He14	4	+++	++	++
He11	5	++	+	++
He5	5	+++	+	++
He15	6	++	++	+

*, **, for explanation see Table 1

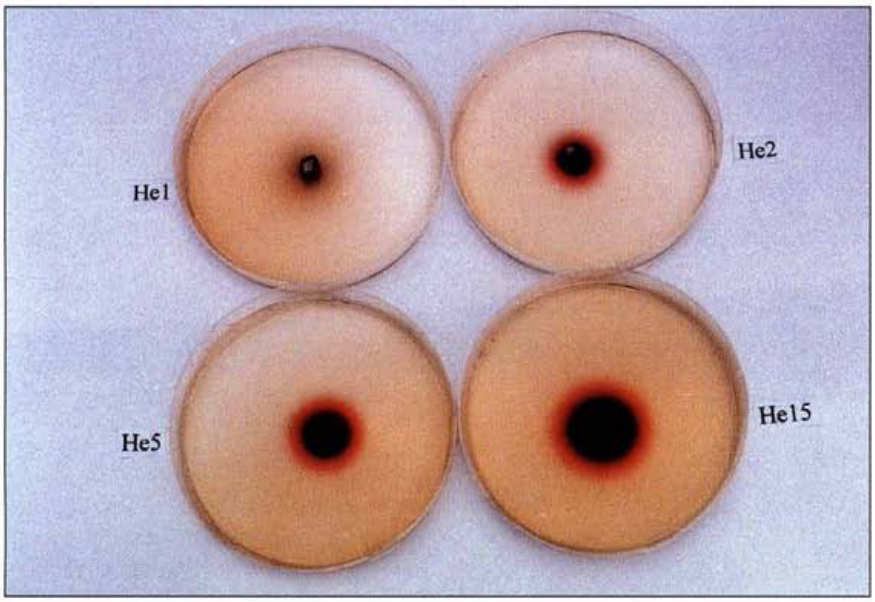


Fig. 3. Laccase reaction with guajacol on agar plates with different strains of *H. erinaceus* (after 4 days, 25 °C).

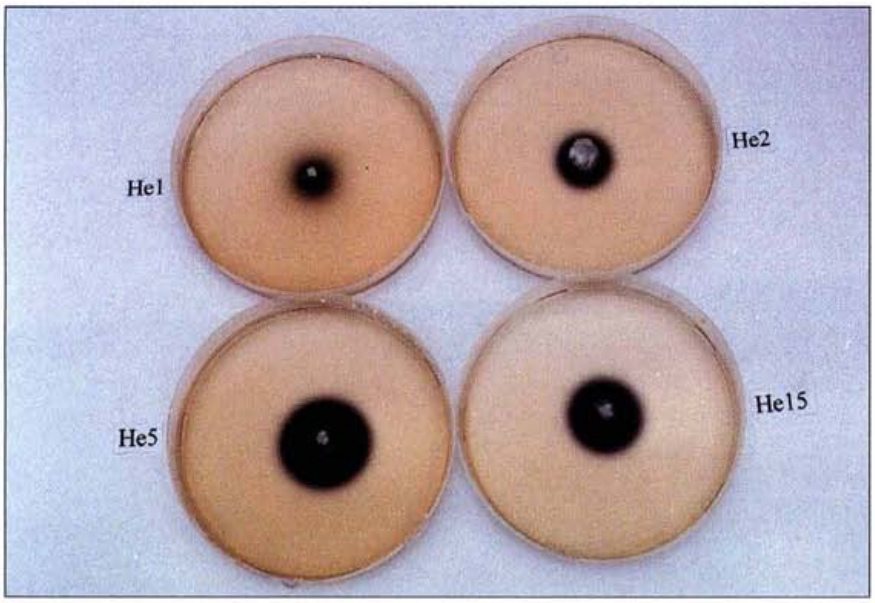


Fig. 4. Laccase reaction with α-naphthol on agar plates with different strains of *H. erinaceus* (after 4 days, 25 °C).

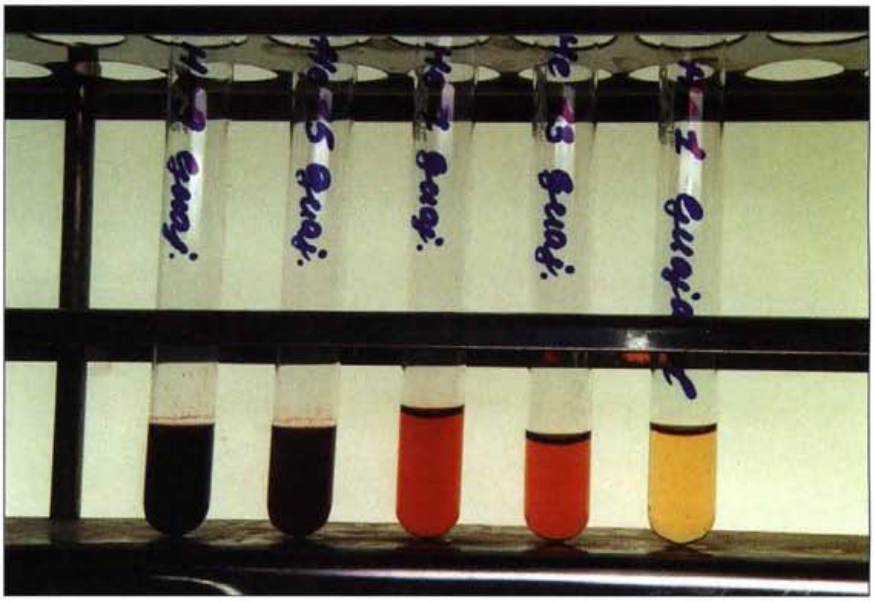


Fig. 5. Qualitative laccase tests (guajacol) with culture filtrate of different strains of *H. erinaceus* (from left to right: He9, He15, He7, He13, He1).

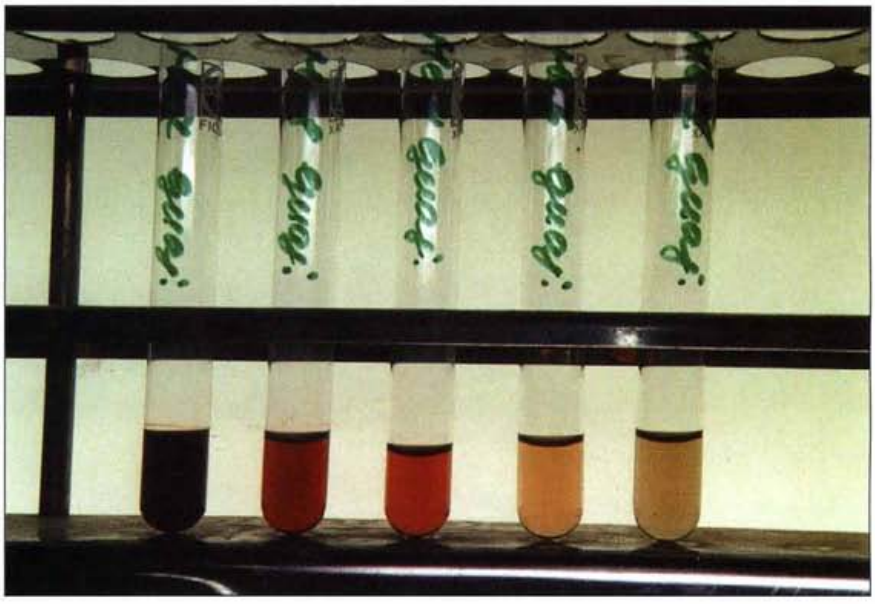


Fig. 6. Qualitative laccase tests (guajacol) with mycelial extracts of different strains of *H. erinaceus* (from left to right: He7, He8, He4, He6, He1).

therefore show a colour reaction with p-cresol as substrate and the rate of lysis of old cells may differ in various strains. There was no correlation between colour of fruitbodies and presence of tyrosinase in the strains investigated.

Except for the homokaryotic strain h6, the tyrosinase tests of all homokaryons were positive.

2. Qualitative enzyme determination in liquid culture

2.1. Laccase

Guajacol

The majority of strains investigated showed a positive laccase reaction with guajacol. Strains producing white fruitbodies showed no laccase reaction in the culture filtrate or the mycelial extract. In contrast, the strains with coloured fruitbodies had distinct, strong or very strong laccase reactions in the culture filtrates (Fig. 5) compared with the mycelial extracts (Fig. 6). Generally, stronger reactions were observed in the culture filtrate than in mycelial extracts (Table 2). The reactions with guajacol became more distinct after 12 hours of incubation. Very often the reaction with guajacol gave an atypical colour, not red-brown but pink or grey. No differences in qualitative laccase tests were found between heat-treated and non-treated liquid samples. No positive laccase reaction was found with culture filtrates of homokaryons.

DMOP

The majority of the strains investigated showed a positive reaction for laccase with DMOP (yellow-orange, Table 2). The reactions with this substrate became more distinct after one hour. Most of the positive strains showed this reaction within 12 hours after addition of the reagent. In a number of cases temperature treatment prevented the laccase reaction (colour production with DMOP). Half of the homokaryons tested showed positice laccase reaction with DMOP.

p-cresol

No positive laccase reaction (white sediment) was found with p-cresol, neither in culture filtrate nor in mycelial extract under the experimental conditions used..

2.2. Tyrosinase

Tyrosine

No positive tyrosinase reaction with tyrosine (red, later changing to black) was found in the culture filtrate of heterokaryons or homokaryons. More than half of the strains investigated had a positive reaction for tyrosinase when the mycelial

extract was investigated. There were more strains with positive tyrosinase reaction after temperature treatment than without treatment. The tyrosinase reaction was more distinct after 12 hours as compared to 1 hr incubation time. Strain He8, He9, He12 and He 14 had no positive reaction for tyrosinase with tyrosine.

p-cresol

With p-cresol no positive tyrosinase reaction (red) in the culture filtrate was observed with heterokaryons or homokaryons. In contrast, mycelial extracts of 10 strains studied showed distinct tyrosinase activity (red with p-cresol). As with tyrosine, the exceptions were the strains He8, He9, He12 and He14, which did not show any positive reaction. In some cases, after 12 hours of incubation the reaction intensity was stronger than after 1 hour, in other cases a positive tyrosinase reaction could be observed only after 12 hours. Generally, temperature treatment increased the intensity of the tyrosinase reaction.

3. Quantitative enzyme determination in liquid culture

Culture filtrate

Except for strains He1 and He6, the heterokaryotic strains investigated showed laccase activity with DMOP. Greatest enzyme activity was found in the strains He5 (0.98 E/ml), He12 (0.85 E/ml) and He14 (0.70 E/ml). Strains with white fruitbodies (colour intensity 1–3) had maximum laccase activity in the culture filtrate of only 0.6 E/ml. Except for the strains He7, He11, He15, strains with yellow and brown fruitbodies (colour intensity 4–6) had a laccase activity in the culture filtrate of more than 0.5 E/ml (Fig. 7).

Homokaryotic strains had no or only very low laccase activity (not higher than 0.10 E/ml).

Mycelial extract

Laccase activity in the mycelial extracts was usually stronger than in culture filtrates. The majority of the strains investigated showed laccase activity with DMOP. Only strain He1 did not possess laccase activity. The highest laccase activity was found in strains He14 (1.25 E/ml), He7 (1.05 E/ml) and He12 (1.00 E/ml). Except for strain He10, the strains with white fruitbodies (colour intensity 1–3) had a laccase activity in the culture filtrate of up to 0.6 E/ml. With exception of strain He4, the strains with yellow-and brown fruitbodies (colour intensity 4–6, Fig. 7) had a laccase activity in culture filtrate of more than 0.6 E/ml.

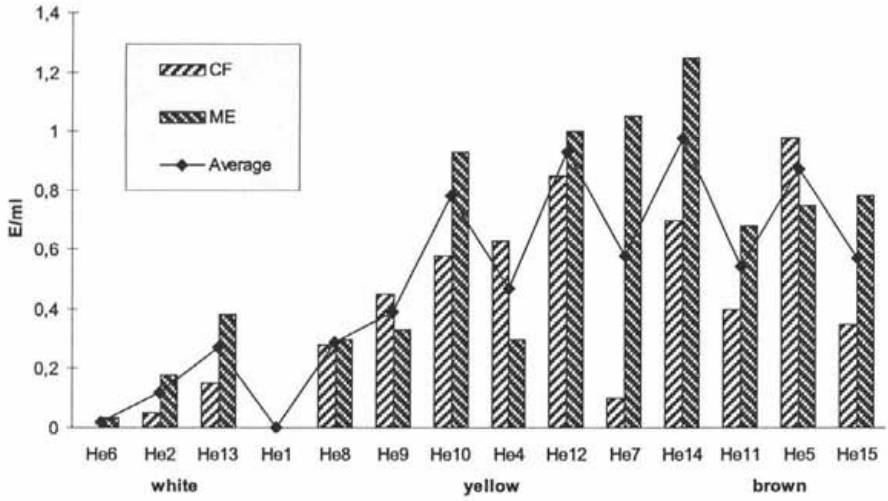


Fig. 7. Correlation between fruitbody colour (arranged for increasing colour intensity: white –> yellow –> brown) and laccase activity of *H. erinaceus* in liquid culture. The line indicates the mean of laccase activity of the culture filtrate(CF) and the mycelial extract (ME).

DISCUSSION AND CONCLUSION

Laccase activity with guajacol as substrate was determined in both agar medium and liquid culture. The results obtained on the agar medium correspond generally with those in liquid culture. Qualitative laccase determinations in agar medium and liquid culture correspond with the quantitative determination of laccase activity with DMOP. An atypical colour of the reaction between laccase and guajacol can be explaned where a pink colour can indicate an early or weak reaction, grey may be the result of (black) precipitated melanin as final reaction product.

Temperature treatment (10 min. at 60 °C.) of enzyme solutions did not destroy the laccase. It may be speculated that the laccase of *H. erinaceus* is more thermostable than in other fungi (Molitoris 1976).

No positive tyrosinase reactions were observed in young colonies on agar medium and in the culture filtrate of liquid cultures. An explanation for this could be the intracellular nature of this enzyme. Another possibility is that this enzyme is produced (and released) only in a later stage of cultivation. Late production of

tyrosinase has been shown in a number of fungi, e.g. by Turner (1974) who found that in *Agaricus bisporus* laccase predominated in the vegetative stage, whereas tyrosinase was found later during fruitbody development. The conditions in our experiments (7 days old liquid cultures) did not lead to the secretion or release of tyrosinase from the mycelium into the culture medium. No correlation between the qualitative tyrosinase tests and the colour of *H. erinaceus* fruitbodies was found. These results for *H. erinaceus* contradict the data obtained for fruitbodies of the white button mushroom *A. bisporus* where it was shown that the tyrosinase is responsible for browning of the fruitbodies resulting in a lower quality of this mushroom (Burton 1988, Rama et al. 1995, Jolivet et al. 1998). A quantitative determination of tyrosinase activity in fruitbodies could be a good method for determining promising strains of *H. erinaceus* with white, well-formed fruitbodies.

In most cases a close correlation between presence and activity of laccase and the colour of fruitbodies of *H. erinaceus* strains was found. However, strains He7, He11 and He15 with brown fruitbodies did not show high laccase activity in the culture filtrate (Fig. 5) although they produced high laccase activity in the mycelial extract. In contrast, strain He4, with brown fruitbodies, showed low laccase activity in the mycelial extract, but had relatively high laccase activity in the culture filtrate. It may be that strains of *H. erinaceum* have different rates of intracellular laccase production and release of this enzyme into the culture medium. This has been shown by Prillinger and Molitoris (1979) for strains of *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. where the secretion of laccase was shown to be under genetic control.

For production of white fruitbodies of *H. erinaceus* strains are recommended where laccase activity in liquid culture (culture filtrate or mycelial extract) is below 0.6 E/ml (in this study strain He1, He2, He6, He8, He9 and He13). An easier way to determine laccase activity would be the use of qualitative tests with guajacol in agar medium. Strains with low overall laccase activity showed no or only weak enzyme reaction in this test. Four strains of *H. erinaceus* with no or weak laccase reaction in qualitative tests (He1, He2, He6 and He13) produced fruitbodies of good quality in cropping experiments. Strains He1 and He2 showed both also rapid mycelial growth and a high yield of fruitbodies (Kirchhoff 1996). The latter two strains we therefore recommend for commercial cultivation.

Determination of the presence of laccase during growth on agar media and/or in liquid culture may constitute a good method to select better strains of *H. erinaceum* more suitable for commercial use. These strains should have good growth characteristics and a first grade fruitbody quality. The enzyme tests could also be important in selection and breeding experiments. In future experiments

for production of new strains that produce white, and thus high grade fruitbodies, the use of homokaryons with low laccase activity could reduce the number of trials and necessary investigations and thereby avoid expensive and time-consuming fruitbody tests.

ACKNOWLEDGEMENTS

The authors thank Ch. Thielke, Bovenden, for isolation and supply of homokaryotic strains used in this investigation, and I. Lauer and I. Kurchenko, Regensburg, for help in the experiments. Thanks are also due to S. T. Moss, Portsmouth, for critically reading the English text.

REFERENCES

BURTON K. S. (1988): The effect of pre- and post-harvest development on mushroom tyrosinase. – J. Hort. Sci. 63: 255–260.

JOLIVET S., ARPIN N., WICHERS H. J. and PELLON, G. (1998): Agaricus bisporus browning: a review. – Mycol. Res. 102 (12): 1459–1483.

KIRCHHOFF B. (1996): Biotechnological Investigations of Hericium erinaceum (Bull.: Fr.) Pers. – In: Rouse D. J. (ed.), Bag-Log Cultivation to Increase Yield. Mushroom Biology and Mushroom Products. – Proceeding of the Second International Conference.: p. 401–406. University Park, Pennsylvania, June 9–12.

MOLITORIS H. P. (1976): Die Laccasen des Ascomyceten Podospora anserina. Beiträge zur Kenntnis von Struktur und Funktion eines Systems multipler Enzyme. – Bibl. Mycol. 52, 81 pp. J. Cramer, Vaduz.

MOLITORIS H. P. and SCHAUMANN K. (1986): Physiology of marine fungi. A screening program for marine Fungi. – In: Moss S. T. (ed.), The biology of marine fungi. Cambridge Univ. Press: p. 35–47., Cambridge.

PETERS E. (1997): Zum Antrag des BDC: Gemeinsame EU-Qualitätsnormen für Champignons. – Der Champignon 5: 203–205.

PRILLINGER H. and MOLITORIS H. P. (1979): Genetic analysis in wood-decaying fungi. I. Geneticvariation and evidence for allopatric specitation in Pleurotus ostreatus using phenoloxidase zymograms and morphological criteria. – Physiol. Plant. 46: 265–277.

RAMA T. K., BURTON S. and VINCENT J. F. V. (1995): Changes of the surface texture of mushrooms during postharvest storage. – In: T. J. Elliott (ed.), Science and Cultivation of Edible Fungi. Balkema: p. 729–732, Rotterdam.

