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Tailoring fungal nomenclature to suit user needs

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Hawksworth D. L. (1995): Tailoring fungal nomenclature to suit user needs. – Czech Mycol. 48: 3–10

The nomenclature of fungi is controlled by the International Code of Botanical Nomenclature, revised at intervals of six years. The latest revision by the XV International Botanical Congress in Tokyo in 1993 signalled a major shift in botanical nomenclature towards increased pragmatism. The "top-ten" changes relevant to mycologists are summarized, and attention is drawn to a resolution of the Congress urging taxonomists to refrain from name changes for non-scientific reasons. Discussions have also been taking place between representatives of the Codes or Rules regulating the names of other organisms with a view to increasing harmonization between their practices and the eventual production of a single Code. Significant common ground has been established and the formation of an International Commission on Bionomenclature has been proposed. The pressure for change comes from both the generators and the users of names, and has targets which if realized will be of benefit to both groups.

Key words: Bionomenclature, code, harmonization, names, nomenclature, taxonomy.

Hawksworth D. L. (1995): Přizpůsobení nomenklatury hub potřebám uživatelů. – Czech Mycol. 48: 3–10

Nomenklatura hub je řízena Mezinárodním kódem botanické nomenklatury, který je upravován každých šest let. Poslední revize přijatá Patnáctým mezinárodním botanickým kongresem v Tokiu 1993 signalizovala velký obrat v botanické nomenklatuře směrem k narůstajícímu pragmatismu. Je zde nastíněno stručně deset nejdůležitějších změn, vztahujících se na mykologii a upozorňuje na resoluci Kongresu vybízející taxonomy, aby upustili od změn jmen z mimovědekých důvodů. Probíhala též jednání se zástuci autorů kódů nebo pravidel regulujících jména ostatních organismů, s vyhlídkou na sladění jejich uplatňování a na případné vytvoření jednotného kódu pro všechny organismy. Významný společný základ byl vytvořen a bylo navrženo zřídit Mezinárodní komisi pro bionomenklaturu. Tlak na navržené změny přichází jak ze strany tvůrců tak i ze strany uživatelů jmen a směřuje k cílům, které kdyby byly uskutečněny, byly by k užitku obou skupin.

INTRODUCTION

The subject of nomenclature is not something which excites the active mycologist. Rather, it is perceived as an unavoidable and too often also a laborious chore by the systematist, and as a cause of irritation by mycologists in general who view name changes as a way of obfuscating communication.

The situation has been aptly summarized by Weresub (1970: 788): "... botanists cherish labyrinthine convolutions of thought and claim the right to burden the future with tortuous mazes".

In the last 6-7 years in particular, the realization that the *status quo* is unsatisfactory to both the generators and users of scientific names has become

widely recognized. Here I wish to draw attention both to some significant changes which have already taken place, and also to new directions currently being pursued. As these changes are already affecting the working practices of systematic mycologists, and will increasingly do so in the future, it is important that they are broadcast widely and fully debated at this time.

BACKGROUND

The nomenclature of fungi (including yeasts and lichen-forming fungi) is covered by the International Code of Botanical Nomenclature (ICBN). This is revised at six-year intervals by each International Botanical Congresses, the most recent being the XVth held in Yokohama, Japan, in August-September 1993 (Greuter *et al.* 1994a).

Proposals to change the way in which the Code operates must first be published in *Taxon*, the official journal of the International Association for Plant Taxonomy (IAPT). These are compiled prior to each Congress and issued together with comments (Greuter and McNeill 1993). A mail ballot of IAPT individual members and those making proposals is conducted, and made available to those present at the Nomenclature Section of the Congress (McNeill 1994).

At the Nomenclature Section, the proposals are debated and the often impassioned debates taped for subsequent transcription and publication (Greuter *et al.* 1994b). Individual botanists registered for the Congress and also institutions which are represented all have votes, the number given to an institution being decided in advance by an IAPT committee. A 60 % majority is required for a proposal to be accepted. Most decisions are effective immediately, although the new Code is not now issued until 10-12 months after the Congress. An Editorial Committee appointed by the Congress decides the final arrangement and in some cases wordings when charged so to do by the Congress.

The Code is now officially published only in English, but translations in French, German, Japanese, and Russian were prepared and authorized by the IAPT after the Berlin Congress in 1987.

The objective of the Code is to promote stability in the names applied to a taxon in a particular taxonomic position and rank. I.e. it does not dictate or impede changes in names for scientific reasons. The reality is rather different. The way the Code now operates repeatedly leads to name changes for nomenclatural rather than scientific reasons. These changes can be due to either the application of the rules themselves or to changes in the rules.

The 1993 Congress was confronted by 320 proposals to change the rules. In the 127 years since the first Code was issued in 1867 (De Candolle 1867), even stable rules have not been attained.

This has become a major cause for concern amongst the users of names and increasingly of taxonomists. Indeed it gives taxonomists a negative and irritating

image and engenders a reluctance to take up name changes generally, and even where they represent new scientific insights.

The International Union of Biological Sciences (IUBS), to which the IAPT and International Botanical Congresses are affiliated, has become increasingly anxious about this situation. IUBS was one of the main sponsors of a meeting held at Kew in 1991 to openly debate various ways of reducing name changes for non-scientific reasons (Hawksworth 1991). Many of the fundamental proposals considered at the Tokyo Congress in 1993 were formulated at that meeting.

CHANGES AT THE TOKYO CONGRESS 1993

It was auspicious that the close of the Nomenclature Session of the Tokyo Congress coincided with a typhoon. The net effect of a raft of proposals which received the necessary majority was a shift towards pragmatism, and has been perceived as placing botanists "on the threshold to a new nomenclature" (Greuter and Nicolson 1993: 925).

The new edition of the Code must be consulted for the numerous changes enacted at this Congress (Greuter *et al.* 1994a). My "TOP TEN" selection of those changes which will impact most on mycologists are summarized in Table 1, and fuller information about these is provided by Greuter and Nicolson (1993), Hawksworth (1993), and Nicolson and Greuter (1994).