TURNER E. M. (1974): Phenoloxidase activity in relation to substrate and development stage in the mushroom Agaricus bisporus. – Trans. Brit. Mycol. Soc. 63: 541–547.

CZECH MYCOL. 52 (3), 2000

Effects of various forestry operations on the fungal flora of fir woods – first results

ELENA SALERNI, ANGELA LAGANÀ, CLAUDIA PERINI
and VINCENZO DE DOMINICIS

Dipartimento di Scienze Ambientali, Università degli Studi di Siena,
Via P. A. Mattioli, 4 – 53100 Siena (Italy)

Salerni E., Laganà A., Perini C. and De Dominicis V. (2000): Effects of various forestry operations on the fungal flora of fir woods – first results. – Czech Mycol. 52: 209–218

The first results of the effects of some parameters on the composition of the fungal flora in *Abies alba* Miller woods are reported. Medium thinning and removal litter seems to have contributed to a slight increase in species diversity. Moreover, the results suggest that this type of study should be continued and extended to other areas, to obtain a large amount of data.

Key words: Species diversity, macromycetes, forestry operations, fir woods.

Salerni E., Laganà A., Perini C. a De Dominicis V. (2000): Vlivy různých lesnických zásahů na houbovou flóru jedlových lesů – první výsledky. – Czech Mycol. 52: 209–218

Předkládají se první výsledky vlivu některých parametrů na skladbu mykoflóry jedlových lesů *Abies alba*. Střední probírka lesního porostu a odstranění opadu se zdá přispívat k slabému nárůstu diversity houbových druhů. Nadto výsledky ukazují, že tento typ studia by měl pokračovat a měl by se rozšířit do dalších oblastí, aby se získalo větší množství dat.

INTRODUCTION

A rapid decline in many species of macrofungi[1)] has recently been reported from many parts of Europe (Arnolds 1987; Fellner and Soukup 1991; Schlechte 1991). The conservation and restoration of fungal biodiversity therefore becomes a major task for mycologists and naturalists.

Forestry operations can be regarded as ecosystem manipulation, or in other words actions aimed at altering certain ecological factors in a controlled and controllable way. Practical indications for more natural forest management can be obtained from a comparison of topsoil responses in permanent control areas and areas subject to various management treatments, using tree growth parameters (increase in height, tree base area, etc.) and ecosystem parameters (biodiversity, bioindicators etc.)

In this study we evaluated the effect of parameters such as tree cover and litter on the composition of the fungal flora of *Abies alba* woods.

[1)] By macrofungi we mean all fungi that produce fruitbodies visible to the naked eye, with dimensions greater than one millimetre (Arnolds 1981).

STUDY AREA

The study area was at Montarioso (Abbadia San Salvatore, Siena – Italy) in forests owned by the Mt. Amiata Mountain Community, at an altitude of 1000–1100 m. Mt. Amiata has an altitude of 1738 m and is an isolated outcrop in southern Tuscany. It consists of volcanic rocks deposited on allochthonous substrates of Cretaceous and early Cenozoic Ligurian facies (Giannini et al. 1972). The volcanic rocks can be distinguished on the basis of petrographic characteristics and altitude. The study area is in the petrographic province of quartz porphyrites of ignimbrites (Carta Geologica d'Italia, Foglio 129).

The area is situated in climate belt A (perhumid), with Im > 100, water surplus 800–900 mm, water deficit < 100 mm and potential evapotranspiration < 650 mm (Carta Climatica della Toscana centro-meridionale, Barazzuoli et al. 1993). Mean annual temperature is less than 10 °C and mean annual rainfall exceeds 1400 mm.

The forest in the study area was planted about 33 years ago and consists prevalently of *Abies alba*, with a minority of *Picea abies*, clusters of *Pinus* spp. and a few broad-leaved trees such as *Acer monspessulanum* L., chestnut and cherry.

METHOD

The study area (7200 m^2) was divided into six 1200 m^2 plots, in four of which the trees were thinned. In each plot, 12 subplots of 250 m^2 were marked out as buffer areas (4200 m^2) to evaluate the effects of thinning and litter removal individually (Fig. 1).

1. Thinning

Analysis of the structure and distribution of the tree showed marked irregularities and dissimilarities in the study area. Since this could have prejudiced the study, as much uniformity as possible was sought for plots undergoing the same type of operation. The parameter on which uniformity was based was tree base area (G).

Thinning was carried out as follows: 20% of trees were cut in plots 2 and 6; 40% of trees were cut in plots 1 and 4; plots 3 and 5 were left as controls.

2. Litter removal

In six of the 12 subplots (total area 1500 m^2), litter and herbaceous layer were removed manually.

The data reported here refer to one year; relevés were made every 2 weeks. All carpophores of macrofungi were recorded and counted by the method of Arnolds (1981) modified for Mediterranean environments by Perini and Barluzzi (1987).

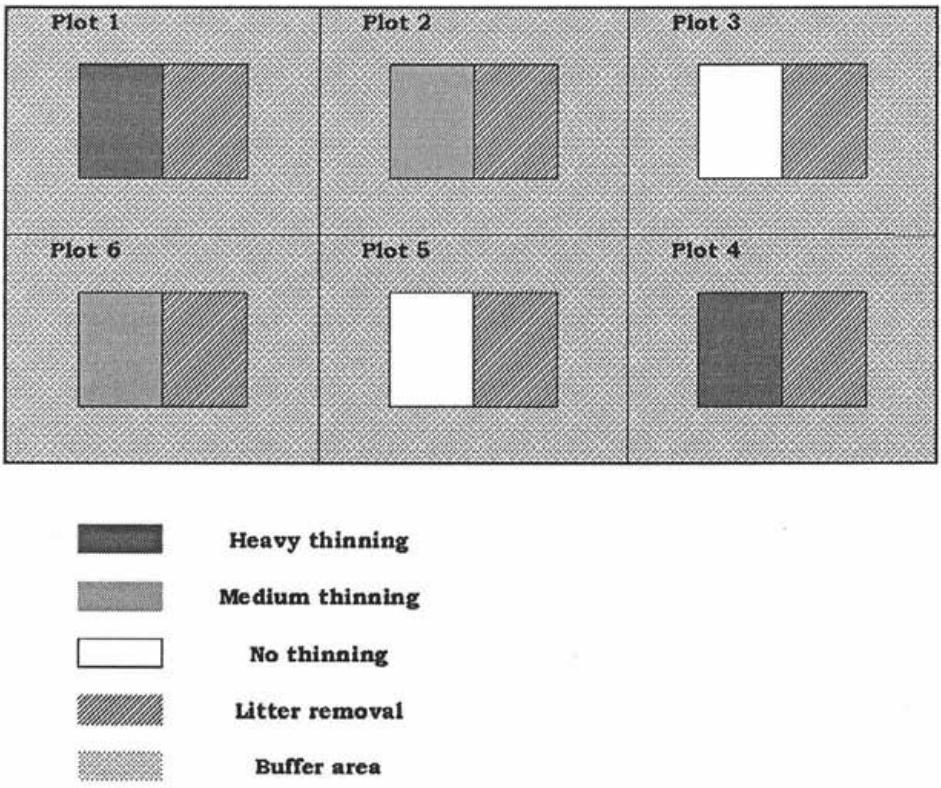


Fig. 1. Map of the studying area.

Nomenclature follows the Dutch fungal check-list (Arnolds et al. 1995); taxa not in this list appear with an asterisk followed by the source of nomenclature in brackets. Authors' names are abbreviated according to Brummit and Powell (1992).

Samples were deposited in the Herbarium Universitatis Senensis (SIENA).

The species were attributed to trophic groups (M = mycorrhizal species; Sh = humicolous saprotrophs; Sl = litter saprotrophs; Sw = lignicolous saprotrophs) according to Arnolds et al. (1995) and personal observations. It is not always easy to determine whether a species lives as a parasite, symbiont or saprotroph, or what the preferred substrate of the latter is. In such cases, the species was attributed to the group that seemed most probable according to the present state of the art.

Table 1. Results

G. t	SPECIES	Plot 1		Plot 2		Plot 3		Plot 4		Plot 5		Plot 6	
	Thinning	heavy		medium		control		heavy		control		medium	
	Litter	P	A	P	A	P	A	P	A	P	A	P	A
Sh	Agaricus sylvaticus Schaeff.										5		
M	Amanita gemmata (Fr.) Bertillon			3									1
M	Amanita muscaria (L.: Fr.) Lam.	5		2							2	3	1
M	Amanita pantherina (DC.: Fr.) Krombh.	1	1		1								
M	Amanita rubescens Pers.: Fr.	32		16	5	6	3	18	2	4	3	30	35
M	Astraeus hygrometricus (Pers.: Pers.) Morgan		12		1		2						
M	Boletus badius (Fr.:Fr.) Fr.	3				5		2	1	14			12
M	Boletus edulis Bull.: Fr. ss. str.	11	3	2	11	3	13	1	2	12	6	6	12
M	Chalciporus piperatus (Bull.:Fr.) Sing.		5	1			1						
Sh(M)	Clavulina coralloides (L.: Fr.) J. Schröt. ss. str.		10	10	15								
Sh(M)	Clavulina rugosa (Fr.) J. Schröt.		5	10									10
Sl	Clitocybe nebularis (Batsch: Fr.) P. Kumm.	1		3	1	70	3	50				10	35
Sl	Clitocybe obsoleta (Batsch) Quél. * (Moser 1983)			1									
Sl	Clitocybe phaeophthalma (Pers.) Kuyper			80		130		2		75	100		
Sh(M?)	Clitopilus prunulus (Scop.: Fr.) P. Kumm.	142	49	251	10	70	48	30	2	70	92	13	63
Sl	Collybia butyracea (Bull.: Fr.) P. Kumm.	11		4		14	2	3		12		3	
Sl	Collybia confluens (Pers.: Fr.) P. Kumm.		20	6	70						30		
Sl	Collybia dryophila (Bull.: Fr.) P. Kumm.			2				1				20	
Sl	Collybia erythropus (Pers.: Fr.) P. Kumm.				23						1		
Sh	Conocybe tenera (Schaeff.: Fr.) Fayod	1			2								
M	Cortinarius praestigiosus (Fr.) M.M.Moser	1		21								2	1
M	Cortinarius variecolor (Pers.:Fr.) Fr.			1									
Sw	Crucibulum crucibuliforme (Scop.) V. S. White											2	
Sh	Cystoderma terrei (B. et Br.) Harmaja		2									4	
Sh	Entoloma juncinum (Kühner et Romagn.) Noordel.									1			
Sh	Entoloma nitens (Velen.) Noordel.					4							
Sw	Galerina marginata (Batsch) Kühner ss. str.			5	1		1			1		2	
M	Gomphidius glutinosus (Schaeff.: Fr.) Fr.	2											
M	Inocybe asterospora Quél.				2	1							
M	Inocybe cfr. fuscidula Velen. var. fuscidula										5		
M	Inocybe cfr. oblectabilis Britz.						3						
M	Inocybe dulcamara (Pers.) P. Kumm. ss. str.				1	1	12			2	7		
M	Inocybe flocculosa (Berk. —›) Sacc.	1		1									
M	Inocybe fuscidula Velen. var. fuscidula			4		25							10
M	Inocybe geophylla (Fr.: Fr.) P. Kumm. var. lilacina (Peck) Gillet	10	13	5	7	10		3	11		6		

Table 1. Results

G. t	SPECIES	Plot 1		Plot 2		Plot 3		Plot 4		Plot 5		Plot 6	
	Thinning	heavy		medium		control		heavy		control		medium	
	Litter	P	A	P	A	P	A	P	A	P	A	P	A
M	Inocybe geophylla (Fr.: Fr.) P. Kumm.	5		2							1	1	
M	Inocybe mixtilis (Britzelm.) Sacc.			1	2		3			4			
M	Inocybe rimosa (Bull.: Fr.) P. Kumm.						1						
M	Inocybe sindonia (Fr.) P. Karst.	40	51	240	4	29	32			212	36	6	18
M	Inocybe soluta Velen.		3										
M	Inocybe splendens R.Heim										8		
M	Inocybe whitei (Berk. et Broome) Sacc.	26	2	33	4		10		1	14	1	20	50
M	Laccaria amethystina (Huds. →) Cooke		2			54		6			12		
M	Laccaria laccata s. l.	70	12	213	13	40	1	10		21		2	12
M	Lactarius salmonicolor R. Heim & Leclair	21		8		5				4		1	
Sh	Lycoperdon molle Pers.:Pers.	1						7					
Sh	Lycoperdon perlatum Pers.: Pers.	60	4	230	45	30			2	57	2	49	106
Sh	Macrolepiota excoriata (Schaeff.: Fr.) Wasser						1						
Sh	Macrolepiota procera (Scop.: Fr.) Singer	5	3	8		5				4			
Sw	Micromphale foetidum (J. Sowerby: Fr.) Singer		20										
Sw	Mycena galericulata (Scop.: Fr.) Gray				3								
Sl	Mycena galopus (Pers.: Fr.) P. Kumm.		1										
Sl/Sw	Mycena pelianthina (Fr.: Fr.) Quél.		20			73		34				20	
Sl	Mycena pura (Pers.: Fr.) P. Kumm.	52	14	20	20	12	1	3		11		86	6
Sw	Mycena renati Quél.		5	2									
Sl	Mycena rosea (Bull.→) Gramberg								5				
Sw	Mycena vitilis (Fr.) Quél.			1									
M?(Sh)	Otidea abietina (Pers.) Fuck.			2									
M(Sh)	Otidea alutacea (Pers.) Massee	4											
Sw	Pluteus cervinus (Schaeff.) P. Kumm.			1									
Sw	Psathyrella candolleana (Fr.: Fr.) Maire						1						
Sw	Psilocybe sublateritia (Fr.)		20										
Sh	Rhodocybe cfr. parilis (Fr.: Fr.) Singer												1
Sh	Rhodocybe gemina (Fr.) Kuyper et Noordel.								4			7	
M	Russula cavipes Britzelm.			1		1	2					1	
M	Russula fragilis (Pers.: Fr.) Fr. ss. str.		1										
M	Russula mustelina Fr.												1
M	Suillus granulatus (L.: Fr.) Roussel							1					
M	Tricholoma portentosum (Fr.: Fr.) Quél.											2	4
Sw	Tricholomopsis rutilans (Schaeff.: Fr.) Singer		15	1	3				1			3	
Sw(P?)	Xerula radicata (Relhan: Fr.) Dörfelt						1						

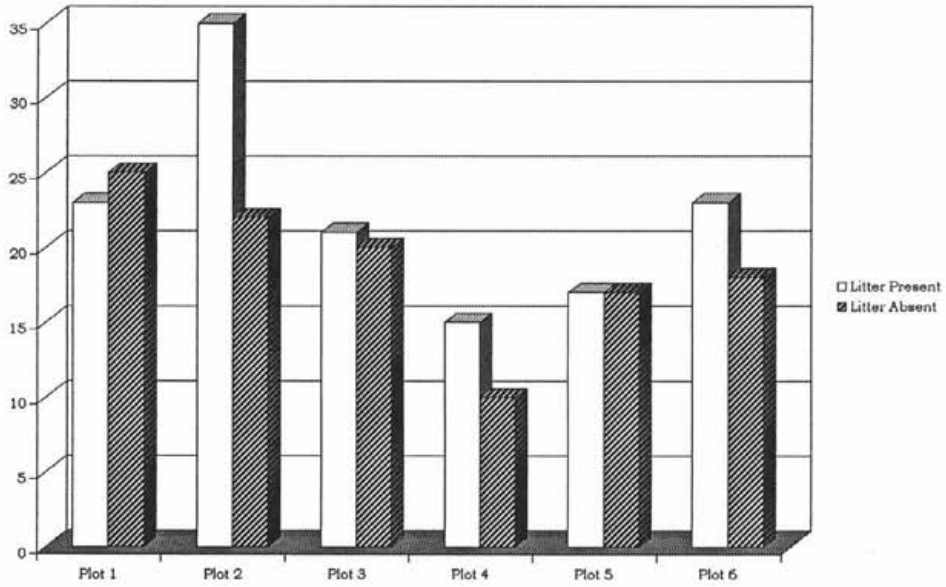


Fig. 2. Number of species recorded in each plot.

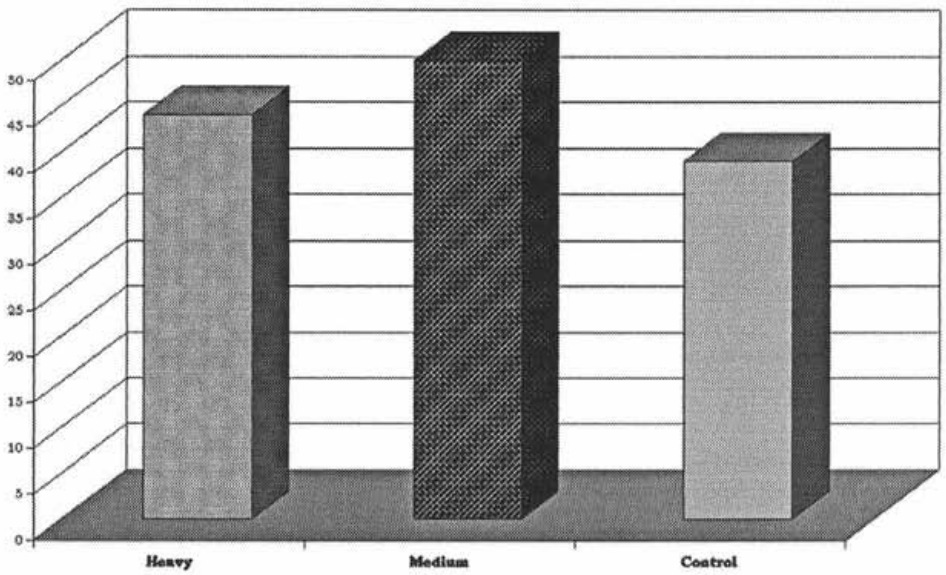


Fig. 3. Mean number of species in plots subject to different degrees of thinning.

Results and Discussion

The results obtained in this first observation period are shown in Table 1. The trophic group is indicated in the first column. The number of fruit bodies observed in each subplot is also indicated. The letters P (present) and A (absent) indicate presence or absence of litter in each subplot.

A total of 71 fungal species were identified: 35 mycorrhizal, 14 humicolous saprotrophs, 11 litter saprotrophs, 11 lignicolous saprotrophs, 0 parasites. Many of the taxa have a broad ecological range. For example *Amanita rubescens*, *Inocybe flocculosa*, *I. rimosa*, *Laccaria laccata*, *Lycoperdon perlatum*, *Mycena galopus* and *M. pura* are found indifferently in conifer and broadleaf forests (Bon 1979; Orsino and Traverso 1986; Stangl 1991; Thoen 1970–71). There were also species linked to *Abies alba*, for example *Lactarius salmonicolor* and *Russula cavipes*, which are reported to be symbionts of this tree (Barkman 1963; Šmarda 1973; Stangl 1991; Thoen 1980) and regarded by Perini et al. (1995) as differential of the fir woods of Mt. Amiata with respect to those of the Casentino.

Species linked to broadleaf forests, such as *Entoloma nitens* and *Inocybe splendens* (Noordeloos 1992; Stangl 1991), may appear due to the presence of *Castanea sativa* Miller, *Acer monspessulanum* L. and *Prunus avium* L. in the area.

The anomalous find of *Astraeus hygrometricus*, a species linked to thermic environments and not found in mycocoenological studies in fir woods by Perini et al. (1995), may be explained by particular microclimatic conditions. In fact, the richest fruiting of this species (12 carpophores) was observed in subplot 1P (heavy thinning with litter removal) which has higher insolation than the other subplots because it is situated at a site where two tracks meet.

The species that produced the greatest number of carpophores was *Clitopilus prunulus* with 840 carpophores over all 12 subplots. The literature indicates that this species is ubiquitous (Darimont 1973; Moser 1983). Also the abundance of *Inocybe sindonia* (668 fruit bodies) in 10/12 subplots (absent in 4P and 4A) is noteworthy. This mycorrhizal species generally grows under conifers (Stangl 1991).

Figure 2 shows the number of species found in each subplot. Subplot 2P (minor thinning without litter removal) had the highest diversity (35 species) and subplot 4P (major thinning without litter removal) had the least (17 species).

Considering the effect of the different operations (Fig. 3), the largest number of species (50) was found in plots undergoing minor thinning. Major thinning did not seem to increase fruiting; in fact, in these plots only 44 species were found, but this number was greater than that of control plots, in which 39 species were found.

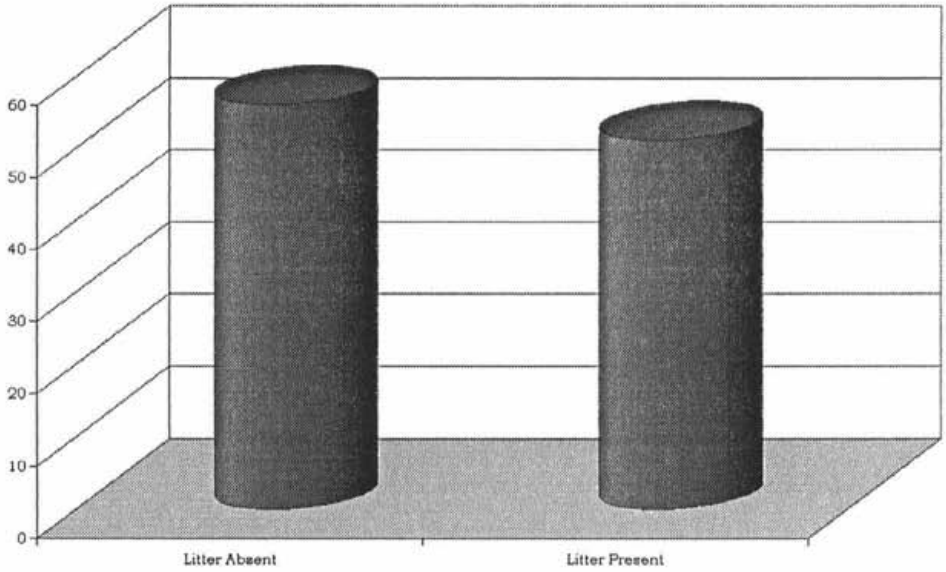


Fig. 4. Number of species in plots with and without litter.

Removal of litter seems to have contributed to a slight increase in species diversity. In subplots in which litter was removed, a total of 56 species were found, whereas in the others there were only 51 (Fig. 4).

Although these results concern only one year of research, they already give indications for forest management if the aim is an increase in diversity of macrofungal flora. The results suggest that this type of study should be continued and extended to other areas, to obtain a large amount of data. In this way, information will be gained on the autoecology of individual species and a comparison can be made with similar data from other environments (Termorshuizen 1990; Keizer and Arnolds 1993; Baar and Kuyper 1993; Keizer 1993). This will provide a relatively complete picture of how to manage and conserve the habitats in which these important forest products grow.

ACKNOWLEDGMENTS

We thank Piergiuseppe Montini, Walter Pascucci, Sauro Visconti, Sergio Antoni, Carlo Saliola and all members of the groups of Castiglion d'Orcia and Radicofani for their collaboration.

REFERENCES

ARNOLDS E. (1981): Ecology and coenology of macrofungi in grassland and moist heathland in Drenthe, the Netherlands. Part 1. Introduction and Synecology. – Bibl. Mycol. 83: 1–410.

ARNOLDS E. (1987): Decrease of ectomycorrhizal fungi in the Netherlands in relation to air pollution. – In: Fellner R. (ed.), Ekologie mykorrhiz a mykorrhizních hub. Imise a mykorrhiza. DT CSWTS, Pardubice. 72–81.

ARNOLDS E., KUYPER TH. W. and NOORDELOOS E. M. (eds.) (1995): Overzicht van de paddestoelen in Nederland. (Wijster, Nederlandse Mycologische Vereniging). – 872 p. Wijster.

BAAR J. and KUYPER TH. W. (1993): Litter removal in forests and effect on mycorrhizal fungi. In D. N. Pegler, L. Boddy, B. Ing and P. M. Kirk (eds.) Fungi of Europe: investigation, recording and conservation. Royal Botanic Gardens, p. 275–286, Kew.

BARKMAN J. J. (1963): Over de standplaats van Cornus suecica in Oostfriesland. – Gorteria 9: 100–105

BARAZZUOLI P., GUASPARRI G. and SALLEOLINI M. (1993): Il Clima. – In: Giusti F. (ed.), La storia naturale della Toscana meridionale, p. 141–171, Cinisello Balsamo.

BON M. (1979): Macromycètes des saulaies fangeuses du bassin inferieur de la Somme. – Doc. Phytosoc. 6: 75–86.

BRUMMITT R. and POWELL C. E. (eds.) (1992): Authors of plant names. – 732 p. Kew.

DARIMONT F. (1973): Recherches mycosociologiques dans les forêts de Haute Belgique. – Mèm. Inst. Roy. Sc. Nat. Belg. 170: XIV + 220 p.

FELLNER R. and SOUKUP F. (1991): Mycological monitoring in the air-polluted regions of the Czech Republic. – Com. Inst. For. Cec. 17: 125–137.

GIANNINI E., LAZZAROTTO A. and SIGNORINI R. (1972): Lineamenti di Geologia della Toscana meridionale. – Rend. Soc. Ital. Miner. Petr. 27: 33–168.

KEIZER P. J. (1993): The ecology of macromycetes in roadside verges planted with trees. Thesis, 290 p. Wageningen.

KEIZER P. J. and ARNOLDS E. (1993): Succession of ectomycorrhizal fungi in roadside verges planted with common oak (Quercus robur L.) in Drenthe, the Netherlands. – In: Keizer P. J. The ecology of macromycetes in roadside verges planted with trees. Thesis, p. 129–149. Wageningen.

MOSER M. (1983): Kleine Kryptogamenflora, Band IIb/2: Die Röhrlinge und Blätterpilze. – 533 p. Stuttgart/New York.

NOORDELOOS M. E. (1992): Fungi Europei: 5. Entoloma s.l. – 760 p. Biella, Saronno.

ORSINO F. and TRAVERSO M. (1986): Ricerche sulla flora micologica della Liguria. I macromiceti della "Pietra di Finale" (Liguria occidentale). – Webbia, 40: 301–322.

PERINI C. and BARLUZZI C. (1987): Considerazioni su aspetti metodologici nello studio delle micocenosi in vari tipi di vegetazione della Toscana centro-meridionale. – In: Pacioni G. (ed.), Studies fungal communities. p. 73–94. L'Aquila.

PERINI C., BARLUZZI C., COMANDINI O. and DE DOMINICIS V. (1995): Mycocoenological research in fir woods in Tuscany (Italy). – Doc. Mycol. 25: 317–336.

SCHLECHTE G. (1991): Zur Struktur der Basidiomyzeten. Flora von unterschiedlich immissionsbelasteten Waldstandorten in Südniedersachsen unter besonderer Berücksichtigung der Mycorrhizabildung. Jahn u. Ernst, p. 224. Hamburg.

ŠMARDA F. (1973): Die Pilzgesellschaften einiger Fichtenwälder Mährens. – Acta Sc. Nat. Brno 7(1): 1–44.

STANGL J. (1991): Guida alla determinazione dei funghi: 3. Inocybe. – 437 p. Saturnia, Trento.

TERMORSHUIZEN A. J. (1990): Decline of carpophores of mycorrhizal fungi in stands of Pinus sylvestris in the Netherlands. Thesis. – 128 p. Wageningen.

THOEN D. (1970): Etude mycosociologique de quelques associations foretières des districts Picardo-Brabançon, mosan et ardennais de Belgique. – Bull. Rechn. Agronom. Gembloux 5: 309–326.

THOEN D. (1971): Etude mycosociologique de quelques associations foretières des districts Picardo-Brabançon, mosan et ardennais de Belgique. – Bull. Rechn. Agronom. Gembloux 6: 215–243.

THOEN D. (1980): Contribution a l'inventaire des mycocénoses des pessières naturelles des Vosges (France). – Bull. Soc. Linn. de Lyon. 49(6): 379–391.

CZECH MYCOL. 52 (3), 2000

Root mycoflora of pepper (Capsicum annuum) antagonistic to Verticillium dahliae

YOUSSUF A. M. H. GHERBAWY and HANSJÖRG PRILLINGER

Institute of Applied Microbiology, University of Agricultural Science, Vienna, Austria

Gherbawy Y. A. M. H. and Prillinger H. (2000): Root mycoflora of pepper (Capsicum annuum) antagonistic to Verticillium dahliae – Czech Mycol. 52: 219–226

Thirty-two species belonging to 19 genera of fungi were collected from 30 soil samples from the rhizosphere of pepper plants. The fungal colonies were characterised using classical morphological methods following identification keys. The most frequently isolated fungi were *Chaetomium globosum, Fusarium oxysporum, Gliocladium roseum, Mucor racemosus, Myrothecium verrucaria, Penicillium aurantiogriseum, P. expansum* and *Trichoderma harzianum*. The crude culture filtrate of *Verticillium dahliae* at 100% concentration caused sharp decrease in pepper seed germination. *Chaetomium globosum, Gliocladium roseum, Myrothecium verrucaria,* and *Trichoderma harzianum* produced a metabolite that retarded radial growth of *Verticillium dahliae*.

Key words: Frequency of root fungi, *Verticillium dahliae*, *Chaetomium globosum Gliocladium roseum, Myrothecium verrucaria, Trichoderma harzianum*, pepper seed germination.

Gherbawy Y. A. M. H a Prillinger H. (2000): Mykoflóra kořenů papriky roční (Capsicum annuum), působící antagonisticky proti Verticillium dahliae. – Czech Mycol. 52: 219–226

Bylo odebráno 30 půdních vzorků z rhizosféry papriky, kde bylo nalezeno 32 druhů hub, které patřily k 19 rodům. Kolonie byly určeny klasickými morfologickými metodami za použití určovacích klíčů. Nejčastěji izolovanými druhy byly *Chaetomium globosum, Fusarium oxysporum, Gliocladium roseum, Mucor racemosus, Myrothecium verrucaria, Penicillium aurantiogriseum, P. expansum* a *Trichoderma harzianum*. Surový 100 % filtrát čisté kultury *Verticillium dahliae* působil prudké snížení klíčivosti semen papriky. Druhy *Chaetomium globosum, Gliocladium roseum, Myrothecium verrucaria* a *Trichoderma harzianum* produkovaly metabolit, který retardoval radiální růst *Verticilium dahliae*.

INTRODUCTION

Verticillium dahliae Kleb. (Fungi imperfecti, Moniliales) is a soil-borne fungus with a world-wide distribution. It caused wilting, stunting and early dying of pepper plants in Israel, resulting in a 22 % reduction in yield (Tsror et al. 1998). It has a wide host range and causes tracheomycosis of many economic plants such as tomato (Osman et al. 1991; Dobinson et al. 1996; Madhosingh 1996; Li et al. 1996; Gold and Robb 1996).

Strategies for the biological control of fungal pathogens are commonly based on direct antagonism like antibiosis or hyperparasitism. Retardation of epidemiological spread offers another promising strategy (Lennartz et al. 1998). Competition

from antagonists for nutrients and space can result in reduced pathogen development, especially in reduced spore production (Lennartz et al. 1998).

Antagonistic microorganisms are able to suppress growth and development of phytopathogenic fungi (Huber et al. 1987; Phillipp 1988). Disease suppression and use of antagonistic microorganisms as biocontrol agents depends on their ability to both colonise roots and produce substances inhibitory to the pathogen (Thomashow et al. 1990). Several antagonistic microorganisms to *Verticillium* wilt have been isolated, most of these fungi, e.g. *Aspergillus flavus, Gliocladium roseum, Penicillium griseofulvum, P. vermiculatum, Pythium* sp., *Trichoderma harzianum* and *T. viride* (Keinath et al. 1981; Ghaffer 1988; Zeise 1990; Osman et al. 1991).

The principal aim of this study was to test fungal species isolated from roots of pepper plants as biological agents for the control of *Verticillium dahliae* causing wilt disease of many crops.

MATERIALS AND METHODS

Estimation of rhizosphere fungi

This part of the study was performed to isolate some fungi that are known to be biological agents and grow saprophytically in pepper field soil also. Samples of rhizosphere soil were collected from the root system of 30 healthy pepper plants growing at a private farm in Stockerau near Vienna City (Austria). From these soil samples, fungi were isolated on potato – dextrose agar (PDA) by the dilution plate method as described by Abdel-Hafez et al. (1990a). Five replications were made for each sample and the developing fungi were identified, counted and the numbers were calculated per g dry soil.

Effect of some root's mycoflora on radial growth of *Verticillium dahliae*

Verticillium dahliae (Isolate MD 15) previously isolated from pepper in Burgenland, Austria, was used during this study. Cultures of *Chaetomium globosum, Gliocladium roseum, Myrothecium verrucaria* and *Trichoderma harzianum* (previously isolated from pepper roots) were maintained on PDA at 28 °C. Two agar discs (1.2 cm in diameter) from a 1–2 wk old culture medium (Mizuno et al. 1974) of the above mentioned fungi were used for production of the inhibitory metabolite. Four replicate flasks of these liquid cultures were maintained at 27 °C ± 0.2 °C on a rotary shaker at 120 rpm. After a 8-day incubation period, samples were filtered through a 0.45 mm filter to remove hyphae and spores of *Chaetomium globosum, Gliocladium roseum, Myrothecium verrucaria* and *Trichoderma harzianum*. The resulting cell-free culture filtrates were separated into two groups. The first group was autoclaved for sterilisation (sterile), the second remained without sterilisation

(normal). The dilutions of both types of filtrate (normal and sterile), uninoculated medium (broth) and sterile distilled water were incorporated into Czapek's solution.

Effect of culture filtrates of *Verticillium dahliae* on pepper seed germination

Seeds of pepper were sterilised by soaking them in 0.1% mercuric chloride for 2 minutes. They were rinsed several times in sterile distilled water. Sterilised Petri dishes (10 cm in diameter) containing sterilised filter paper were prepared for seed germination. Twenty seeds were germinated in 10 ml of the normal and autoclaved fungal filtrates (100%, 75%, 50% and 25% concentrations), respectively. Control treatments received 10 ml of sterilised distilled water. All plates were incubated at 25 °C for 5 days, after which the germination rate n each treatment was recorded.

RESULTS AND DISCUSSION

Thirty–two species belonging to 19 fungal genera (Table 1) were isolated from 30 rhizosphere soil samples of pepper plants.

The most prevalent genera were *Chaetomium, Fusarium, Gliocladium, Mucor, Myrothecium, Penicillium* and *Trichoderma*. They were recovered from 56.7, 50, 63.3, 60, 63.3 and 66.7% of the total number of samples comprising 8.95, 8.08, 9.96, 7.88, 21.96 and 15.54% of total fungi, respectively. They were represented by *Chaetomium globosum, Fusarium oxysporum, Gliocladium roseum, Mucor racemosus, Myrothecium verrucaria, Penicillium aurantiogriseum, P. expansum* and *Trichoderma harzianum*. Gherbawy and Abdelzaher (1999) isolated *Chaetomium globosum, Fusarium oxysporum, Mucor racemosus, Myrothecium verrucaria, Penicillium aurantiogriseum* and *Trichoderma harzianum* from the rhizosphere of tomato plants in Egypt. They reported that these fungal species were recovered from 10, 35, 30, 40, 20, and 15% of the samples comprising 1.0, 3.0, 1.5, 3.7, 0.5 and 1.3% of total fungi, respectively. Most of the above mentioned species were previously isolated but with different incidences from the rhizosphere of several plants cultivated or grown in many parts of the world (Abou El-Souod et al. 1988; Abdel-Hafez et al. 1990a,b, 1995; Rajendra and Saxena 1991; Abdelzaher et al. 1999). The remaining genera and species were less frequently or rarely isolated (Table 1).

Effect of the culture filtrates of *Verticillium dahliae* on pepper seed germination

The crude filtrate (100%), either autoclaved or not, showed an inhibitory effect on the germination of pepper seeds (Fig. 1). The pepper seed germination rates were 50%, 56%, 53.1% and 59% when allowed to germinate in 25%, 50%, 75%

Table 1. Average total count (ATC), number of isolation cases (NIC), occurrence remarks (OR) and percentage of total count (TC) of various fungal genera and species recovered from 30 rhizosphere soil samples of pepper plants on PDA medium at 28 °C.

Genera & species	ATC	NIC & OR	TC %
Acremonium strictum	15300	10 M	6.7
Alternaria alternata	2500	5 L	1.09
Aspergillus sp.	1570	5 L	0.69
A. flavus	500	3 R	0.22
A . fumigatus	420	1 R	0.18
A. ochraceus	650	2 R	0.28
Chaetomium globosum	20500	17 H	8.95
Cladosporium cladosporioides	3500	6 L	1.53
Fusarium oxysporum	18500	15 H	8.08
Gliocladium roseum	22800	19 H	9.96
Mucor sp.	18040	18 H	7.88
M. circinelloides	1500	8 M	0.65
M. hiemalis	500	4 L	0.22
M. pusillus	440	2 R	0.19
M. racemosus	15600	16 H	6.81
Myrothecium verrucaria	21400	15 H	9.34
Nectria haematococca	7500	8 M	3.27
Nigrospora spherica	360	1 R	0.16
Paecilomyces variotii	450	2 R	0.20
Penicillium sp.	50300	19 H	21.96
P. aurantiogriseum	15000	15 H	6.55
P. brevicompactum	450	4 L	0.20
P. citreonigrum	6000	7 L	2.62
P. citrinum	7500	9 M	3.27
P. corylophilum	300	2 R	0.13
P. expansum	15400	16 H	6.72
P. griseofulvum	350	1 R	0.15
P. montanense	5000	8 M	2.18
P. waksamanii	300	2 R	0.13
Rhizopus stolonifer	3200	5 L	1.4
Stachybotrys chartarum	400	1 R	0.17
Trichoderma harzianum	35600	20 H	15.54
Gross Total counts	229020		
Number of genera	19		
Number of species	32		

Occurrence remarks: H = high occurrence, between 15–30 cases (out of 30); M = moderate occurrence, between 8–14 cases; L = low occurrence, 4–7 cases; R = rare occurrence, 1–3 cases.

and 100% of the normal filtrate of *Verticillium dahliae*. On the other hand, these percentages were 43.3%, 54.1%, 51.1% and 32.1%, in the case of the autoclaved filtrate. Vilich et al. (1998) reported that barley seeds treatments with *Chaetomium globosum* and *C. funicola* resulted in an increase in root fresh weight. They also reported that after seed inoculation and 3 weeks of cultivation, the fungi were re-isolated initially from the roots and later from leaves without causing any symptoms. Khallil and Ammer (1994) reported that sunflower seed germination rates were 0, 55 and 85% respectively, when allowed to germinate in the normal and autoclaved filtrate of *Fusarium solani* and distilled water. Variation of germination rates is probably due to the fungal filtrate effect on either plant germination enzymes or growth hormones.