The net effect of this suite of changes is that it is now possible to avoid name changes for non-scientific reasons in almost all ranks, although this appears to have come as rather a shock to at least one participant at the Tokyo Congress (Brummitt 1994).

Especially important and symptomatic of the changed attitude was an all-Congress Resolution which: "urges ... taxonomists, while such work continues, to avoid displacing well established names for purely nomenclatural reasons, whether by change in their application or by resurrection of long-forgotten names; ...". This is a clear instruction from the body from which the Code derives its authority, and one which transcends the Code itself. It does not mean that the Code should not be followed, but rather that the full power of the new possibilities should be tried first. If they have not, making changes for non-scientific reasons should be delayed until at least the next International Botanical Congress to be held in St Louis in 1999.

The type of statement which will increasingly seen in the work of responsible taxonomists is exemplified by that of Vitikainen (1994: 217) who did not take up an earlier name for *Peltigera laciniata* as "Its adoption would... be a disadvantageous nomenclatural change, and a proposal to include it [*i.e.* the earlier name] in the list of nomina rejicienda will accordingly be made elsewhere".

The President of IUBS wrote to its national and scientific members in March 1994 to request them to alert systematists, editors and referees to this changed position – to discourage them from accepting papers proposing name changes for non-scientific reasons. In order to enhance the standing of our subject, it is important that all taxonomists take due note and act accordingly in response to this dramatically changed situation.

NAMES IN CURRENT USE

One important series of proposals submitted to the Congress, but which did not receive the requisite majority, was the provision to grant a protected status to Lists of Names in Current Use (NCUs). It had been proposed that names on suitably checked lists would be protected against earlier unlisted names and homonyms, with spellings, places of publication, dates, and types as listed.

Three Lists has been prepared for consideration by the Tokyo Congress (Greuter *et al.* 1993a, b, c), that on generic names (Greuter *et al.* 1993a) being especially significant in covering 28 041 names and having received input from 219 taxonomists, and in being prepared and published in only five years.

While the NCU proposals failed by a whisker, gaining 55 and not the required 60% of the vote at the Nomenclature Section, the Congress did agree to establish a Permanent Committee on NCUs with a mandate "to initiate, assist, coordinate and vet the production of lists and updatings of the existing lists of NCU".

However, sympathy for the concept of adopting well-researched lists was evidenced by a special resolution on the list of species and varietal names within one family, the *Trichocomaceae* (which includes *Aspergillus* and *Penicillium* anamorphs; Greuter *et al.*, 1993c): the "Nomenclature Section, noting that the List of Names in Current use for the *Trichocomaceae*, which has already been approved by the International Commission on *Penicillium* and *Aspergillus* of IUMS, urges taxonomists not to adopt names that will compete with or change the application of any names on that List".

With this precedent established, it is anticipated that further well-researched lists and internationally mandated lists produced before and submitted the 1999 Congress are also likely to be the subject of special resolutions.

HARMONIZATION BETWEEN CODES OF NOMENCLATURE

Biological nomenclature as a whole is under greater scrutiny than at any time in its history, and the five different internationally mandated Codes or Rules have common problems to confront; apart from botany (Greuter *et al.* 1994), these are concerned with bacteria (Sneath 1992), cultivated plants (Brickell *et al.* 1980),

viruses (Francki *et al.* 1990, Mayo 1994), and zoology (International Commission on Zoological Nomenclature 1985).

The International Union of Microbiological Societies (IUMS) and IUBS sponsored an inter-code Exploratory Meeting on "Harmonization between Codes of Nomenclature" with 2-3 representatives of each Code in March 1994. The need to work towards increased harmonization, and to a unified Biological Code was embraced for the first time. Common ground was identified, and different practices were discussed in depth.

Highlights amongst the conclusions of this Exploratory Meeting are summarized in Table 2. A full account of the pioneering discussions held on that occasion is provided in Hawksworth *et al.* (1994). In the event that the proposed International Commission on Bionomenclature is established, significant developments towards a more effective system for the nomenclature of all organisms can be anticipated, so that appropriate proposals can be developed and submitted to the International Botanical Congress in 1999.

Attention is also drawn to a draft glossary of official and unofficial terms used in bionomenclature developed from one of the background documents prepared for the Exploratory Meeting (Hawksworth 1994a).

WHY THE DIRECTION HAS CHANGED

The drive for change comes from both the generators and the users of names. The generators of taxonomies need to be freed from as much nomenclatural drudgery as possible. A poll in 1991 indicated that this was on average about 20 % of the research time available (Hawksworth 1992). In the case of mycology, this is critical in the light of our level of ignorance and the limited and declining taxonomic workforce. There is a need to concentrate on the 95 % or so unknown fungi and not continually rework the "known" 5 %.

The user community has become irritated with the numbers of name changes for reasons they do not understand. In the case of *Aspergillus* and *Penicillium*, 38% (41 of 205) and 53% (73 of 150) respectively of names used in previous monographs were not used in the more recent (Hawksworth 1994b).

The result is that such work is often ignored, as indicated by the take-up of names in the *Biological Abstracts* (BIOSIS) database (Hawksworth 1992, 1994b). For example, the change in name from *Aspergillus nidulans* to *A. nidulellus* has for all practical purposes been ignored (1 usage out of 1026 since 1985), as has that of *Podospora anserina* to *P. pauciseta* (2 usages out of 318 since 1970). Even after over 20 years, *Cephalosporium acremonium* still predominates over *Acremonium chryosgenum* and *A. strictum* in use (337 out of 449 usages since 1970). Changes in names which reflect advances in science, and which add to the predictive value and utility of names, should be enthusiastically embraced by users, yet they are

Tab. 1 – A "TOP TEN" of changes to the International Code of Botanical Nomenclature pertinent to mycologists in adopted at the International Botanical Congress in 1993

1	Rejection of names extended to any name that could cause a disadvantageous change.
2	Conservation of species names without restriction.
3	Metabolically inactive cultures accepted as types.
4	The ability to designate an "epitype" when the original material is not diagnostic
5	Establishment of a list of "suppressed works" not to be used as a source of names.
6	Registration of names to be a condition of valid publication from 2000.
7	"Phylum" approved as an acceptable alternative to "Division".
8	Clarification that taxa traditionally treated in the Botanical Code remain covered even if now referred to other kingdoms.
9	The word "in" not to be used as a part of author citations.
10	The "Committee for Fungi and Lichens" renamed as the Committee for Fungi".