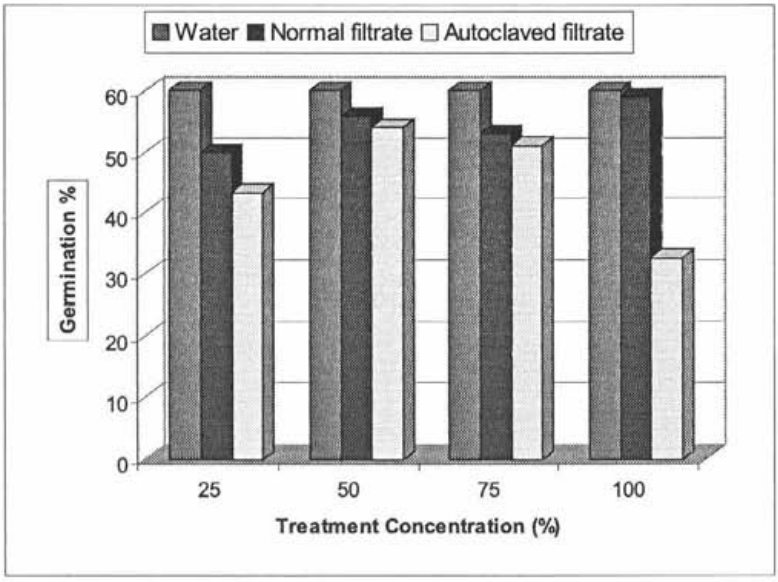


Fig. 1. Effect of *Verticillium dahliae* culture filtrates on the germination rate of pepper seeds.

Effect of root mycoflora on radial growth of *Verticillium dahliae*

The culture filtrate (normal and autoclaved) of all tested fungi showed a great effect on radial growth of *Verticillium dahliae* when they amended with medium. The filtrate of *Chaetomium globoseum* demonstrated the greatest effect in comparison with water and broth. The radial growth of *Verticllium dahliae* was 2.5, 2.3 and 2 cm when the medium was amended with a 25, 50, 100% concentration of the normal filtrate of *Chaetomium globosum,* receptively. On the other hand,

the radial growth was 2.75, 2.4 and 2.2 cm in the case of the autoclaved filtrate. (Fig. 2). Osman et al (1991) reported that filtrates of *Pythium* sp. and *Aspergillus flavus* suppressed radial growth and conidial production of *Verticillium dahliae*. Farvel *et al.* (1987) reported that *Talaromyces flavus* produced a metabolite that not only retarded the radial growth but also killed the sclerotia of *Verticillium dahliae*. This supports the possibility of biocontrol of *Verticillium* wilt of pepper, using other soil inhabiting fungi.

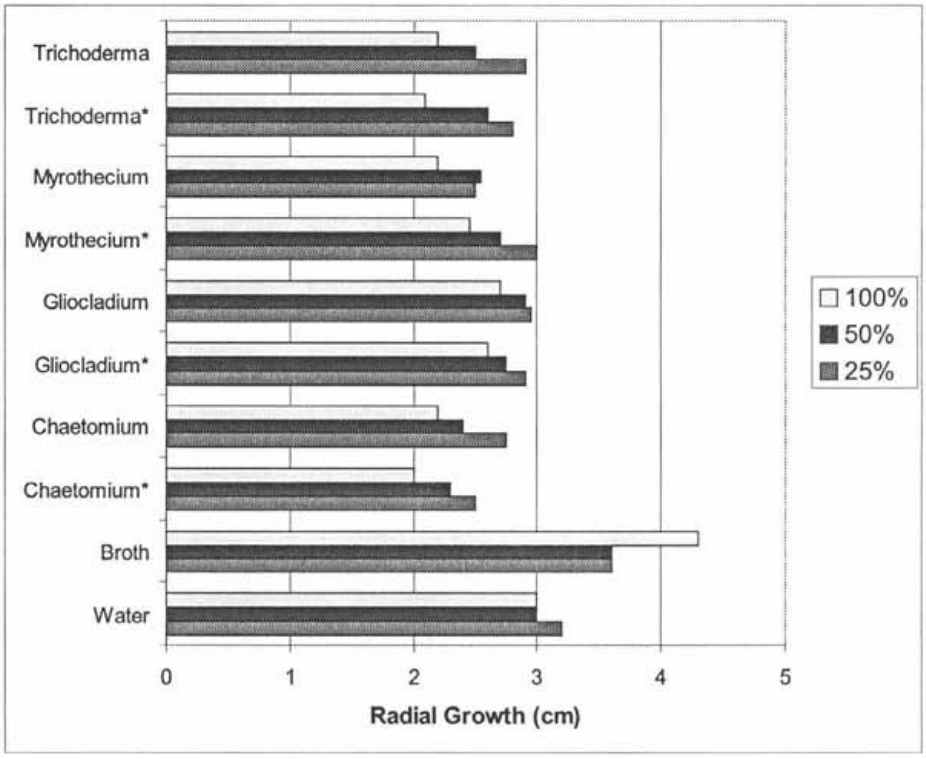


Fig. 2. Radial growth of Verticillium dahliae on Czapek's solution agar amended with 25, 50, 100% dilution of culture filtrate (normal* and autoclaved) of tested fungal species, broth and water.

Vilich et al. (1998) observed that culture filtrates of *Chaetomium globosum* and *C. funicola* inhibited the germination of mildew conidia on solid media in vitro. Mathew and Gupta (1998), during their study on biological control of root rot of French bean caused by *Rhizoctonia solani*, used *Chaetomium globosum*, *Coniothyrium mintans*, *Gliocladium virens*, *Trichoderma hamatum*, *T. harzianum*

and *T. viride*. They reported that *Gliocladium virens* and *Trichoderma harzianum* proved superior to other antagonists in reducing pre-emergence root rot to 6.7% and 13.3% respectively, as compared to 36.7% in the control.

ACKNOWLEDGEMENTS

We wish to thank Dr. K. Lopandic and Mag. J. Kraus for their helpful discussion. This work was supported by ÖAD (Österreichischer Akademischer Austauschdienst).

REFERENCES

ABDEL-HAFEZ A. I. I., ABDEL-HAFEZ S. I. I., MOHAWED S. M. and AHMED T. A. M. (1990a): Fungus flora of air, root and leaf surface of lentil plant cultivated in Upper Egypt. – Bull. Fac. Sci., Assiut Univ. 15(2): 140–47.

ABDEL-HAFEZ S. I. I., MAZEN M. B. and SHABAN G. M. (1990b): Seasonal fluctuations of rhizosphere and rhizoplane fungi of Egyptian wheat plant. – Bull. Fac. Sci., Assiut Univ. 19(1 – D): 173–184.

ABDEL-HAFEZ S. I. I., EL-SAID A. H. M. and GHERBAWY Y. A. M. H. (1995): Seasonal fluctuations of soil and root surface fungi of sugarcane in Egypt.– Bull. Fac. Sci., Assiut Univ. 24(2 – D) 131–151.

ABDELZAHER H. M. A., GHERBAWY Y. A. M. H. and ELNAGHY M. A. (1999): Damping-off disease of ize caused by Pythium deliense Meurs in El – Minia, Egypt and its possible control by some antagonistic soil fungi. – Egyptian J. Microbiology (in press).

ABU EL-SOUOD S. M., EL-SHOURBAGY M. N. and EL-REFAI E. M. (1988): Effect of different growth stages on the rhizosphere and rhizoplane fungi of Thymelaea hirsuta at different localities. – Ann. Microbiol. 38: 15–28.

DOBINSON K. F., TENUTA G. K. and LAZAROVITS G. (1996): Occurrence of race 2 of Verticillium dahliae in processing tomato fields in southwestern Ontario. – Canadian J. Plant Pathology – Revue Canadienne de Phytopathologie. 18: 55–58.

FARVEL D. R., KIM K. K. and PAPAVIZAS G. C. (1987): Viability of Microsclerotia of Verticillium dahliae reduced by a metabolite produced by Talaromyces flavus. – Phytopathology 77: 616–619.

GHERBAWY Y. A. M. H. and ABDELZAHER H. M. A. (1999): Isolation of fungi from tomato rhizosphere and evaluation of the effect of some fungicides and biological agents on the production of cellulase enzymes by Nectria haematococca and Pythium ultimum var. ultimum. – Czech Mycology 51(2–3): 157–170.

GHAFFER A. (1988): Biological control of sclerotial diseases. – In: K. G. Mukerji and K. L. Garg (eds.), Biocontrol of Plant Diseases 1: 153–175.

GOLD J., LEE B. and ROBB J. (1996): Colonization of tomatoes by Verticillium dahliae: Determinative phase II. – Canadian J. Botany – Revue Canadienne de Botanique. 74: 1279–1288.

HUBER J., BOCHOW H. and JUNGE H. (1987): Selektion und biotechnische Herstellung von Kulturlosungen mikrobieller Antagonisten zur Unterdruckung phytopathogener Bodenpilze. – J. Basic Microbiol. 27: 497–503.

KHALLIL A. M. and AMMAR M. M. (1994): Water and terrestrial fungal flora in Misurata (Libya).– Bull. Fac. Sci., Assiut Univ. 23(2 – D): 39–65.

KEINATH A. P., FRAVEL D. R. and PAPAVIZAS G. C. (1981): Potential of Gliocladium roseum for biocontrol of Verticillium dahliae. – Phytopathology 81: 644–648.

LENNARTZ B., SCHOENE P. and OERKE E. C. (1998): Biocontrol of Botrytis cinerea on grapevine and Septoria spp on wheat. – Mededelingen-Faculteit-Landbouwkundige-en-Toegepaste-Biologische-Wetenschappen-Universiteit-Gent. 63(3B): 963–970.

LI J. J., ZINGEN SELL I. and BUCHENAUER H. (1996): Induction of resistance of cotton plants to Verticillium wilt and of tomato plants to Fusarium wilt by 3 – aminobutyric acid and methyl jasmonate. – Zeitschrift Fur Pflanzenkrankheiten und Pflanzenschutz – Journal of Plant Diseases and Protection 103: 288–299.

MADHOSINGH C. (1996): A rapid tomato seedling assay for Verticillium wilt.– Journal of Phytopathology – Phytopathologische Zeitschrift 144: 131–134.

MATHEW K. A. and GUPTA S. K. (1998): Biological control of root rot of French bean caused by Rhizoctonia solani. – Journal of Mycology and Plant Pathology 28(2): 202–205.

MIZUNO K., YAGI A., TANAKA M., MATSUURA K., YAMAGUCHI K. and ASANO K. (1974): A new antibiotic, talaron. – J. Antibiot. 7: 560–563.

OSMAN A. R., SATOUR M., SABET K. K. and ABDEL-GHAFOUR S. M. E. (1991): Epidemiological aspects of tomato wilt caused by Verticillium dahliae. – Egypt. J. Agric. Res. 69 (3): 673–683.

PHILLIPY W. D. (1988): Biologische Bekampfung von Pflanzenkrankheiten. – Ulmer, Stuttgart, Germany.

RAJENDRA S. and SAXENA V. C. (1991): Wilt of cauliflower: Rhizosphere and rhizoplane studies. – Int. J. Trop. Plant Sci. 9 (1): 71–82.

THOMASHOW L. S., WELLER D. M., BONSALL R. F. and PIERSON L. S. (1990): Production of the antibiotic phenazine– 1– carboxyclic acid by fluorescent Pseudomonas species in the rhizosphere of wheat. – Appl. Environ. Microbiol. 56: 908–912.

TSROR L., ERLICH O., AMITAI S. and HAZANOVSKY M. (1998): Verticillium wilt of paprika caused by a high virulent isolate of Verticillium dahliae. – Plant Disease 82(4): 437–439.

VILICH V., DOLFEN M. and SIKORA R. A. (1998): Chaetomium spp. colonization of barley following seed treatment and its effect on plant growth and Erysiphe graminis f. sp. hordei disease severity. – Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 105(2): 130–139.

ZEISE K. (1990): Erste Untersuchungen zu einer durch Verticillium dahliae Kleb. verursachten Fruchtfolgekrankheit an Winterraps (Brassica napus L. var. oleifera Metzger). Diss., Rostock, Germany.

CZECH MYCOL. 52 (3), 2000

The nematophagous hyphomycete Esteya vermicola found in the Czech Republic

ALENA KUBÁTOVÁ[1], DAVID NOVOTNÝ[2], KAREL PRÁŠIL[1] and ZDENĚK MRÁČEK[3]

[1] Culture Collection of Fungi (CCF), Department of Botany,
Faculty of Science, Charles University, Benátská 2, 128 01 Praha 2, Czech Republic,
e-mail: kubatova@natur.cuni.cz
[2] Czech Collection of Microorganisms,
Faculty of Science, Masaryk University, Tvrdého 14, 602 00 Brno, Czech Republic
[3] Laboratory of Insect Pathology, Institute of Entomology,
Czech Academy of Sciences, Branišovská 31, 370 05 České Budějovice, Czech Republic

Kubátová A., Novotný D., Prášil K. and Mráček Z. (2000): The nematophagous hyphomycete Esteya vermicola found in the Czech Republic – Czech Mycol. 52: 227–235

During a study of surface mycoflora of the bark beetle *Scolytus intricatus* (Coleoptera: Scolytidae), the hyphomycetous microfungus *Esteya vermicola* was found associated with *Bursaphelenchus eremus* (Nematoda: Aphelenchoididae). *Esteya vermicola* and *Bursaphelenchus eremus* were recorded in the Czech Republic for the first time. The find of *E. vermicola* is considered to be the second one in the world. *Esteya vermicola* is a nematophagous species with two types of conidia, described in 1999 from the pinewood nematode *Bursaphelenchus xylophilus* in Taiwan. Our new records are from the surface of larvae and adult beetles of *Scolytus intricatus* and their galleries under bark of branches of three species of oak (*Quercus petraea, Q. polycarpa,* and *Q. robur*) on several localities in the Polabí and Křivoklátsko regions, Czech Republic. A description of morphological features and drawings of the fungus are given. Examined strains are maintained in the Culture Collection of Fungi (CCF), Faculty of Science, Charles University, Prague and Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno.

Key words: conidial fungi, *Esteya vermicola, Bursaphelenchus eremus, Scolytus intricatus,* nematodes, bark beetles

Kubátová A., Novotný D., Prášil K. a Mráček Z. (2000): Nematofágní hyfomycet Esteya vermicola nalezen v České republice – Czech Mycol. 52: 227–235

Během studia povrchové mykoflóry bělokaza dubového (*Scolytus intricatus*, Coleoptera: Scolytidae) byl zjištěn výskyt mikroskopické vláknité houby *Esteya vermicola* ve spojení s háďátkem *Bursaphelenchus eremus* (Nematoda: Aphelenchoididae). *Esteya vermicola* i *Bursaphelenchus eremus* byli v České republice zaznamenáni poprvé. Nález *E. vermicola* je považován za druhý nález na světě. *E. vermicola* patří mezi nematofágní houby, tvoří dva typy konidií a byla poprvé popsána roku 1999 jako parazit háďátka *Bursaphelenchus xylophilus*, škůdce borovic na Taiwanu. Naše nálezy pocházejí z povrchu larev a dospělců bělokaza dubového (*S. intricatus*) a jejich chodbiček pod borkou větví tří druhů dubu (*Quercus petraea, Q. polycarpa, Q. robur*) na několika lokalitách v Polabí a na Křivoklátsku. V článku je uveden popis morfologických znaků, perokresba a mikrofotografie. Vybrané kmeny jsou uloženy ve Sbírce kultur hub (CCF) na katedře botaniky přírodovědecké fakulty UK v Praze a v České sbírce mikroorganismů (CCM) přírodovědecké fakulty MU v Brně.

INTRODUCTION

Esteya vermicola J. Y. Liou, J. Y. Shih and Tzean is a nematophagous endoparasitic fungus recently described by Liou et al. (1999) from Taiwan. They found it attacking the pinewood nematode *Bursaphelenchus xylophilus* which causes the wilting disease of pine trees (e.g. *Pinus densiflora, P. thunbergii, P. luchuensis*) in eastern Asia. It is noteworthy that the life cycle of this nematode is associated with beetles. After Mamiya (1983), the dauerlarvae of the pinewood nematode enter tracheas of the just-pupated longhorn beetle *Monochamus alternatus*. Adults of the beetle fly from the dead trees to healthy ones for post-maturation feeding. On pine twigs, the nematodes leave the beetles and enter twig tissue through wounds caused by the beetles. Nematode feeding leads to the destruction of epithelial and parenchyma cells and infected trees die within 2–3 months. Liou et al. (1999) revealed the strong parasitic potential of *Esteya vermicola*. Infection by adhesive lunate conidia led to the killing of almost 100 % of the nematode population within 8–10 days.

There is wide range of relationships between bark beetles and nematodes, from simple phoresis to facultative or obligate parasitism. In the Czech Republic, the oak bark beetle *Scolytus intricatus* damages different species of oak (e.g. *Quercus robur, Q. petraea, Q. polycarpa*) and contribute to their dying. From 1997 to 1999, the surface mycoflora of *Scolytus intricatus* and its galleries was studied. The results of this study will be published elsewhere. During the study, the rare hyphomycete *Esteya vermicola* was found in connection with *Scolytus intricatus* (Coleoptera: Scolytidae) and its phoretic nematode *Bursaphelenchus eremus* Rühm (Nematoda: Aphelenchoididae). Both fungus and nematode were yet unknown from the Czech Republic. For the fungus it seems to be the first record in Europe. Therefore, a description of the fungus and notes on the significance of these species are given.

MATERIAL AND METHODS

Study sites

Oak branches (*Quercus petraea, Q. robur* and *Q. polycarpa*) infested with *Scolytus intricatus* were collected on four localities in central Bohemia, Czech Republic: Mlynářův luh and Kohoutov in the Křivoklátsko region, Libický luh and Bačov in the Polabí region. All localities are characterised by deciduous forest with prevailing oaks.