Tab. 2 – Highlights from the IUBS/IUMS Exploratory Meeting on Inter-code Harmonization 1994 (Hawksworth *et al.* 1994)

1	To work towards a unified system of biological nomenclature.
2	There is considerable scope for harmonization, even though differences in procedures could not be fully reconciled for the nomenclature of the past.
3	The availability of lists of published names, and the registration of new names, will facilitate the harmonization of procedures.
4	Author citations should be made optional (and be recommended only in a strictly taxonomic context).
5	The nomenclatural problems posed by ambiregnal organisms, can be accommodated by modifications to the existing Codes.
6	Authors of new generic names should avoid proposing a name established under another Code, and provisions be introduced into each Code to disallow new generic names that are junior homonyms under any Code.
7	The use of different type faces for scientific names in all ranks is desirable.
8	A unified *Glossary of Terms Used in Bionomenclature* to be produced.
9	An IUBS/IUMS *Commission on Bionomenclature* to be established.

being ignored along with the research that provided the basis for those changes in classification. The baby is being thrown out with the bathwater.

FUTURE PROSPECTS

I find it remarkable that so much progress has been made towards making nomenclature a pragmatic servant of science, rather than a historical and pseudolegalistic endeavour, in so short a time. The issue only started to be raised in earnest in 1987, prior to the XIV International Botanical Congress held in Berlin.

Well-established changes in working practices are never easy to accept, but if the benefits are sufficient, we should not be deterred and "grasp the nettle". The key benefits being targeted are:

(1) An increasingly unified approach to nomenclature across all biology.
(2) A reduction in the nomenclatural burden of biosystematists to an acceptable level.
(3) A reduction in name changes for non-scientific reasons.
(4) A rise in the standing of biosystematics within biology.

I believe these four targets are well-worth aiming at. We may not score bulls-eyes first time, and any new procedures require very full debates before implementation. However, we can be certain that if no arrows are fired, no target will be hit.

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Worldwide occurrence of psychoactive mushrooms – an update

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Stijve T. (1995): Worldwide occurrence of psychoactive mushrooms – an update. – Czech Mycol. 48: 11–19

An update is given on the recorded psilocybin – and ibotenic acid containing mushrooms on a worldwide scale. Many new psilocybian species have been discovered during the last 15 years, including representatives of the genera Psilocybe, Panaeolus, Inocybe, Gymnopilus and Pluteus, whereas only Amanita regalis was identified as a new and potent source of ibotenic acid. Recreational use of psychoactive mushrooms has spread from the USA to Europe, but here, like anywhere else, it remains a marginal phenomenon. The mushrooms commonly used are limited to a few species: in Europe it is almost invariably Psilocybe semilanceata, whereas in the USA Ps. cubensis, is widely used. Locally, Ps. stuntzii and Panaeolus subbalteatus have also gained some popularity. Misuse in South America or Asia is virtually unknown, in spite of the easy availability of psychoactive fungi. The trade in "magic mushrooms" e.g. Ps. cubensis and Copelandia cyanescens on the Thai island of Koh Samui, or in Indonesian Bali only caters to European and American tourists. Finally, young people and members of the drug-using subculture in Australia and New Zealand have also become aware of the psychoactive fungi growing in their respective countries

Key words: Psychoactive mushrooms, psilocybin, ibotenic acid, recreational use

Stijve T. (1995): Současný stav výskytu psychoaktivních hub na světě. – Czech Mycol. 48: 11–19

Je podán přehled o současném výskytu hub obsahujících psilocybin a kyselinu ibotenovou. V posledních 15 letech bylo zjištěno mnoho druhů obsahujících psilocybin zahrnujících zástupce rodů Psilocybe, Panaeolus, Inocybe, Gymnopilus a Pluteus. Pouze Amanita regalis byla identifikována jako nový a silný zdroj kyseliny ibotenové. Rekreační užívání psychoaktivních hub se rozšířilo z USA do Evropy, ale zde, jakož i kdekoli jinde zůstávají psychoaktivní houby okrajovým fenoménem. Houby všeobecně užívané jsou omezeny na několik druhů: v Evropě je to většinou Psilocybe semilanceata, zatímco v USA je široce užívaná Psilocybe cubensis. Místně je populární Ps. stuntzii a Panaeolus subbalteatus. Zneužívání halucinogenních hub v Jižní Americe nebo Asii je prakticky neznámé, přestože jsou zde psychoaktivní houby snadno dostupné. Obchod s „kouzelnými houbami" např. Ps. cubensis a Copelandia cyanescens na thajském ostrově Ko Samui (Kosamui) a indonéském Bali slouží k uspokojení turistů z Evropy a Ameriky. Mládež a členové komunit užívajících drogy v Austrálii a na Novém Zélandě jsou si vědomi, že i v jejich zemi rostou psychoaktivní houby.

The subject matter of this short review will be confined to mushrooms containing psilocybin and psilocin, and to those *Amanitaceae* that are characterised by isooxazole derivatives such as ibotenic acid. This excludes the *Russulaceae* and *Boletaceae* which are ritually used in New Guinea, but of which the active principle (if any) has not yet been isolated, and the Pyrenomycetes containing the well-known ergot alkaloids.

Already in 1978, Singer contributed a very good review article to the now classic CRC book on "Mushroom Poisoning" by Rumack and Salzman (Singer 1978). Singer listed then not less than 38 hallucinogenic *Psilocybe* species, mostly from

Mexico, Meso-America and the USA. In addition, he cited *Conocybe cyanopus*, and two *Coprinaceae, Copelandia cyanescens* and *Panaeolus subbalteatus* as hallucinogenic mushrooms, while reserving his opinion on a few blue-staining *Gymnopilus* in which the occurrence of psilocin/psilocybin had not yet been convincingly demonstrated.

As for psychoactive *Amanitae*, Singer mentioned of course *A. muscaria* and *A. pantherina* as species containing ibotenic acid and muscimol, but he rightly pointed out that this group of *Amanitae* is taxonomically rather complex, and that many varieties, on both sides of the Atlantic have not yet been studied in this respect. We may expect that appreciable progress has been made since 1978 and indeed, many more psychoactive mushrooms, especially psilocybian species have been discovered. Not long ago, a very useful index was published by Allen et al. (1992) who listed not less than 130 psilocybin-containing species from 12 genera, belonging to 6 families. These authors have considerably extended the afore-mentioned list by Singer (1978), and also that given in Guzman's monograph (Guzman 1983). Allen et al. (1992) pretend that 90 of the about 300 recognised *Psilocybe* sp. are hallucinogenic.

We have critically examined this by checking the evidence available in the literature, which yielded the following results: for 40 taxons we found reports on chemical analysis proving the presence of the indole alkaloids. For another 39 species no analysis was available, but the original papers and Guzman's monograph reported blueing tissues and/or hallucinogenic action. For seven species no such information could be found and an additional four were unknown in the literature.

This means that about 80 *Psilocybes* are psychoactive, but unfortunately this does not help much in the taxonomy!

Curiously enough, the number of hallucinogenic European *Psilocybes* has not increased since the early seventies. The ubiquitous *Ps. semilanceata* (Fr.) Kummer (Hofmann et al. 1963, Repke and Leslie 1977, Christiansen and Rasmussen 1982, Stijve 1984, Stijve and Kuyper 1985), also known as the Liberty Cap is still by far the most important. *Ps. bohemica* Šebek (Semerdžieva and Wurst 1986), *Ps. cyanescens* Wakefield (Benedict et al. 1962, Beug and Bigwood 1982) and *Ps. liniformans* Guzman et Bas (1977) are rare or, at least, not widely distributed.

On the other hand, many more psilocybin-containing mushrooms belonging to other genera have been described, especially by European authors. Quite unexpectedly, the hallucinogen turned up in the genus *Inocybe*, as was discovered by accident in Germany by Drewitz (1983) who described a curious case of poisoning by *Inocybe aeruginascens*, which was mistaken for edible *Marasmius oreades*. Soon afterwards, the presence of psilocybin and baeocystin was confirmed by chemical analysis (Stijve et al. 1985, Gartz and Drewitz 1985), and 4 other *Inocybe* species, mostly rare ones, were also found to contain those hallucinogens (Stijve and Kuyper 1985, Stijve et al. 1985).

In *Gymnopilus*, another genus belonging to the Cortinariaceae, several sp. have been suspected to contain psilocybin/psilocin (Hatfield and Valdes 1978), but this has only been confirmed for *G. purpuratus* (Cooke et Massee) Sing., an exotic species from South America and Australia, which was accidentally introduced into Germany during the 80 ies (Kreisel and Lindequist 1988, Gartz 1989).

It is often thought that there are many hallucinogenic species among the *Panaeoloideae*. However, in Europe there is only *P. subbalteatus* (Berk. et Br.) Sacc., that has been unequivocally established to contain psilocybin in significant concentrations (Beug and Bigwood 1982, Ola'h 1968, Fiussello and Scurti Ceruti 1972, Stijve 1985).

In popular handbooks *Panaeolina foenisecii* is said to be psychoactive, in spite of the negative reports that have been published during the last ten years (Beug and Bigwood 1982, Stijve et al. 1984, Allen and Merlin 1991). The error can be explained by confusion with *P. subbalteatus*, a taxon superficially resembling *P. foenisecii*, and in the USA both mushrooms often grow together on well-manured lawns, which is not or seldom the case in Europe (Stijve 1987).

In the tropics there are a number of *Copelandia* sp. of which *C. cyanescens* (Berk. et Br.) Sing., also known as *Panaeolus cyanescens* (Berk. et Br.) Sacc. is the most widely distributed. This blue-staining mushroom is strongly hallucinogenic, because of its high psilocin content, which may exceed 1 percent on dry weight. It is interesting to note that collections from Hawaii also contain significant levels of psilocybin, whereas this compound is virtually absent in collections from Australia and Thailand (Stijve 1992).

Some uncertainty still exists about Ola'h's latent psilocybian species (Ola'h 1969), but it could well be that the phenomenon exists. Indeed, although *Panaeolus campanulatus* and *P. fimicola*, both from the USA and Europe, have generally been found not to contain any psilocybin, the presence of this compound was recently unquestionably established in collections of both *Panaeoli* from Southern Brazil (Stijve and de Meijer 1994).

Since 1981 we know that the genus *Pluteus* also comprises some taxons containing psilocin/psilocybin and some as yet unidentified tryptamine compounds (Saupe 1981, Stijve and Bonnard 1986, Stijve and de Meijer 1993). The most common species in Europe is the slightly greenish staining *Pluteus salicinus*. Another one, *Pl. nigroviridis* Babos is most rare (Stijve and Bonnard 1986). We recently demonstrated the presence of the hallucinogens in *Pl. glaucus* Sing. and in a closely related taxon, both collected in Southern Brazil (Stijve and de Meijer 1994).

Lately, Besl discovered in a hothouse of the botanical institute at Regensburg a new species of *Galerina*, that was described as *Galerina steglichii* spec. nov. This tiny mushroom had blueing tissues and was found to contain psilocybin (Besl 1994).