Methods

The micromycete was isolated using two methods. (1) Larvae and mature beetles were excised from bark and separately washed with sterile water with

a Tween 80 in sonicator. Washed beetles, 1 ml of the suspension and detritus from galleries were inoculated each on a separate Petri dish with 1.5 % malt extract agar (MA1.5) and streptomycin (0.1 g/l). Most *Esteya* isolates were detected by this method. (2) Pieces of branches infested by *Scolytus* were superficially sterilised (96 % ethanol: 1 min., sodium hypochlorite: 3 min., 96 % ethanol: 30 s), divided into bark and wood, and inoculated onto Petri dishes with 2 % malt extract agar (MA2). After several days of incubation at 25 °C the fungi were isolated. *Esteya* strains were identified according to Liou et al. (1999). For identification, potato-glucose agar (PGA), potato-carrot agar (PCA), 2 % malt extract agar (MA2) and incubation in the dark at 25 °C were used. In addition, incubation at different conditions (room temperature: daylight/dark or UV light/dark) were tried. Representative strains were freeze-dried in skimmed milk and are deposited in the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague and in the Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno.

Photographs were taken on Olympus BX-50 microscope with a DIC.

RESULTS AND DISCUSSION

Description of *Esteya vermicola* on the basis of our isolates

Representative strains:

CCF 3115: ex dead female of *Scolytus intricatus* under bark of branch of *Quercus petraea*, Mlynářův luh, Křivoklátsko region, Czech Republic, isol. A. Kubátová as No. 116/97, VIII. 1997

CCF 3116: ex male of *Scolytus intricatus* before emergence from *Quercus polycarpa*, Libický luh, Polabí region, Czech Republic, isol. A. Kubátová as No. 50/98, V. 1998

CCF 3117: ex gallery of female of *Scolytus intricatus* before emergence from *Quercus polycarpa*, Libický luh, Polabí region, Czech Republic, isol. A. Kubátová as No. 51/98, V. 1998

CCF 3118: ex gallery of larva of *Scolytus intricatus* before wintering in *Quercus robur*, Bačov, Polabí region, Czech Republic, isol. A. Kubátová as No. 218/98, XI. 1998

CCF 3131: ex male of *Scolytus intricatus* before emergence from *Quercus petraea*, Kohoutov, Křivoklátsko region, Czech Republic, isol. A. Kubátová as No. 67/97, IV. 1997

CCF 3132: ex larva of *Scolytus intricatus* after wintering in *Quercus polycarpa*, Libický luh, Polabí region, Czech Republic, isol. K. Prášil as No. 17A/98, V. 1998

CCM 8247: ex male of *Scolytus intricatus* after emergence from *Quercus robur*, Bačov, Polabí region, Czech Republic, isol. D. Novotný as No. B12D12, VII. 1999

CCM 8251: ex bark of twig of *Quercus robur* with gallery of *Scolytus intricatus*, Bačov, Polabí region, Czech Republic, isol. D. Novotný as No. B3K2, VII. 1999

Macroscopic features

PGA, 7 days, 25 °C, in the dark: colonies 24–30 mm diam., greyish green, reverse dark greyish to olive green. Colonies after 10 days 39–48 mm diam. and after 14 days 65–71 mm diam.

PCA, 7 days, 25 °C, in the dark: colonies 20–30 mm diam., grey, reverse dark grey. Colonies after 10 days 40–50 mm diam. and after 14 days 62–72 mm diam.

MA2, 7 days, 25 °C, in the dark: colonies 26–32 mm diam., dark olive green, reverse dark olive green. Colonies after 10 days 41–46 mm diam. and after 14 days 60–68 mm diam.

MA2, 7 days, 30 °C, in the dark: colonies 7–12 mm diam. Colonies after 10 days 10–21 mm diam. and after 14 days 18–36 mm diam.

Growth at 37 °C is nil.

Microscopic features (Figs. 1 and 2)

Hyphae subhyaline, grey green to olive green, smooth to roughened, sometimes with slime sheath. Conidiophores, conidiogenous cells and conidia of two types, often growing on the same hypha.

Conidiogenous cells of the first type sessile, smooth to roughened, with olive green inflated base ca 3.1–5.4 μm wide, abruptly tapering into a thin subhyaline neck of varying length, ca 1.5 μm wide, often crooked, sometimes percurrent. Conidia one-celled, lunate, subhyaline, smooth-walled, 9.3–12.4 × 3–3.2 μm. They are formed very often on hyphae submerged in agar, too. Conidiophores of the second type loosely branched or simple, olive green, often roughened. conidiogenous cells about 30–40 μm long, straight, mostly with swollen base (3–4.7 μm). Conidia cylindrical, one-celled, hyaline, smooth, 4.8–6.2 × 1.3–1.5 (-2) μm.

The ability and mode of conidia germination were examined on MA2. Both types of conidia germinated the second day after inoculation of a water suspension. The lunate conidia formed one or more germ tubes in the centre of a concave side. The cylindrical conidia formed the germ tube at the end (Fig. 3).

Some details on a conidiogenesis are given by Liou et al. (1999): reportedly phialidic in the first type and phialidic enteroblastic in the second type. The unusual way of development, especially the forming of lunate conidia, needs further study using TEM.

The microscopic observations of our strains fit well with the description given by Liou et al. (1999). However, Liou et al. reported that on enriched media, cylindrical conidia were formed first. Our strains produced both types of conidia on nutrient media very early, mostly the third day of cultivation. Sporulation under different light conditions (daylight/dark, UV light/dark) seems to be nearly the same.

The teleomorph is not known and was not observed in this study. In strain CCF 3116, dark multicellular bodies of irregular shape were observed after 2 month of cultivation on malt extract agar (see Fig. 2 k).

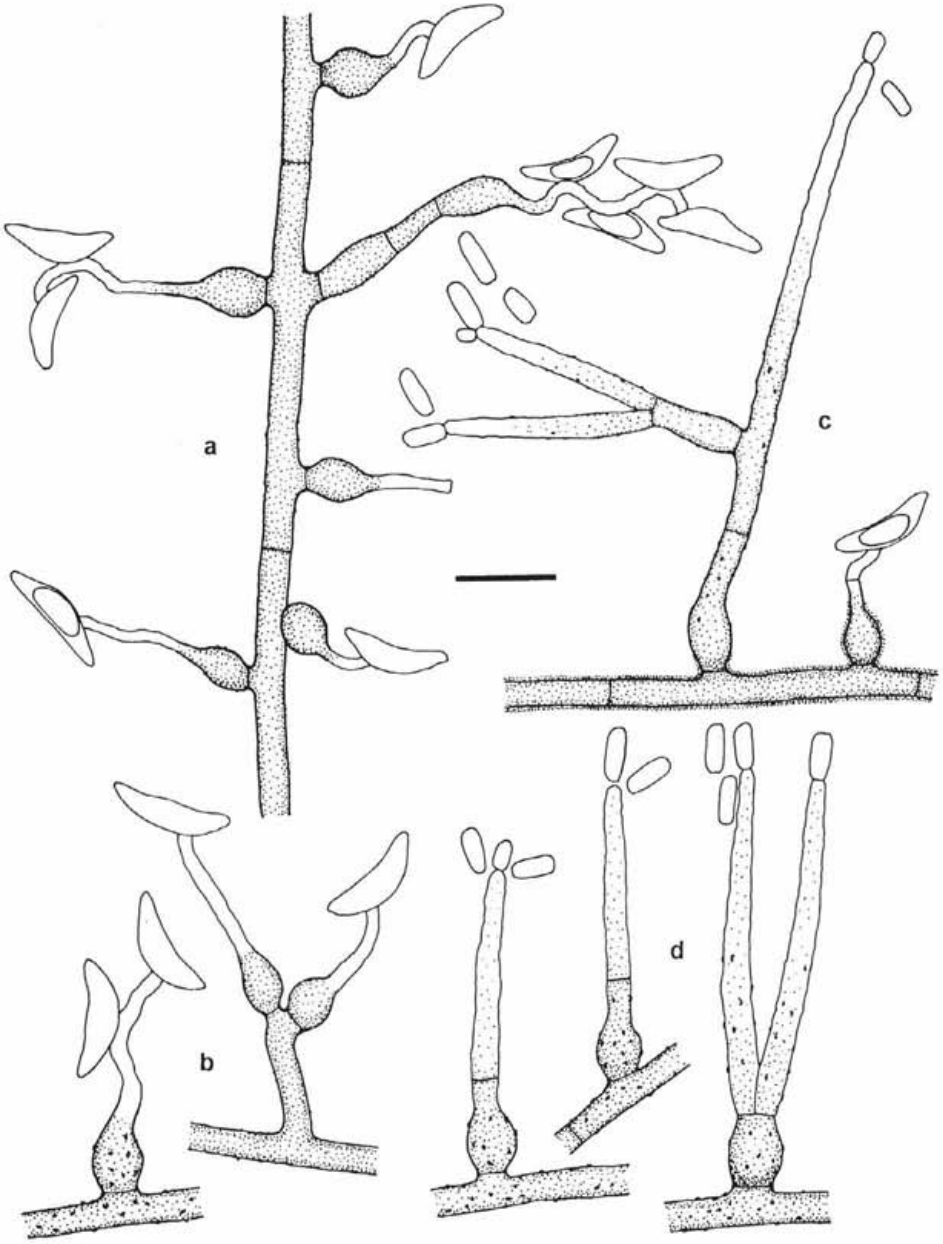


Fig. 1. *Esteya vermicola.* a, b: conidiophores with lunate conidia, c: hypha with two types of conidiophores and conidia, d: conidiophores with cylindrical conidia. Bar = 10 μm.
A. Kubátová and K. Prášil del.

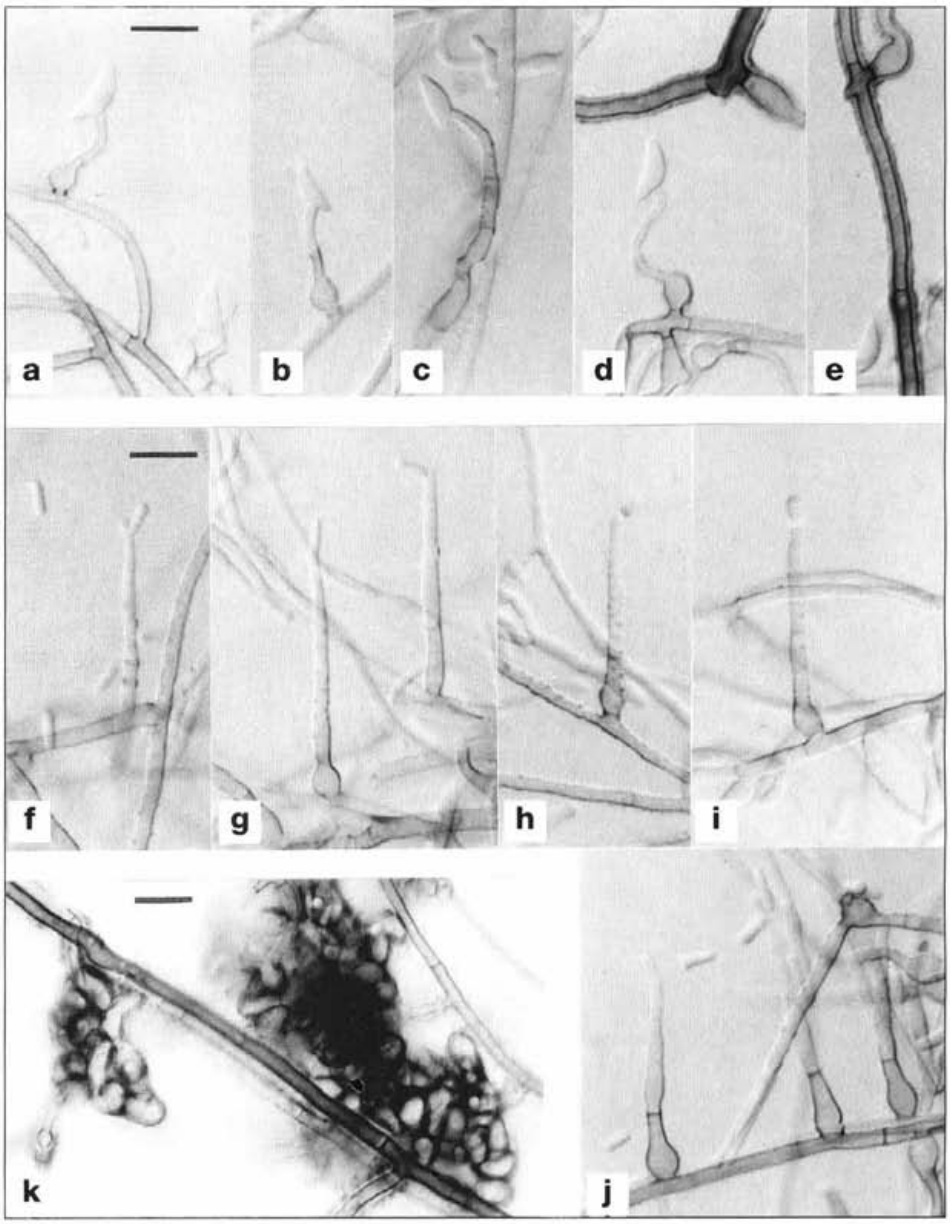


Fig. 2. *Esteya vermicola.* a - d: conidiogenous cells with lunate conidia, e: dark hypha with sheath, f - j: conidiogenous cells with cylindrical conidia, k: dark multicellular bodies. Bars = 10 μm. Interference contrast.

Photo A. Kubátová

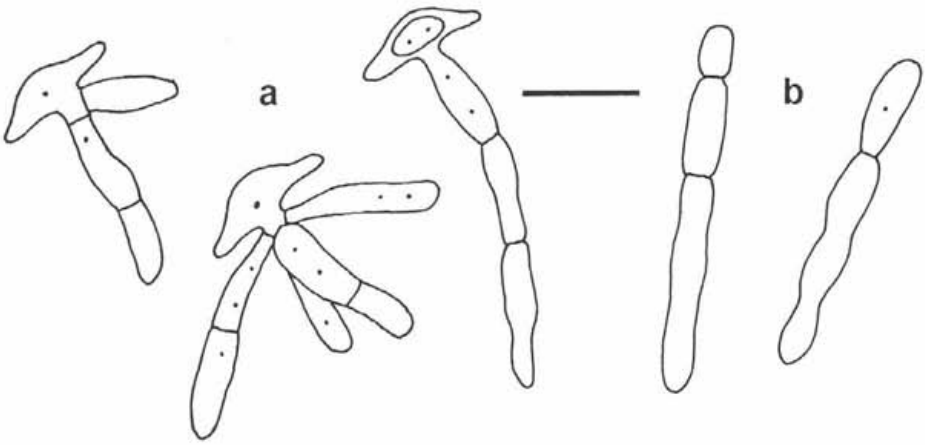


Fig. 3. *Esteya vermicola,* the strain CCF 3116. a: germinating lunate conidia, b: germinating cylindrical conidia. Bar = 10 μm.

A. Kubátová del.

Occurrence and significance for forest pathology

Strains of *Esteya vermicola* were isolated from the surface of larvae and adult beetles of *Scolytus intricatus* and their galleries under bark of three species of oak (*Quercus petraea, Q. polycarpa,* and *Q. robur*) on four studied localities in the Polabí and Křivoklátsko regions, Czech Republic. The total number of finds reached 24 but not all records were isolated in pure culture. Most records of *Esteya vermicola* were from young beetles before emergence and their galleries on *Quercus polycarpa* at Libický luh, Polabí region. *Esteya* was observed on this locality associated with nine beetle bodies from a total of 25 beetles studied (see Table 1). It is obvious that *Esteya* was found in different stages of the life cycle of *Scolytus intricatus*, however predominantly on adults.

Interestingly, free-living nematodes – males, females, and larvae – were observed on several pieces of oak branches infested or non-infested with *Scolytus* and incubated in moist chambers as well as on some Petri dishes during isolation of micromycetes from *Scolytus intricatus*. The nematodes had a stylet, characteristic indistinct bursa and spicules with a prominent rostrum. It was identified as *Bursaphelenchus eremus* (Rühm 1956, Yin et al. 1988). The fourth larval stage and females of the nematode are known as a phoretic living under elytra or in intersegmental folds, while the facultative entomoparasitism of this nematode species was not proved. Young nematode larvae, males and egg laying females live in a frass of bark beetle galleries feeding on fungal hyphae. *B. eremus* may play a certain role as vector of micromycetes similarly as *Scolytus intricatus*. This is the first record of *Bursaphelenchus eremus* from the Czech Republic.

Table 1. List of records of *Esteya vermicola* associated with *Scolytus intricatus*

region	locality	species of oak	number of records of *Esteya vermicola* associated with *Scolytus intricatus*
Křivoklátsko	Kohoutov	*Quercus petraea*	• on 1 male before emergence (of 20 adults total)
	Mlynářův luh	*Q. petraea*	• on 1 male and 2 dead females (of 100 adults total) • on 1 larva (of 25 larvae total)
Polabí	Libický luh	*Q. polycarpa*	• on 6 males and 3 females before emergence and in their galleries (of 25 adults total)
	Bačov	*Q. robur*	• in galleries of 2 larvae before wintering (of 60 larvae total) • on 4 females in maturation feeding and on 1 female (of 30 adults total) • in 3 samples of bark from oak branch with beetle galleries (1 branch of 3 total)

Liou et al. (1999) recorded a strong killing potential of *Esteya vermicola* for the nematode *Bursaphelenchus xylophilus*. Its lunate conidia adhere to the nematode and penetrate the cuticle and muscle layer. Infection experiments with *Esteya vermicola* and *Bursaphelenchus eremus* were not carried out in our study. However, during incubation of a mixture of micromycetes from the surface of *Scolytus intricatus* on Petri dishes, coiled cadavers of nematodes overgrown by *Esteya vermicola* were observed.

In Fig. 4 a scheme is presented to demonstrate relations of the micromycete *E. vermicola* to nematode *Bursaphelenchus eremus* and bark beetle *Scolytus intricatus*, all organisms living in branches of *Quercus* spp.

Concluding, the study resulted in new data on the ecology of *Esteya vermicola*, a so far little known hyphomycete. It is evident that this fungus is not restricted to the subtropic climate of Taiwan, the nematode *Bursaphelenchus xylophilus*, the longhorn beetle *Monochamus alternatus* and *Pinus* spp., but is also living in the temperate climate of the Czech Republic, associated with the nematode *Bursaphelenchus eremus* and bark beetle *Scolytus intricatus* feeding on *Quercus* spp. trees. While nematode *Bursaphelenchus xylophilus* is the causal agent of pine wilt disease in eastern Asia (Mamiya 1983), *B. eremus* is probably not damaging oak tissues and feeding on microfungi in bark beetle galleries. The parasitic potential of *Esteya vermicola* for *Bursaphelenchus* nematodes is noteworthy and may be of value for plant pathologists. Therefore, virulence studies for other nematode genera should be carried out.

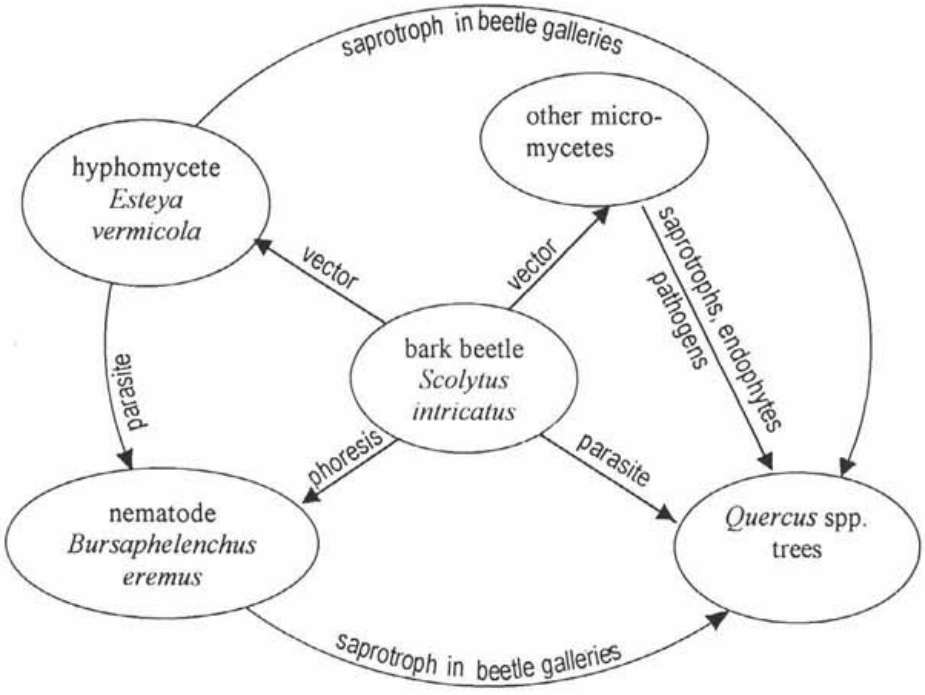


Fig. 4: Relations of the studied organisms: *Esteya vermicola*, *Bursaphelenchus eremus*, *Scolytus intricatus*, and *Quercus* spp.

ACKNOWLEDGEMENTS

We wish to thank Ing. P. Šrůtka (Czech University of Agriculture, Prague) for providing the infested oak material. Dr. J. Kocourková (Mycol. Dept., National Museum, Prague) is thanked for her photographic assistance. The work was supported by the Grant Agency of the Czech Republic (Project No. 203/97/0037).

REFERENCES

LIOU J. Y., SHIH J. Y. and TZEAN S. S. (1999): Esteya, a new nematophagous genus from Taiwan, attacking the pinewood nematode (Bursaphelenchus xylophilus). – Mycol. Res. 103: 242–248.

MAMIYA Y. (1983): Pathology of the pine wilt disease caused by Bursaphelenchus xylophilus. – Ann. Rev. Phytopathol. 21: 201–220.

RÜHM W. (1956): Die Nematoden der Ipiden. – Parasitol. Schriftenreihe 6: 1–435.

YIN K., FANG Y. and TARJAN C. (1988): A key to species in the genus Bursaphelenchus with a description of Bursaphelenchus hunanensis sp. n. (Nematoda: Aphelenchoididae) found in pine wood in Hunan province, China. – Proc. Helminthol. Soc. Wash. 55: 1–11.

CZECH MYCOL. 52 (3), 2000

Xeromphalina brunneola (Tricholomataceae), a new member of the European mycoflora

VLADIMÍR ANTONÍN

Moravian Museum, Dept. of Botany,
Zelný trh 6, CZ-659 37 Brno, Czech Republic
(E-mail: vantonin@mzm.cz)

Antonín V. (2000): Xeromphalina brunneola (Tricholomataceae), a new member of the European mycoflora – Czech Mycol. 52: 237–242

A new fungus originally described from North America, *Xeromphalina brunneola* O. K. Mill., is recorded as a new element of the European macromycetous mycoflora. A description of macrofeatures and microfeatures is given in detail, and recently known European localities are summarized.

Key words: Tricholomataceae, *Xeromphalina brunneola*, distribution

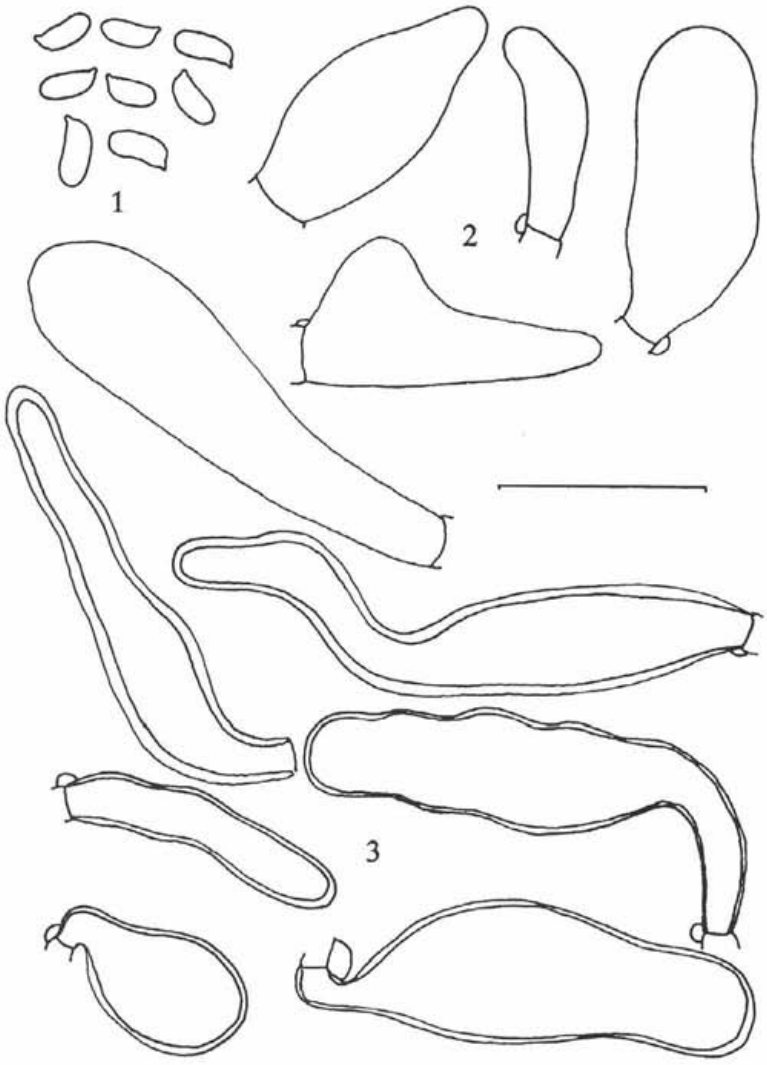
Antonín V. (2000): Xeromphalina brunneola (Tricholomataceae), nový prvek evropské mykoflóry – Czech Mycol. 52: 237–242

Xeromphalina brunneola O. K. Mill., původně popsaná ze Severní Ameriky, byla poprvé zaznamenána z materiálu v evropských herbářích. Je podán makroskopický a podrobný mikroskopický popis a jsou shrnuty dosud známé evropské lokality.

The small genus *Xeromphalina* Kühner et Maire usually includes only three or four species in European monographs and keys (*X. campanella, X. cauticinalis, X. fellea, X. cornui*, e.g. Klán 1984, Moser 1983). Some other new species were described during the last few years, e.g. *X. junipericola* G. Moreno et Heykoop (Moreno and Heykoop 1996) and *X. minutissima* Esteve-Rav. nom. prov. (Esteve-Raventós 1995). Redhead (1988) studied the genus *Xeromphalina* of the northern hemisphere, and found specimens of some North American species also in European material. *Xeromphalina brunneola* O. K. Mill. is one of them.

During the preparation of the European monograph of the genus *Xeromphalina* and revision of specimens from various herbaria, I found specimens of this species collected in some European countries. This species is not included in the new monograph by Bon (1999). Because they represent the first recognized records in Europe, I decided to write this paper.

The macrodescription is based on the original description by Miller (1968). Microscopical features are described from examined material mounted in Melzer's reagent, Congo Red, and KOH. For the basidiospores the following factors are used: E (quotient of length and width in any one spore); Q (mean of E-values). Authors of fungal names are cited according to Kirk and Ansell (1992).



Figs. 1–3. *Xeromphalina brunneola*. 1. basidiospores, 2. circumcystidia, 3. caulocystidia. Scale bar = 20 μm.

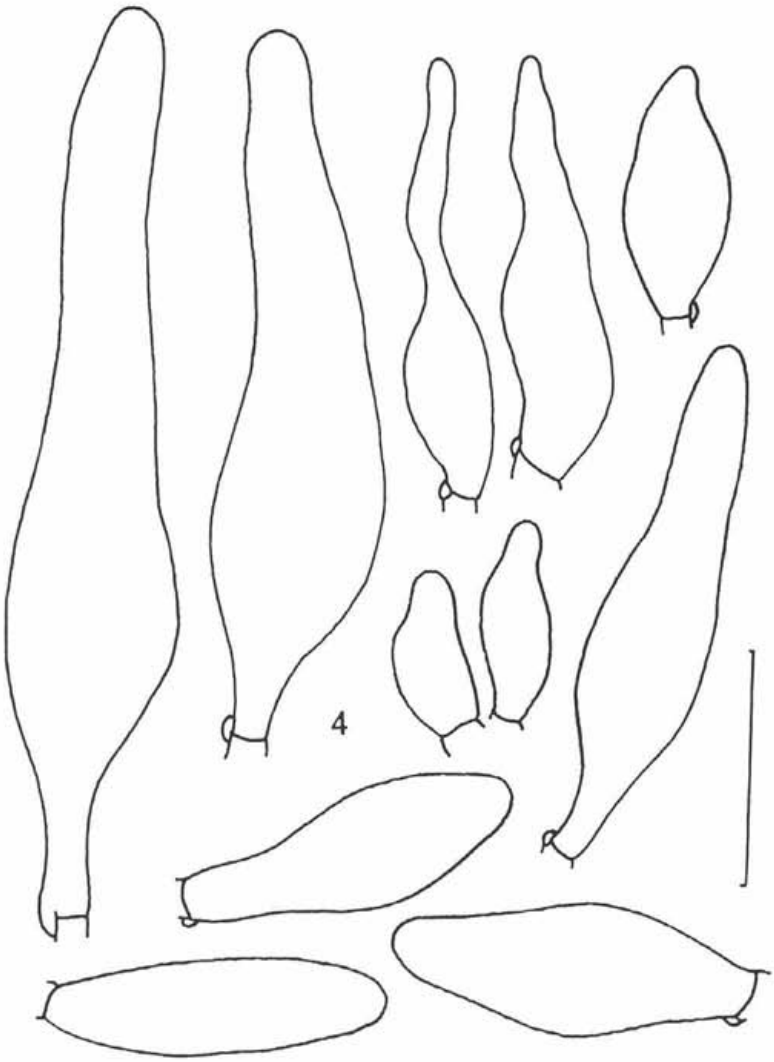


Fig. 4. *Xeromphalina brunneola*. 4. cheilocystidia. Scale bar = 20 μm.

Xeromphalina brunneola O. K. Mill., Mycologia 60: 167. 1968.

Pileus 6–15 mm broad, convex-depressed to nearly plano-depressed, glabrous, moist, margin opaque when moist, evenly dull orange overall. Lamellae close, decurrent, narrow, not intervenose when young, sometimes somewhat intervenose with age, orange-buff, with even, dull edges. Stipe 30–60 × 1–2.5 mm, tapering somewhat towards base, nearly bulbous at base, fistulose, cartilaginous, smooth and orange buff at apex, ochraceous pubescent, darker rusty brown downward. Context thin, brownish, cartilaginous, with persistently disagreeable taste, and at first disagreeable smell.

Basidiospores (5.0-)5.5–7.0(-7.5) × 2.5–3.5 μm, E = 1.7–2.3, Q = 1.9–2.1, narrowly ellipsoid, cylindrical-ellipsoid, often suballantoid, thin-walled, amyloid, hyaline, smooth. Basidia 21–29 × 5.0–6.5 μm, 4-spored, clavate. Basidioles 10.0–27 × 2.5–7.0 μm, cylindrical to clavate. Cheilocystidia 25–85 × (6.5-)8.0–16.5(-19) μm, lageniform, lanceolate, (sub)utriform, clavate, subfusoid, sometimes pedicellate, thin-walled, hyaline, non-amyloid. Tramal hyphae of cylindrical to fusoid cells, thin– to slightly thick-walled, incrusted in grains or plaques, yellow-brown in KOH, up to 15 μ wide; mixed with thick-walled, subhyaline, refractive, up to 20 μm wide hyphae. Pileipellis a cutis of radially arranged, cylindrical, slightly thick-walled, incrusted, dark-yellow-brown in KOH, up to 8 μm wide hyphae; hyphae at pileus margin both smooth and incrusted. Pileus trama of hyphae yellow-brown in H_2O and in KOH. Circumcystidia not frequent, 25–50 × 14–16 μm, clavate, broadly clavate, subfusoid, sometimes irregular, thin– to slightly thick-walled; cylindrical, often irregular to subcoralloid terminal cells or lateral projections present. Stipitipellis a cutis, of parallel, cylindrical, slightly thick-walled, coarsely incrusted, brownish orange in KOH, up to 10 μm wide hyphae. Stipe medulla hyphae yellow-brown in H_2O and in KOH; thick-walled hyphae similar to pileus trama also present. Caulocystidia (23-)30–65 × (6.0-)11–18 μm, clavate, broadly clavate, utriform, cylindrical, sometimes irregular, not or rarely branched, never coralloid, thick-walled, obtuse, yellow– to orange-brown in KOH. Clamp connections present. No hyphae amyloid or dextrinoid.

Growing from July to November, saprotrophic, in clusters or in groups on coniferous wood, especially of *Picea*.

Collections examined:

AUSTRIA: Lunz, Aug., leg. C. Keissler, in:, No. 949. (BPI 757880; PRM 5980, a short-spored form – "var. *myriadea*"). – Carinthia, Turracher Hoke, 18 Aug. 1974 leg. D. A. Reid [K(M) 68765]. – Carinthia, St. Lorenzen, 13 Aug. 1974 leg. D. A. Reid [K(M) 75587].

FINLAND: Etelä-Pohjanmaa, Zyteri, Rantasipi, 15 Aug. 1983 leg. J. Stordal 22920 (O 120213).

ITALY: Varena, Aug. 1923 leg. G. Bresadola (BPI 757835). – ditto, Aug. 1921 leg. G. Bresadola (BPI 757849, a short spored form – "var. *myriadea*").

NORWAY: Akershus, Ås, 18 Aug. 1978 leg. G. Gulden 891/78 (O 120170). – Vestfold, Sem, Eik, 18 Aug. 1981 leg. S. Aase (O 120214). – Hedmark, Stor-Elvdal, Sørnesset, 14 July 1973 leg. G. Gulden 653/73 (O 120180). – Buskerud, Modum hd., 26 Aug. 1986 leg. J. Stordal 25063 (O 199). – Østfold, Halden, Solli kapell, 16 Sept. 1975 leg. G. Gulden 370/75 (O 120178). – Akershus Co., Eidsvoll Municipality, 13 Sept. 1994 leg. B. Spooner [K(M) 31833].

POLAND: Białowieża forest, 17 Sept. 1981 leg. D. N. Pegler 3400 [K(M) 68763].

SWEDEN: Öland, Böda, 2 Oct. 1980 leg. M. Korhonen and R. Tuomikoski (H).

U. S. A.: Idaho, Priest River, 25 Sept. 1964 leg. O. K. Miller 2866 (MICH, holotype). – Pennsylvania, Butler Co., Monroe, Little Buffalo Creek, 26 Sept. 1936 leg. L. K. Henry (PRM 832209).

Xeromphalina brunneola is characterized in having relatively small, rather dark coloured carpophores, rather small ellipsoid to cylindric-ellipsoid spores which are often suballantoid, presence of unbranched and often voluminous caulocystidia and presence of thick-walled hyphae in tissues.

It is close to *X. campanella* (Batsch: Fr.) Kühner et Maire. According to Miller (1968) it should differ in having darker coloured carpophores, disagreeable taste, reddish brown pileipellis and stipitipellis at apex in KOH, pigmented caulocystidia and smaller spores. Redhead (1988) discussed those features and stated, that the characters for distinguishing both species in herbarium specimens are not only size but also shape of the spores and the presence of thick-walled hyphae in tissues. My studies supported this conclusion with one addition – the disagreeable taste is distinct also in dry carpophores. Other European species differ especially in having branched to coralloid circum– and caulocystidia.

In comparison with the literature, the original microdescription by Miller (1968) differs in slightly smaller spores (5.5–6.6×2.5–3.0 μm) and narrower cheilocystidia (× 5.5–8.5 μm).

A short-spored form was recognized in some collections from the Alps (Austria, Italy). It differs from the typical form only in having smaller spores: 4.5–6.0 × 2.5–3.5 μm (E = 1.6–2.1, Q = 1.8). It is known only from some exsiccata of Keissler's Kryptogamae exsiccatae (as *X. campanella* var. *myriadea*) and two of Bresadola's collections. However, some other studied herbarium specimens called var. *myriadea* (e.g. Rabenhorst, Fungi europaei, No. 2001) represent the typical *X. campanella*. According to the diagnosis, the original var. *myriadea* Kalchbr. in Fr. (Hymenomycetes europaei: 162. 1874) should be two times smaller, densely caespitose, pale fulvous, with pale brick-fleshy lamellae (Fries 1874: "Varietas

insignior est *myriadea* Kalchbrenner in litteris, vulgari dimidio minor, densissime caespitosa, truncis pinnum denso vellere obducens, dilute fulva, lamellis pallide testaceo-carneis").

Xeromphalina brunneola seems to be a boreal-montane species, known from Europe (Austria, Finland, Italy, Norway, Poland, Sweden), North America (Canada, U. S. A.) and Asia (Japan).

In modern literature, only Veikko and Hintikka (1957) published a description of var. *myriadea* from some localities in Finland. According to them, macroscopically, it resembles a smaller (pileus 5–8 mm broad) and paler coloured (pileus pale yellow-brown, slightly darker at centre) *X. campanella*; microscopically it is identical with typical variety of *X. campanella*. The authors also mentioned a different nature of cultures of both varieties. However, both *X. brunneola* and especially its short-spored form have distinctly smaller spores than *X. campanella*. A revision of herbarium specimens (herb. H) showed that var. *myriadea* sensu Veikko and Hintikka (1957) represents a small form of *X. campanella*.

ACKNOWLEDGEMENTS

The author wishes to thank the curators of herbaria BPI, C, H, MICH, K, O, and PRM for kindly sending types and other herbarium specimens. The study of this group belongs to a larger taxonomic grant supported by the Grant Agency of the Czech Republic (No. 206/98/0257).

REFERENCES

BON M. (1999b): Les Collybio-Marasmioïdes et ressemblants. – Fl. mycol. Eur. 5, Doc. mycol. Mém. hors sér. 5: 1–171.

ESTEVE-RAVENTÓS F. (1995): Xeromphalina minutissima nom. prov., una nueva especie a Ser Validada. – Bol. Soc. Micol. Madrid 20: 313–316.