It is not unthinkable that psilocybin and related compounds will turn up in other mushrooms, but there have been erroneous reports on the subject, as

was pointed out by Stijve and Kuyper (Stijve and Kuyper 1988) *Mycena pura* and/or closely related taxons have been recognised as hallucinogenic (Heim 1963, Giacomoni 1984), but the active principle has not yet been isolated. Moreover, some exotic *Hydnaceae* such as *Sarcodon atroviridis* were found to contain not less than 4 unidentified tryptamine derivatives (Stijve 1993). On the other hand, a publication about a hallucinogenic *Lepiota* from Florida (Anonymous 1983) was in all probability a hoax. We can be brief about ibotenic acid – containing *Amanita* sp. Although Ott (1978) has claimed widespread recreational use of both *A. muscaria* and – *pantherina*, this is somewhat hard to believe. Indeed, ingestion of these mushrooms prompts mostly severe nausea, drooling, vomiting, whereas feelings of elevated mood and euphoria, followed by deep sleep and vivid dreams are far more rare.

Tab. 1 Ibotenic acid content of psychoactive Amanita species.

	Ibotenic acid determined as muscimol in percentage on dry weight
– Amanita muscaria	
ex Chamonix, Fr.	0.15
ex Lally, CH	0.16 – 0.22
ex Puidoux, CH	0.12
ex Paranã, Brazil	0.08 – 0.13
– Amanita regalis	
ex Gavle, Sweden	0.62 !!
– Amanita pantherina	
ex Gamburg, GFR	0.19
ex Puidoux, CH	0.31
ex Haute Savoie, Fr.	0.25

Few people who have tried it want to renew the experience (McDonald 1978). Chemical investigation of these mushrooms seems to have made little progress since Müller's and Eugster's discoveries (Müller and Eugster 1965, Good et al. 1965). The results of a limited survey carried out in our laboratory on the active principle in psychoactive *Amanita* species are given in table 1. It is noteworthy that

Amanita regalis, a stout variety of the fly agaric, was found to contain not less than 0,6 percent ibotenic acid, which is far more than ever reported for the other two *Amanitae* (Stijve 1982). This rare mushroom was said to have caused a pleasant delirium with a minimum of bodily discomfort. More collections should be analysed to check whether the high content is a distinguishing feature of *A. regalis*.

Modern recreational use of psilocybin-containing mushrooms both in the USA and Europe has been stimulated by popular and semi-scientific publications (Oss and Oeric 1976, Cooper 1980, Schreibler 1987, Stamets 1978). However, in spite of alarmist medical publications (Young et al. 1982, Jansen 1988) and lurid newspaper articles, the phenomenon remains marginal. Psilocin and psilocybin are not habit-forming and few individuals persist in using these mushrooms beyond the first experience of what is called a bad trip.

In Europe, from Italy to Finland (Semerdžieva and Nerud 1973, Christiansen et al. 1981, Michaelis 1977, Gartz 1993, Flammer and Horak 1983, Samorini and Festi 1988, Ohenoja et al.), it is invariably *Psilocybe semilanceata* that is used. There is indeed some selling of this dried mushroom (mostly at the equivalent of 2 – 3 Swiss francs/piece) in the big cities (Turberg 1984, Unsigned 1990), but it is really a minor street drug compared to heroin. The authorities in most European countries are aware of this. When the phenomenon reached Switzerland, the police used helicopters to chase the mushroom hunters on the alpine meadows. Now, some ten years later, such extreme measures have been abandoned, but the authorities still keep an eye on publications encouraging consumption of these fungi (Scheibler 1993).

Concerning recreational use of psilocybin mushrooms in the USA, the information contained in Jonathan Ott's article (Ott 1978) is still valid, although he exaggerates the number of species (21!) actually used for that purpose. The most widely used mushroom, gathered in the wild or cultivated (Stijve 1982) is *Psilocybe cubensis* (Earle) Sing., often called "golden tops", because of the golden yellow coloured pileus. *Psilocybe stuntzii* Guzman & Ott which was only discovered in the early seventies has also gained some popularity as has *Ps. cyanescens* Wakefield, which can also be grown both indoors and outdoors (Stamets and Chilton 1983). According to Ott, *Panaeolus subbalteatus* was commonly used in the Pacific Northwest 15 years ago. It could well be that its popularity has decreased now. In many persons it causes an upset stomach (Bigwood 1984). A recent report by Merlin and Allen (1993) discusses recreational use of *Copelandia (Panaeolus) cyanescens* in the Hawaiian islands.

Both *Ps. (sub) cubensis* and *Copelandia* sp. are sold to tourists in several resort areas of Thailand, notably on the islands Koh Samui and Koh Pha-ngan (Allen and Merlin 1992). Restaurants on these islands serve hallucinogenic omelettes, stews, soups, and even pizza's containing so-called "magic mushrooms", that is coprophilic *Ps.cubensis* grown in "farms" which mainly consist of well-manured rice paddies.

This practice is, of course, not without danger, because consumption of these dishes has sometimes induced bizarre and dangerous behaviour in tourists. Worse still, some restaurants serve omelettes containing more powerful synthetic hallucinogens, such as LSD! Similar practices using the same mushrooms are said to represent a minor tourist attraction on the Indonesian island of Bali (Hollander 1981).

There are no confirmed reports on abuse of psychoactive mushrooms in other Asian countries, even where legislation is absent. In Japan possession of such mushrooms is illegal, and one must even be cautious when transporting botanical specimens (Baumann 1990).

Gymnopilus spectabilis (Fr.) Sing. became first known as a hallucinogenic fungus in Japan (Romagnesi 1964). It is called there "Ohwaraitake", which means big laughter mushroom. It does not contain any psilocybin, but its active principle was recently characterised as a group of neurotoxic oligoisoprenoids (Tanaka et al. 1993).

We do not know anything about the possible use of psychoactive mushrooms in Africa, but the occurrence of those fungi there is more than likely. On the other hand, we are better informed about Australia and New Zealand, where the public at large has also become aware of psilocybian fungi growing in their respective countries (Shepherd and Hall 1973, Hall 1973). There is no evidence that the aboriginies were or are familiar with this kind of mushrooms. Recreational use seems to be limited to descendants of European immigrants who must have learned about the mind-altering effects by reading American literature. McCarthy (1971) has suggested that abuse of psychoactive fungi has been introduced by visiting American surfers at Australian beach resorts. Be this as it may, interest in hallucinogenic fungi was, as elsewhere, widely promoted by popular press articles describing the use of these mushrooms. Allen et al. mention that three agarics, *Psilocybe cubensis, Ps. subaeruginosa* Cleland, and *Copelandia cyanescens* are recreationally used in Australia (Allen et al. 1991), but the number of users is still small, and it is unlikely that hallucinogenic mushrooms will replace traditional stimulants.