FRIES E. M. (1874): Hymenomycetes europaei sive epicriseos systematis mycologici. – Uppsala.

KIRK P. M. and ANSELL A. E. (1992): Authors of fungal names. – International Mycological Institute, Kew.

KLÁN J. (1984): The genus Xeromphalina (Tricholomataceae) in Europe. – Čes. Mykol. 38: 205–217.

MILLER O. K. (1968): A revision of the genus Xeromphalina. – Mycologia 60: 156–188.

MORENO G. and HEYKOOP M. (1996): Xeromphalina junipericola sp. nov. (Tricholomataceae, Agaricales) from Spain. – Z. Mykol. 62(1): 37–41.

MOSER M. (1983): Die Röhrlinge und Blätterpilze. – In: Kleine Krypt.-Fl., 2b/2, Jena, 532 pp.

REDHEAD S. A. (1988): Notes on the genus Xeromphalina (Agaricales, Xerulaceae) in Canada: biogeography, nomenclature, taxonomy. – Can. J. Bot. 66: 479–507.

VEIKKO E. and HINTIKKA K. (1957): Über die finnische Arten und Varietäten der Gattung Xeromphalina Kühner et Maire. – Karstenia 4: 5–9.

CZECH MYCOL. 52 (3), 2000

The taxonomy of Pholiota fusus – a critical evaluation

JAN HOLEC

National Museum, Mycological Department,
Václavské nám. 68, 115 79 Praha 1, Czech Republic

Holec J. (2000): The taxonomy of Pholiota fusus – a critical evaluation. – Czech Mycol. 52: 243–251

Pholiota fusus (Batsch) Singer based on *Agaricus fusus* Batsch is included in most floras of the 19th century and appears in some works of the 20th century. Recent records documented by herbarium material were published by Bon, Tjallingii-Beukers and Noordeloos. A careful revision of these collections showed that they most probably represent an aberrant robust form of *Hypholoma sublateritium*. The original *Agaricus fusus* Batsch is hard to interpret. The species probably does not belong to *Pholiota* or not even to brown-spored fungi. Later interpretations of *Pholiota fusus* are dubious and cannot be verified due to the lack of any herbarium material. The concept of *Pholiota fusus* used by some authors of the 20th century follows that of Ricken. However, *Flammula fusa* sensu Ricken is probably a non-existing entity based on a mixture of characters taken from various species. There is no reliable evidence that a separate species of *Pholiota* corresponding to Batsch's original description or various later interpretations really exists. Consequently, the name *Pholiota fusus* must be considered a nomen dubium and should be rejected.

Key words: Fungi, basidiomycetes, *Agaricales*, *Agaricus fusus*, *Pholiota*, *Hypholoma*, taxonomy

Holec J. (2000): Taxonomie druhu Pholiota fusus – kritické zhodnocení. – Czech Mycol. 52: 243–251

Pholiota fusus (Batsch) Singer založená na *Agaricus fusus* Batsch je druhem zahrnutým do většiny významných mykologických flór 19. století a objevuje se i v některých pracích z 20. století. Nálezy doložené herbářovým materiálem publikovali v poslední době Bon, Tjallingii-Beukers a Noordeloos. Revize tohoto materiálu prokázala, že se s největší pravděpodobností jedná o atypickou robustní formu druhu *Hypholoma sublateritium*. Původní *Agaricus fusus* je obtížně interpretovatelný druh, který zřejmě nepatří do rodu *Pholiota* a dokonce ani mezi hnědovýtrusé houby. Pozdější interpretace jména *Pholiota fusus* jsou pochybné a navíc je nelze seriózně ověřit, protože chybí jakýkoli herbářový materiál. Pojetí druhu *Pholiota fusus* u většiny autorů 20. století vychází z Rickena. Bohužel, *Flammula fusa* sensu Ricken je zřejmě neexistující druh, protože v jeho popisu se mísí znaky několika různých druhů. Nemáme také žádný spolehlivý důkaz, že existuje samostatný druh rodu *Pholiota*, který by odpovídal Batschovu původnímu popisu nebo některé z pozdějších interpretací. V důsledku toho je nutno považovat druh *Pholiota fusus* za pochybný a toto jméno je třeba zavrhnout.

INTRODUCTION

Pholiota fusus (Batsch) Singer or *Flammula fusus* (Batsch) P. Kumm. based on *Agaricus fusus* Batsch is included in most floras of the 19th century and appears in some works of the 20th century (the epithet is sometimes cited as

"*fusa*"). Records documented by herbarium material were recently published by Bon (1971), Tjallingii-Beukers (1987) and Noordeloos (1999). This paper aims to critically evaluate the taxonomic position and value of this less known species based on collections of the aforementioned authors.

RESULTS

Pholiota fusus (Batsch) Singer

Agaricus fusus Batsch, Elench. fung., Cont. secunda: column 13, 1789. – *Flammula fusus* (Batsch) P. Kummer, Führer Pilzk.: 82, 1871, "*fusa*". – *Dryophila fusus* (Batsch) Quél., Enchir. fung.: 70, 1886. – *Pholiota fusus* (Batsch) Singer, Lilloa 22: 516, 1951 ("1949"), "*fusa*".

Holotype: Batsch, Elench. fung., Cont. secunda, tab. 32, fig. 189a-c, 1789 (illustration serving as the holotype). Type locality: Germany, hill near Jena, in a pine wood, 20 Sep. 1788, leg. A. J. G. C. Batsch.

Description of recent collections identified as *Pholiota fusus*

The description of the macrocharacters is based on records annotated and published by Bon (1971), Tjallingii-Beukers (1987) and Noordeloos (1999) as I did not see any fresh collection of this taxon.

Pileus up to 10 cm, convex with strongly involute margin, expanding with age, sometimes trapezoidal in profile, finally slightly concave at centre, fleshy, not hygrophanous, not translucently striate, not scaly, smooth. Pileus cuticle slightly viscid when moist, slightly lustrous when dry, dark rusty-brown to red-brown or rather paler red-brown to brick-brown; fulvous to cream-rusty towards the margin, with fibrillose veil patches at margin (veil colour – Bon 1971: whitish to silvery, Noordeloos 1999: lemon to sulphur-yellow). Lamellae crowded, L=40–70, l=1–5, thin, adnate-emarginate or subdecurrent, greyish-yellowish to olivaceous-ochre when young, then grey-brown to chocolate-brown ("bistre-chocolaté" according to Bon 1971), edge remaining yellow, lemon yellow or olivaceous. Stipe 10–12 × 2.5–4 cm, cylindrical in upper part but distinctly tapering in basal part or the whole stipe gradually tapering downwards, with whitish evanescent fibrillose-submembranaceous annular zone, yellowish in upper part, towards the base gradually pale fulvous to dark brown-red or brownish-black at base (bistré), sometimes with several armillate traces at base. Context white, whitish or pale lemon-yellow in inner part of pileus, red brown in cortex of pileus, whitish to yellow in stipe, spongy, finally brownish in stipe base. Taste mild, sweetish, then

slightly bitterish after chewing, smell slightly aromatic on cross-section (like beer according to Bon 1971). Spore print colour unknown.

The description of the microcharacters is based on personal study of collections mentioned below. Spores 5.2–6.0 × (3.4-)3.7–4.0(-4.3) μm, ellipsoid-ovoid, ovoid or almost subamygdaliform-ovoid, slightly inequilateral, smooth, rather pale, yellowish-brownish to greyish-ochre in KOH, wall yellow-brown, moderately thick, germ pore well distinct, 0.6–1.0 μm broad, looking like a gap at the spore apex, the apex sometimes almost truncate but covered with a fine convex "cap" over the gap filled with a gelatinous substance. Basidia 14–16 × 5–6 μm, narrowly clavate, 4(2)-spored. Cheilocystidia forming a sterile band on the edge, 23–34 × 6–8 μm, narrowly cylindrical-clavate, narrowly clavate, sometimes also narrowly fusiform-lageniform or narrowly utriform, often constricted at several places and with a subcapitate apex, thin-walled, mostly hyaline but sometimes partly filled with a pale yellow homogeneous pigment. Pleurocystidia of the chrysocystidia type, very abundant, 24–38 × 10–15 μm, clavate or clavate-fusiform with a conical, mucronate to rostrate apex, with a refractive inclusion colouring yellow in a KOH solution or NH_4OH or almost completely filled with a yellow-rusty to rusty-brown refractive substance, thin-walled, absent on lamellae edge. Lamellar trama regular to slightly subregular, made up of hyphae 3–15 μm broad, cells cylindrical to narrowly fusiform, sometimes slightly yellow-rusty membranal pigmented or filled with a pale yellow substance. Pileus cuticle a cutis, 2-layered, upper layer relatively pale, sometimes slightly gelatinised, made up of rather densely arranged, parallel to slightly interwoven cylindrical hyphae 1.5–6.0 μm broad, with yellow-rusty membranal pigmentation and strong rusty-brown incrustations, gradually passing into the lower layer which is much darker (brown), made up of densely arranged parallel hyphae 5–15(-23) μm broad, cells cylindrical, fusiform, barrel-shaped, broadly ellipsoid, ovate to almost subglobose ("subcellular hypodermium"), with yellow membranal pigment and strongly developed rusty-brown incrustations forming small "plates" on hyphae surface. Stipe cuticle a trichoderm made up of upturned, straight, curved or interwoven hyphae 3–6 μm broad, terminal elements not cystidia-like, cell wall with a yellow membranal pigment and rusty-brown incrustations. Clamp connections present in all tissues.

Growing as a saprophyte on wood of deciduous trees, for instance *Betula* (Noordeloos 1999). Fructification: September-October.

Brief survey of the most important characters of *Pholiota fusus* sensu Bon, Tjallingii-Beukers and Noordeloos

Fruitbodies moderately large to large, pileus fleshy, smooth, not scaly, dark rusty-brown to red-brown, paler red-brown or brick-brown; fulvous to cream-rusty towards the margin, lamellae at first greyish-yellowish, olivaceous-ochre or greyish

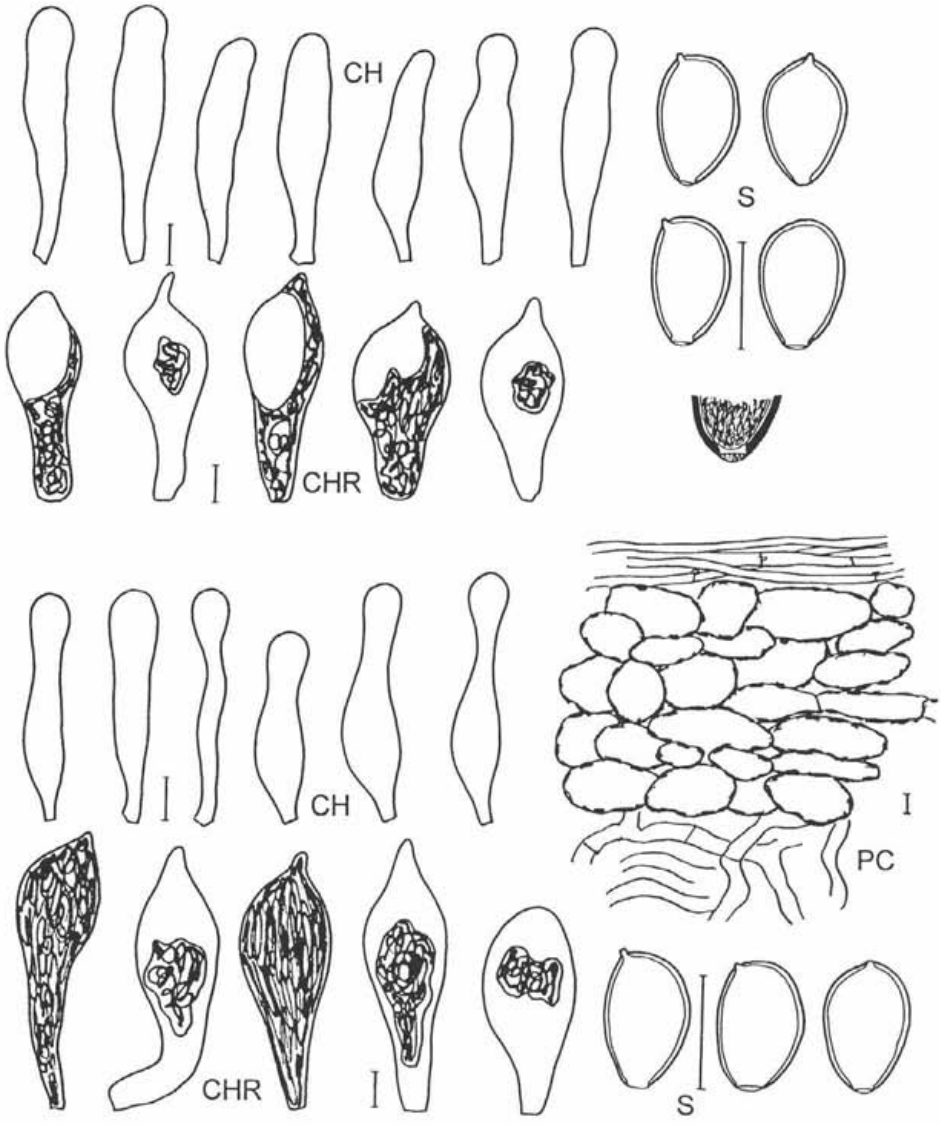


Fig. 1. France: Fresnes-sur-Escaut, near Bon Secours, on trunk (deciduous tree?), Oct. 1969, leg. Coppin (herb. Bon 91090).
The Netherlands: Prov. Utrecht, Huis ter Heide, on stump of *Betula*, 20 Oct. 1956, leg. J. A. R. v. Stolk (L 956.140 380).
CH: cheilocystidia, CHR: chrysocystidia, S: spores, PC: pileus cuticle. Scale bar = 5 μm. Ill. J. Holec.

-greenish, then grey-brown to chocolate-brown, stipe cylindrical in upper part but distinctly tapering in basal part or the whole stipe gradually tapering downwards. Spores 5.2–6.0 × (3.4-)3.7–4.0(-4.3) μm, ellipsoid-ovoid, ovoid or almost subamygdaliform-ovoid, germ pore well distinct, 0.6–1.0 μm broad, cheilocystidia cylindrical-clavate, narrowly clavate, sometimes also narrowly fusiform-lageniform or narrowly utriform, often constricted at several places and with a subcapitate apex, chrysocystidia present at lamellae surface, pileus cuticle a cutis, 2-layered, upper layer formed by cylindrical, fusiform, barrel-shaped, broadly ellipsoid, ovate to almost subglobose cells 5–15(-23) μm broad ("subcellular hypodermium"). Growing as a saprophyte on wood of deciduous trees.

DISCUSSION

1. Recent records named *Pholiota fusus* (Batsch) Singer from France (Bon 1971) and the Netherlands (Tjallingii-Beukers 1987, Noordeloos 1999; these two descriptions are based on the same collection, see Collections studied) are quite identical in macro- and microcharacters. The main diagnostic characters are summarized above. These characters are in contradiction to the original description of *Agaricus fusus* Batsch. His fungus is small, has a rimose pileus surface and pale lamellae (see also Figs. 189 a, b, c by Batsch). It was found on soil among needles in a pine wood. These discrepancies are so distinct that the name *A. fusus* cannot be used as a basionym for the aforementioned collections which represent a rather fleshy lignicolous fungus with glabrous pileus and rather dark coloured lamellae.

2. In my opinion, the records published by Bon (1971), Tjallingii-Beukers (1987) and Noordeloos (1999) do not belong to the genus *Pholiota*. This opinion is based mainly on the presence of ellipsoid, ovate to almost subglobose cells in the lower layer of the pileus cuticle. Bon (1971: 51) called this structure a "hypocutis pseudoparenchymateux". This character is quite atypical of the genus *Pholiota* where the lower layer of the pileus cuticle is formed by cylindrical or at least narrowly barrel-shaped cells without coarse incrustations. The "hypodermium forming a subcellular layer" is even an important diagnostic feature of the genus *Hypholoma* sensu Singer (1986, as *Naematoloma*). If compared with species of this genus (considered a subgenus of *Psilocybe* by Noordeloos 1999), the recent records named *Pholiota fusus* are almost identical with *Hypholoma sublateritium* (= *Psilocybe lateritia* sensu Noordeloos 1999). I found no substantial difference in microcharacters. Concerning the macrocharacters, the colours also are identical and the robust stature and distinctly tapering stipe in "*Pholiota fusus*" are the only differences. Unfortunately, the spore print colour was not observed in recent records of "*P. fusus*". However, the lamellae colour given by Bon (1971: ochre-olivaceous or grey-greenish, then dark chocolate-brown) and Noordeloos (1999: greyish-yellowish then grey-brown) better corresponds to a *Hypholoma* than to *Pholiota*. The

tapering stipe is known from *H. sublateritium* (see e.g. Noordeloos 1999: 70). I compared several collections of *H. sublateritium* with specimens of "*P. fusus*" from France and the Netherlands. They were proven to be completely identical in microcharacters. Based on all these facts, I consider *Pholiota fusus* sensu Bon, Tjallingii-Beukers and Noordelos an aberrant robust form of *Hypholoma sublateritium* (Fr.) Quél.

3. There are various interpretations of the name *Pholiota fusus* (Batsch) Singer in literature. The original *Agaricus fusus* by Batsch is hard to interpret (see above, point 1). Most probably it does not belong to *Pholiota* and not even to brown-spored fungi because the lamellae are described as "pallentes" or "von blasser Farbe" and the habitus is quite atypical of *Pholiota*. Figure 189 by Batsch is also difficult to interpret.

4. Plate 398 by Bulliard (Herb. France, vol. 9, 1789) is often mentioned as typical of *Pholiota fusus* (e.g. Fries 1838: 186, 1874: 247; Gillet 1874: 535; Bon 1971: 51; Noordeloos 1999: 91). Bulliard himself named the fungus in this plate *Agaricus hybridus*. It is also difficult to interpret this illustration. The fungus is rather robust, has yellow subdecurrent lamellae, white membranaceous partial veil, a distinctly downwards tapering stipe and grows on soil or in caves of old trunks. It is said to be extremely rare. I have no idea which species the table represents. It is worth mentioning that *P. fusus* is not included in the Flore analytique by Kühner and Romagnesi (1953). There is only a short discussion on page 332 and Bulliard's plate is not mentioned at all.

5. The species is included in old British floras (Cooke 1883: 169, Massee 1893: 134, Smith 1908: 151, Rea 1922: 317) and depicted by Cooke in plate 433 and 434 (Ill. Brit. fung., 1884). Plate 433 is cited by Bon (1971) as characteristic of his record. In my opinion, this illustration somewhat resembles old robust fruitbodies of *Pholiota lubrica*. The second one (pl. 434) certainly is something else because the lamellae are pure yellow and deeply decurrent. In his description, Cooke (1883) described the lamellae as ferrugineous when mature so that his *A. fusus* cannot be a *Hypholoma* species. Concerning the later descriptions, it is not clear if the authors saw fresh material (except for that by Rea who added "v.v." = vidi vivo; however, his description is partly taken from Ricken). The descriptions seem to be a mixture of data taken from other works (Batsch, Bolton, Fries, Cooke; see Massee 1893: 134). The spore print colour is never mentioned. With regard to these obscurities and lack of any herbarium material these interpretations cannot be identified with certainty. Similarly, in the British check-list (Dennis et al. 1960), *Flammula* or *Pholiota fusus* is "excluded pending clearer definition". However, it is clear that the British *Flammula fusus* is something else than the original *Agaricus fusus* Batsch.