Finally, it would seem that the "mushroom pandemic" (Pollock 1975) has not yet reached South America. Although psilocybin-containing fungi occur widely in countries like Argentina, Uruguay and Brazil (Guzman 1983, Stijve and de Meijer 1993) the local population is either unaware of and/or not interested in their psychoactive properties.

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Merits and limitations of immunodiagnostic assays for systemic mycoses

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Kaufman L. (1995): Merits and limitations of immunodiagnostic assays for systemic mycoses. – Czech Mycol. 48: 21–29

The incidence of systemic fungal diseases has increased significantly over the last decade. During that time considerable work has been done on isolating and characterizing new antigens and developing technology. However, few new immunodiagnostic tests for the mycoses have come into routine use.

Most of the currently used immunodiagnostic tests are designed to detect antibodies to specific fungal pathogens. These tests, though far from optimal, have proved useful for diagnosing aspergillosis, blastomycosis, candidiasis, histoplasmosis and other mycotic infections mainly in the immunocompetent host. They may, however, exhibit cross-reactivity, and fail to distinguish active form past infection, and colonization from invasive disease. More recently, attention has been devoted to developing antigen detection procedures. While such procedures have been successfully developed for cryptococcosis and histoplasmosis, those for the opportunistic mycoses, i.e. aspergillosis and candidiasis have been generally unsatisfactory. Their insensitivity, resulting from the transient nature of the antigen(s) detected or failure to test for a battery of diagnostic antigens. To overcome these problems, current research has focused on the use of more purified antigens, monoclonal or adsorbed polyclonal antibodies, and the refinement or introduction more sensitive assays. An overview of the immunodiagnostic tests currently used, their value and shortcomings will be presented.

Key words: Systemic mycoses, immunodiagnostic tests, aspergillosis, blastomycosis, candidiasis, histoplasmosis

Kaufman L. (1995): Výhody a omezení metod pro imunodiagnózu systémových mykóz. – Czech Mycol. 48: 21–29

Výskyt systémových houbových infekcí se v posledním desetiletí podstatně zvýšil. V této době bylo vykonáno mnoho na izolaci nových antigenů a vývoji nových technik. Do rutinní praxe se však dostalo jen málo nových imunodiagnostických testů. Většina v současnosti užívaných testů je zaměřena na průkaz protilátek proti specifickým houbovým antigenům. Tyto testy nejsou zdaleka optimální, osvědčily se však v diagnostice aspergilózy, blastomykózy, kandidózy, histoplasmózy a dalších infekcí, zvláště u imunokompetentních hostitelů. Mohou však být ztíženy křížovými reakcemi a nejsou schopny rozlišit probíhající infekci od dříve prodělané či pouhou kolonizaci od invazívního onemocnění. V novější době byla pozornost věnována rozvoji technik k průkazu samotných antigenů. Úspěšné metody byly vyvinuty pro kryptokokózu a histoplasmózu; pro oportunní mykózy, t.j. aspergilózu a kandidózu, však jsou výsledky převážně neuspokojivé. Malá citlivost testů je výsledkem přechodnosti výskytu dokazovaných antigenů v těle hostitele nebo nemožnosti testovat současně větší počet diagnostických antigenů. K překonání těchto problémů byl současný výzkum zaměřen na použití dokonaleji purifikovaných antigenů, monoklonálních protilátek a zjemnění dosavadních nebo zavedení nových citlivých testů. Je podán přehled výhod a nevýhod v současnosti používaných imunodiagnostických metod.

The incidence of systemic fungal diseases has increased significantly over the last decade. During that time considerable research has been done in isolating and characterizing new antigens and developing new technology. However, few new immunodiagnostic tests for the mycoses have come into routine use, due to lack of appropriate evaluations or availability.

Most of the immunological tests currently in use have some limitations and proper interpretation of test results is enhanced if the laboratory has access to information on the patient's clinical history, symptoms, treatment, occupation, history of travel, and residence. In many situations where newly isolated and characterized antigens or their homologous antibodies have been incorporated into tests, the tests have demonstrated inadequate sensitivity. Accordingly, emphasis has been placed on interpreting the clinical relevance of acceptable conventional tests, and on improving the quality of the antigens or antibodies used therein. Although the established tests demonstrate moderate to good sensitivity and specificity, more rapid and accurate tests are needed. To assure their widespread use, such tests would have to be extensively evaluated, standardized, and made commercially available. Use of such tests would lead to early diagnosis of fungal infections and to the prompt delivery of therapy. Furthermore, such tests might allow accurate monitoring of the course of the disease and the effects of therapy.

Most fungal immunodiagnostic tests are designed to detect antibodies to specific pathogens. These tests though far from optimal have proven useful for diagnosing mycotic infections mainly in the immunocompetent host. Some, however, exhibit cross-reactivity, and fail to distinguish active from past infection, and colonization from invasive disease. The development of assays specific for antibodies associated with early active infection are needed. More recently, researchers have direct their efforts to the development of antigen detection procedures. Whereas such procedures have been successfully developed for cryptococcosis and histoplasmosis, those for the opportunistic mycoses, i.e. aspergillosis and candidiasis have been generally unsatisfactory (de Repentigny and Reiss 1984). The main problem is insensitivity, apparently resulting from the transient nature of the targeted antigen(s).To overcome the aforementioned problems, current immunologic research has focused on the use of more purified antigens, monoclonal adsorbed polyclonal antibodies, and the refinement or introduction of more sensitive assays. The purpose of this presentation is to present an overwiev of the immunodiagnostic tests currently used, and to target any obvious shortcomings.

Aspergillosis

Invasive aspergillosis is difficult to diagnose ante mortem. It is however, being diagnosed post mortem with increasing frequency in immunocompromised patients. Specific antibodies to *Aspergillus* spp. can readily be detected in the serum of the patients with non-invasive aspergillosis.

Precipitin and counterimmunoelectrophoresis (CIE) tests have proven to be practical and reliable. A review of the literature indicates that the percent of antibody-positive immunosuppressed aspergillosis cases detected by ID varied from 0 to 70% (Kaufman 1983), and that sensitivity could be increased to 79-90%, through the use of radio- and enzyme- immunoassays (Kaufman 1983). For improved sensitivity a battery of *Aspergillus* spp. antigens, i.e. *A. fumigatus, A. flavus,* and *A. niger* should be used in enzyme immunoassay (EIA) and immunodiffusion (ID) tests. Antibody tests have demonstrated limited diagnostic value and have not replaced biopsy as the definitive means to establish an antemortem diagnosis of invasive disease. The fact, however, that *Aspergillus* spp. antibodies can be detected in sera from immunocompromised patients suggests that development of more sensitive assays for unique antibodies could lead to increased diagnosis of invasive aspergillosis.

Radio- and enzyme-immunoassays have been used with moderate success for the detection of aspergillosis antigenemia, but none have gained universal acceptance. A competitive binding radioimmunoassay (RIA) for galactomannan is potentially useful for detecting *Aspergillus* spp. antigenemia. The method has a sensitivity of 74% and a specificity of 90% (Talbot et al. 1987). However, since antigenemia is not always evident, frequent monitoring of granulocytopenic patients is important. A latex agglutination (LA) test with a sensitivity of 93% for detecting serum galactomannan in invasive aspergillosis has received mixed reviews and awaits further evaluation (Dupont et al. 1990). An EIA inhibition test for aspergillosis antigenemia and antigenuria has been developed using either a polyclonal or monoclonal antibody to galactomannan antigen (Rogers et al. 1990).The test reportedly provides 95% or greater sensitivity, specificity, and predictive values for invasive disease. Serial specimens, however, had to be tested to achieve such values.

As indicated earlier histologic studies are important in diagnosing invasive aspergillosis. The histologic recognition of an *Aspergillus* sp. in biopsy specimens, however, can be difficult, because *Fusarium* spp., *Pseudallescheria boydi*, and other hyaline opportunistic fungi are morphologically similar. Specific antibodies are needed to enable an unequivocal identification of Aspergillus spp. in tissue (Kaufman 1992a).

Blastomycosis

In recent years serologic tests for the diagnosis of blastomycosis have improved substantially as a result of increased purification of the *Blastomyces dermatitidis* A antigen. Complement fixation (CF) and ID tests are specific but demonstrate poor to moderate sensitivity, ranging from 40 – 65% (Kaufman 1992b). Increased sensitivity ranging from 80 – 88% accompanied by excellent specificity was ac-

complished through development of EIA, western blot and RIA tests. Cross-reactions, when apparent, are mainly with histoplasmosis case sera (Kaufman 1992b). Recently Klein and Jones (1990) described a RIA for antibody to 120-kDa cell wall protein of *B. dermatitidis*. This assay using the 120-kDa antigen which is identical or very similar to the A antigen, demonstrates a sensitivity of 85 % and total specificity when sera are diluted above 1:40. The aforementioned tests appear very promising and await further evaluation.

Candidiasis

Candidiasis is the most common life-threatening systemic mycotic infection. Non-culture methods are needed to complement culture techniques. Current immunodiagnostic methods suffer from problems with sensitivity and or specificity. Immunodiffusion and counterelectrophoretic antibody detection methods while demonstrating reasonable sensitivity frequently do not allow distinction between infected and colonized cases. A variety of antigen assays have been developed but none have been accepted for widespread use. The Cand-Tec latex agglutination test for an uncharacterized heat-labile antigen has a sensitivity of 55% (Ness et al. 1989). The sandwich mannoprotein EIA is reliable for detecting 65-70% of candidiasis cases among human cancer patients (Meckstroth et al. 19). The poor to moderate sensitivity exhibited by both tests does not provide an optimum negative predictive value for diagnosing invasive candidiasis.

Candida-enolase a 48 kDa cytoplasmic antigen, has also proven useful as a diagnostic marker of candidiasis in neutropenic patients. The test demonstrates a sensitivity of 85% and a specificity of 96% when multiple serum specimen are studied, and a sensitivity of approximately 54% when only single specimens are studied (Walsh et al. 1991). The test is poor with non-granulocytopenic patients. To achieve the improved sensitivity and specificity it may be best to perform tests for both antigens and antibodies.

Cryptococcosis

A variety of diagnostically and prognostically useful tests for cryptococcosis have been developed during the last two decades. These procedures are an indirect fluorescent antibody (IFA) technique, and a tube agglutination (TA) test for cryptococcal antibodies, and a LA test and a EIA for cryptococcal antigen. Antibody tests are of value in the detection of early or localized cryptococcosis. They are, however, less specific than the antigen tests (Kaufman and Reiss 1992).

The EIA detects cryptococcal antigen earlier and at lower concentrations than the LA test. It is also not subject to prozone reactions and detects about 6 ng of the capsular polysaccharide per ml, in contrast to 35 ng/ml detected by the LA

test. The sensitivity of the EIA has been further increased as a result of using monoclonal rather then polyclonal antibody in the detection system.

The LA test with serum is limited by the occurrence of false-positive reactions by RF as well as false negative reactions by soluble immune complexes, problems which can be eliminated by treatment with pronase (Kaufman and Reiss 1992). Inspite of these problems, LA assays for cryptococcal antigen have proven invaluable in the diagnosis of chronic meningitis. Antigen has been detected in the CSF of over 90% patients with such disease. The test, however, is less sensitive for detecting non-meningeal cryptococcosis. Because the LA test with pretreated specimens is rapid, very specific, diagnostically and prognostically valuable, and simple to perform, it remains the most widely used procedure for detecting cryptococcal antigen. At present, effective diagnosis of cryptococcosis is best achieved through the concurrent use of antigen and antibody tests.