6. The concept of *Pholiota fusus* used by some authors of the 20th century follows that of Ricken (1915: p. 206, Fig. 58/4, as *Flammula fusa*). A careful review of his description shows that it is probably based on fruitbodies of more than one

species. Based on my experience with *Pholiota* and *Hypholoma*, it is impossible that within one species the colour of mature lamellae is either rusty-yellow ("schliesslich rostgelb") or grey-olive to brown-olive ("aber auch grauoliv schl. braunoliv"). This is a mixture of pholiotoid and hypholomoid characters. The pholiotoid ones are represented by rusty-yellow lamellae and the spore print colour which is not purple according to Ricken. Hypholomoid characters are the grey and olive tinge of the lamellae and the nature of the veil on the pileus surface. In my opinion, figure 58/4 by Ricken represents dull coloured fruitbodies of *Hypholoma sublateritium* with grey-olive lamellae (compare Ricken's almost identical picture of *H. sublateritium* in Fig. 65/2). However, the spore size given by Ricken (8–9 × 4–5 μm) does not fit any of the species mentioned. During the work on *Pholiota* I did not find any fruitbodies or herbarium collections corresponding to Ricken's description of *Flammula fusus*. Herbarium specimens labelled with this name (from M and IB) appeared to be either *P. lubrica* or *P. pinicola*. Considering all the facts summarized here, *F. fusa* sensu Ricken is a non-existing entity based on a mixture of characters taken from various species. The possibility that his *F. fusa* is an unknown new species is unlikely.

7. Ricken's concept of *Pholiota fusus* has been used by Moser (1953, 1955, 1967, 1978, 1983; as *P. fusa*) in all editions of his key. No specimen of *Pholiota* corresponding to this concept was found among Moser's collections kept in IB. Due to the facts summarised in the previous paragraph, the real existence of this taxon is doubtful. The "species" is included in the flora by Kreisel et al. (1987) with a note that the fungus originally depicted by Batsch is certainly another species than *P. fusa* sensu modern authors (= Ricken, Moser etc.).

8. The illustration in Fries (Ic. hymenomyc. 2, Tab. 117: fig. 1, 1878) is designated as typical of *P. fusa* by Tjallingii-Beukers (1987) and Noordeloos (1999). Fries himself named this fungus *Agaricus fusus* Batsch * *filius* Fr. In my opinion, this taxon has nothing in common with the original *A. fusus* Batsch and with *Pholiota fusa* sensu Bon (1971), Tjallingii-Beukers (1987) and Noordeloos (1999). It is a clear *Pholiota* with a slender fistulose stipe and buff ("gilvus") pileus with rufous centre. Orton transferred the Friesian name to *Pholiota* as *P. filia* (Fr.) P. D. Orton. In my opinion, *Agaricus fusus * filius* is very close or even identical with *Pholiota mixta* (Fr.) Kuyper et Tjall.-Beuk.

CONCLUSIONS

The characters of recent records of *Pholiota fusus* (Batsch) Singer published by Bon (1971), Tjallingii-Beukers (1987) and Noordeloos (1999) do not agree with the original *Agaricus fusus* Batsch. The records most probably represent an aberrant robust form of *Hypholoma sublateritium* (Fr.) Quél.

The original *Agaricus fusus* Batsch is hard to interpret. The species probably does not belong to *Pholiota* or not even to brown-spored fungi.

Later interpretations of *Agaricus* (*Flammula*, *Pholiota*) *fusus* are dubious. It is not clear if the authors saw fresh material. Due to the lack of any herbarium material, their identity can not be verified.

The concept of *Pholiota fusus* used by some authors of the 20th century (Moser, Kreisel etc.) follows that of Ricken which is probably based on fruitbodies of more than one species. During my work on *Pholiota* I did not find any fruitbodies or herbarium collections corresponding to Ricken's description. Consequently, *Flammula fusa* sensu Ricken is considered a non-existing entity based on a mixture of characters taken from other species.

There is no reliable evidence (herbarium specimens or well-documented descriptions) that a separate species of *Pholiota* corresponding to Batsch's original description or various later interpretations really exists. Consequently, the name *Pholiota fusus* (Batsch) Singer must be considered a nomen dubium.

Collections studied: France: Fresnes-sur-Escaut, near Bon Secours, on trunk (deciduous tree?), Oct. 1969, leg. Coppin (herb. Bon 91090). – The Netherlands: Prov. Utrecht, Huis ter Heide, on stump of *Betula*, 20 Oct. 1956, leg. J. A. R. v. Stolk (L 956.140 380, L 973.123 503).

ACKNOWLEDGEMENTS

I thank Prof. M. Bon and Dr. M. E. Noordeloos for providing herbarium material for this study. The work was financially supported by the Grant Agency of the Czech Republic (Project No. 206/97/0273), the final elaboration also by Research Project (F02/98:NMPM00001) of the Ministry of Culture of the Czech Republic.

REFERENCES

BON M. (1971): Macromycetes du nord de la France. – Bull. Soc. Bot. Nord France 24: 43–60.

COOKE M. C. (1883): Handbook of British fungi. – 398 p. London.

DENNIS R. W. G., ORTON P. D. and HORA F. B. (1960): New check list of British Agarics and Boleti. – Trans. Brit. Mycol. Soc., suppl.: 1–225.

FRIES E. (1838): Epicrisis systematis mycologici, seu synopsis Hymenomycetum. – 610 p. Uppsala.

FRIES E. (1874): Hymenomycetes europaei sive epicriseos systematis mycologici editio altera. – 756 p. Uppsala.

JACOBSSON S. (1991) "1990": Pholiota in northern Europe. – Windahlia 19: 1–86.

KREISEL H. et al. (1987): Pilzflora der Deutschen Demokratischen Republik. – 281 p. Jena.

KÜHNER R. and ROMAGNESI H. (1953): Flore analytique des champignons supérieurs. – 557 p. Paris.

MASSEE G. (1893): British fungus-flora. Vol. 2. – 460 p. London.

MOSER M. (1953): Die Blätter- und Bauchpilze. – In: Gams H. (ed.), Kleine Kryptogamenflora von Mitteleuropa, vol. 2: 1–282, Jena.

MOSER M. (1955): Die Röhrlinge, Blätter- und Bauchpilze. – In: Gams H. (ed.), Kleine Kryptogamenflora , ed. 2, vol. 2b: 1–327, Jena.

MOSER M. (1967): Die Röhrlinge und Blätterpilze. – In: Gams H. (ed.), Kleine Kryptogamenflora, ed. 3, vol. 2b/2: 1–443, Stuttgart.

MOSER M. (1978): Die Röhrlinge und Blätterpilze. – In: Kleine Kryptogamenflora, ed. 4, vol. 2b/2: 1–532, Jena.

MOSER M. (1983): Die Röhrlinge und Blätterpilze. – In: Kleine Kryptogamenflora, ed. 5, vol. 2b/2: 1–533, Stuttgart.

NOORDELOOS M. E. (1999): Strophariaceae Sing. & Smith. – In: Bas C. et al., Flora agaricina neerlandica, vol. 4: 27–107, Rotterdam.

REA C. (1922): British Basidiomycetae. – 799 p. Cambridge.

RICKEN A. (1915): Die Blätterpilze. Vol. 1. – 480 p. Leipzig.

SMITH W. G. (1908): Synopsis of the British Basidiomycetes. – 531 p. London.

TJALLINGII-BEUKERS. D. (1987): Het geslacht Pholiota (Bundelzwammen). – Wetensch. Mededeling Koninkl. Nederl. Natuurhistor. Vereniging 185: 1–75.

Journal Review

Field Mycology, vol. 1, part 1.

New mycological magazine
published quarterly by Cambridge University Press for the British Mycological Society, Royal Botanic Gardens, Kew, Surrey TW9 3AE, Great Britain.
Edited by Geoffrey Kibby. E-mail of the Editorial: FieldMycol@aol.com. ISSN 1468-1641.
Price: 16 pound sterling for individuals, 26 for institutions.

The first number of the new mycological journal Field Mycology was published in January 2000. According to its editors, it should be a full colour journal providing articles of interest about fungi to the field mycologists, covering all aspects of identification, conservation, recording and collection, for all ages and educational levels. The editorial board consists mainly of non-professional mycologists, members of the British Mycological Society (for instance G. Kibby – editor in chief, A. Henrici, R. Phillips etc.). Professional mycologists are represented by P. Roberts from the Herbarium of the Royal Botanic Gardens, Kew. Geographically, the articles deal with all aspects of field mycology with an emphasis on British and northern European fungi.

The papers in the first volume can be divided into several main groups: 1. methods of collecting, identification and documentation in the mycology of macromycetes, 2. portraits, comments and colour photographs of interesting fungal species, 3. user friendly identification keys and introductions to several genera or fungal groups, 4. book reviews, 5. a profile of an mycological group, 6. reports on new finds of selected species of fungi.

The first impression of the new journal is good and pleasant. It is printed on quality paper and the reproduction of colour photographs is true. The layout is well-arranged and makes the text easy to follow. In my opinion, it is very important that so much space is devoted to methodology. What we need is to improve our skills and knowledge to be able to understand the specifics and difficulties of mycology. For beginners it is always better to understand the variability of macromycetes and be able to recognise the important macro- and microcharacters more than superficially know hundreds of species. In future volumes the amount of pages devoted to identification keys could even be reduced (they are often misleading if not based on thorough monographic knowledge of a particular genus or group) in favour of methodological papers (for instance: how to recognise different type of hyphae, cystidia, how to use stains and microchemical reagents, what are the important diagnostic characters of individual genera, how to document interesting records etc.). Consequently, the reader will become familiar with the conceptional background of present mycology and then be able to work independently. The new journal is set up this way which is likeable.

A journal like Field Mycology certainly is very interesting for people from all parts of Europe. Regarding some minor inaccuracies (e.g. p. 12: *Gloiothele lactescens*: amyloid ornamented spores, wrong!, p. 17: the same species: spores smooth, correct!), the editorial work should be done more carefully to ensure correctness of the information.

Finally, I wish Field Mycology a lot of success, many satisfied readers and good weather for collecting fungi that will be presented on its pages!

Jan Holec

Book Review

J. Heilmann-Clausen, A. Verbeken and J. Vesterholt (1998)

The genus Lactarius

In: Fungi of Northern Europe, vol. 2, 287 p.
Published by the Danish Mycological Society.
ISBN 87-983581-4-6.

A large gap after the *Lactarius* monograph by Neuhoff was recently filled in by Belgian and Danish mycologists. Although the authors did not aim to make a critical revision of all North European species (here defined as the area north of Paris, the Alps and the Carpathians mountains and west of the Ural mountains), the book is not only a modern identification guide (expression used by the authors themselves). It is a critical evaluation of the *Lactarius* flora of the mentioned area based on their own fresh collections, observations and original syntheses. Each of the 97 species recognised by the authors are presented in a brief, clear and practical way. The descriptions of macro- and microcharacters are based on fresh material cited in a special chapter and deposited in herbaria (mainly C or some private herbaria). All species are depicted in colour photographs. The quality of the photographs and their reproduction is excellent in most cases and makes the book very attractive both for professional and amateur mycologists. Colour terms used in descriptions are based on colour codes by Kornerup and Wanscher (1974), which is very important and practical in the case of tiny differences between related species (pileus and lamellae colour). Generally, the work is excellent concerning its scope and adequate methods of study. All facts can be checked which is not always common.

For natural reasons, there are some minor mistakes or questionable points which, however, do not principally influence the high quality of the book. The holotype of *Lactarius pilatii* Z. Schaefer was collected in the Šumava Mountains in southwest Bohemia (not Karlštejn in Central Bohemia). *Lactarius intermedius* is not included in the book. In *Lactarius decipiens* the host tree species are not mentioned. The photograph of *Lactarius subdulcis* is too pale although the dull brownish colour is stated as a distinguishing character of the species. Similarly, *Lactarius helvus* has usually more ochraceous fruitbodies (not so grey as in the photograph). Concerning this species, white milk is mentioned in the original description by Fries. In the Czech Republic, we know this species with hyaline watery milk (the same as given in the book of Heilmann-Clausen et al.). Therefore, the name *L. helvus* can not be used for the species and the name *Lactarius aquifluus* Peck should be used instead of it. The photograph of *L. trivialis* does not reflect the huge variability of that species. According to M. Beran, *Lactarius syringinus* is merely a form of *L. vietus*.

Methodologically, it would be interesting to study more macrochemical reactions, especially those of the milk (see e.g. Herink, Čes. Mykol. 10: 148-159, 1956; 11: 119-124, 1957). Similarly, the condensability of milk was not observed by the present authors - there are important differences among various species that can be used in their delimitation. In the identification keys, it would be better to use morphological and anatomical characters instead of ecological ones - in stands with mixed vegetation (mixed forests, parks) the identification would be more reliable. In key H, point 10, *Lactarius trivialis* should be included into the group of species with milk turning greenish-grey.

In our opinion, the descriptions and photographs of *L. romagnesii* and *L. ruginosus* are so similar that it is almost impossible to distinguish them. The concept of *Lactarius fulvissimus* seems to be rather broad (it is said to grow even under conifers in Central Europe) and inludes e.g. *Lactarius subsericatus* (Kühner et Romagn.) ex Bon, which is a good species. The concept of *L. aurantiacus* is also extremely broad (including *Lactarius mitissimus* (Fr.: Fr.) Fr. and *Lactarius aurantiofulvus* Blum ex Bon). The species is said to grow mainly on

rich or calcareous soils. However, in the Czech Republic we collect a species of this group for instance on acid soils in spruce forests (it is identified as *Lactarius aurantiofulvus*).

On the other hand, we fully agree with synonymisation of two of Romagnesis species - *L. hemicyaneus* and *L. quieticolor* which is well documented by the published photographs. The same concerns the synonymisation of *Lactarius zonarioides* Kühner et Romagn. and *L. bresadolanus* Singer, which is in agreement with observations by M. Beran.

The debatable points mentioned above do not change the positive impression of the book. The authors have produced a practical, well-documented publication which surely will become a handbook for all mycologists dealing with this interesting genus as well as with biodiversity, ecology and mapping of agarics.

Jan Holec and *Miroslav Beran*

Czech Mycology, published by the Czech Scientific Society for Mycology. Graphic design by B. Bednář, PISCES. Typeset by TEX. Printed by Čihák Press, Praha 10. Distributed by the Czech Scientific Society for Mycology, P.O.Box 106, 111 21 Praha 1, and Kubon & Sagner, P.O.Box 340108, 80328 München, Germany. Annual subscription: Vol. 52, 1999–2000 (4 issues), US $ 86,–, DM 136,–

INSTRUCTIONS TO AUTHORS

Preparation of manuscripts. Manuscripts are to be submitted in English, German or French. The text of the manuscript should be written on one side of white paper (A4, 210 × 297 mm) with broad margins (maximum 30 lines per page). Each manuscript must include *an abstract* (in English) not exceeding 100 words and a maximum of five key words. The paper will be followed by an abstract in Czech (or Slovak). The journal is responsible, however, for the translation of abstracts into Czech for foreign authors. Please send *two copies* of the typescript. The authors are asked to submit diskettes with *the accepted manuscripts* prepared on personal computers. The files should be in ASCII format, graphs in Excel. Both HD and DD/3.5″ and 5.25″ diskettes are acceptable.

Illustrations and tables. All tables, black and white photographs and figures (in black indian ink on a separate sheet) combined with the legends should be self-explanatory. Legends to the figures must be typed on a separate sheet. Colour photographs can be accepted but the authors will be responsible for the costs. All drawings or a photographs of microstructures should be provided with a scale. All illustration should be submitted as *the original drawing and one clear* copy. Output from computer graphics programmes produced on plotters or laser printers is quite acceptable. The dimension of any figure should not exceed 180 × 260 mm in size. References to illustrative matter in the text should normally in parentheses, e.g. ... spore sizes (Table 1) and ... as shown in Fig. 2 ... Figs. 1–5 ... Map 1 ...

Nomenclature. Latin names should conform to the International code of botanical nomenclature. New taxa must be substantiated by a Latin diagnosis including a reference to the public herbarium where the type specimen is deposited. The authors are asked to use only the acronyms listed in the Index Herbariorum.

References. References are to be listed in alphabetical order according to the surnames of the first authors. The bibliography should be written as follows:

Moravec J. (1984): Two new species of Coprobia and taxonomic remarks on the genera Cheilymenia and Coprobia (Discomycetes, Pezizales). – Čes. Mykol. 38: 146–155. (*journal article*)

Ryvarden L. (1978): The Polyporaceae of North Europe, Vol. 2. Inonotus-Tyromyces. – 507 p. Oslo. (*book*)

Tommerup I. C., Kuek C. and Malajczuk N. (1987): Ectomycorrhizal inoculum production and utilization in Australia. – In: Sylvia D. M., Hung L. L., and Graham J. H. (eds.), Proceedings of the 7th North American Conference on Mycorrhizae, p. 93–295, Gainesville.

The references *in text* should be Moravec (1984), or (Moravec 1984); or Kühner and Romagnesi (1974); When there are three or more authors use the form Tommerup et al. (1987).

Manuscript evaluation. All manuscripts will be reviewed by two reviewers, and the authors informed about their acceptance, rejection or necessary revisions within two months. If a manuscript is returned for revision, the authors should submit a revised version within three months.

Authors should preferably have their English language texts approved by a native – English speaker.

Proof corrections. Proofs of the paper will be sent to authors together with the original manuscript. If not returned within three weeks, the proof correction will be carried out by the editor. The principal author will receive 30 reprints free of charge.

Correspondence. All correspondence concerning the journal should be sent to the following address: Czech Mycology / Česká mykologie, National Museum, Department of Mycology, Václavské náměstí 68, 115 79 Praha 1, Czech Republic. Phone: 02/24497259 or 96151284

Czech Mycology, published by the Czech Scientific Society for Mycology. Graphic design by B. Bednář, PISCES. Typeset by TEX. Printed by Čihák Press, Praha 10. Distributed by the Czech Scientific Society for Mycology, P.O.Box 106, 111 21 Praha 1, and Kubon & Sagner, P.O.Box 340108, 80328 München, Germany. Annual subscription: Vol. 52, 1999 – 2000 (4 issues), US $ 86,–, DM 136,–

CZECH MYCOLOGY / ČESKÁ MYKOLOGIE

is an international scientific journal publishing papers in all aspects of mycology including taxonomy, ecology, physiology and mycofloristics as well as mycological topics in forestry, agriculture and medicine. Czech Mycology will publish full length papers and short communications reporting original research which make a significant contribution to mycology. Review articles are also published.

CONTENTS