Histoplasmosis

Serologic evidence is often the prime factor in the definitive diagnosis of histoplasmosis. Such evidence may be obtained through commercially available CF, ID, and LA antibody tests, used singly or in some combination, or by the double-antibody sandwich RIA for *H. capsulatum* antigen (Kaufman 1992c). With the currently available yeast-form and histoplasmin antigens, the CF test, although sensitive, is not entirely specific. The *H. capsulatum* yeast-form antigen in particular, may cross-react with sera from patients with blastomycosis, coccidioidomycosis, and other mycoses.

The histoplasmosis ID and CF tests, with histoplasmin as antigen, will react with about 80% of serum specimens from patients with histoplasmosis. Because the histoplasmin H and M antigens are specific for *H. capsulatum*, the ID test provides a more accurate diagnosis with sera that have low titres or cross-react in CF tests (Kaufman 1992c).

The histoplasmin LA test although satisfactory for detection of early acute primary infection, yields negative results with sera from many persons with chronic histoplasmosis.

Detection of *H. capsulatum* polysaccharide antigen provides a rapid means of diagnosing disseminated histoplasmosis in persons with AIDS, in those otherwise immunosuppressed, and in non-immunocompromised patients. The HPA test is particularly important in testing AIDS patients residing in the histoplasmosis endemic areas, since up to 20% of them develop disseminated histoplasmosis. The antigen is found in the urine of 90% and in the blood of 50% of patients with disseminated histoplasmosis. However, 50 to 75% of the patients with less severe disease (non-disseminated) may be negative for antigenuria (Kaufman 1992c). Antigenuria detection offers an opportunity for early diagnosis of histoplasmosis

since it is usually present at the time of clinical manifestations, whereas cultures may not become positive until 2-4 weeks later. The RIA test is not without limitations. Prior to disseminating disease, tests for *H. capsulatum* polysaccharide may be negative. The test is also not entirely specific, false-positive results have been reported with urine or serum samples from patients with disseminated blastomycosis, paracoccidioidomycosis, and a patients with coccidioidal meningitis. Furthermore, the test is complex, reagents and kits are not yet commercially available, and for the present testing is performed at only one laboratory. Obviously a non-isotopic variation of the method would contribute to its more extensive use.

Pythiosis

Pythiosis is a disease of animals and humans caused by *Pythium insidiosum*. The clinical symptoms of the disease are not pathognomonic and diagnosis is based upon isolation and identification of the etiologic agent. Unfortunately, the etiologic agent is not always successfully cultured and histopathologic studies do not always allow a definite diagnosis. The serodiagnosis of pythiosis could circumvent the need for extensive, time-consuming, costly cultural and histopathological studies in humans and animals with suspected pythiosis. Preliminary studies indicate that the ID test with culture filtrate antigens of *P. insidiosum* specifically diagnoses the disease in animals. Todate a decline in precipitins suggests successful immunotherapy or surgery (Mendoza et al. 1986).

Specific FA reagents may be used to identify this fungus-like organism in histopathologic sections (Mendoza et al. 1987).

Paracoccidioidomycosis

CF, EIA, ID, and CIE tests are useful in the diagnosis of paracoccidioidomycosis and for monitoring responses to treatment. The ID and CIE are simple to perform and are among the most specific procedures. CF, EIA, and other tests using nonpurified antigens are sensitive but less specific.

The CF test will detect antibodies in 79 to 96% of patients with paracoccidioidomycosis (Kaufman and Reiss 1992). However, the CF results with filtrate antigens prepared from multiple or single strains of the yeast form of *Paracoccidioides brasiliensis* are not always specific, and cross-reactions may occur with sera from patients with other diseases, particularly histoplasmosis. It is generally accepted that the ID test is the most practical and specific test for diagnosing paracoccidioidomycosis. With reference sera, it is entirely specific and has a sensitivity of 95% (Kaufman and Reiss 1992). An initial serodiagnosis of paracoccidioidomycosis can be obtained in over 98% of cases with the concomitant use of the ID an CF tests.

A 43 kDA glycoprotein is the major precipitinogen (Puccia et al. 1986) and appears to be identical to the E2 antigen described by Yarzabal et al. (1977) and antigen 1 described earlier by Restrepo and Moncada (1974). The 43 kDa antigen, however, demonstrates cross-reactivity in the EIA and its utility in EIA and other quantitative assays awaits further study. Recently the 43 kDa glycoprotein was demonstrated by the immunoblot technique (Mendes-Giannini et al. 1989) in the sera of patients with paracoccidioidomycosis. The diagnostic and prognostic value of this and other *P. brasiliensis* antigens in antigenemia or antigenuria tests require further study.

In the absence of multiple budding yeasts forms the histological characteristics of *P. brasiliensis* may not be specific and the fungal elements may be confused with those of *B. dermatitidis, C. immitis* and *H. capsulatum.* Polyclonal FA reagents though specific must be adsorbed. Preliminary studies (Figureoa et al. 1994) suggest that *P. brasiliensis* can be specifically immunohistologically identified with monoclonal antibodies directed against a 22- to 25- kDa cytoplasmic antigen. Additional studies with blastomycosis, coccidioidomycosis and histoplasmosis case tissues are needed to confirm the specificity of these antibodies.

Zygomycosis

Zygomycetes are the third leading cause of fungal infection in patients with hematologic malignancies. Greater than 90% of disseminated zygomycosis cases were diagnosed post mortem. Early diagnosis may lead to greater survival and effective treatment. Development of sensitive and specific immunologic tests could provide rapid and noninvasive diagnostic tools.

A 2 hr. EIA using homogenate antigens of *Rhizopus arrhizus* and *Rhizomucor pusillus* was evaluated against an ID test using the *R. arrhizus* antigen (Kaufman et al. 1989). In tests with 43 proven zygomycosis case sera, the sensitivity of the EIA was 81% and the ID 66%. The specificity of the EIA was 940 whereas that of the ID test was 91%. The sensitivity of these tests are far from optimal and non-specific reactivity was particularly evident with sera from patients with aspergillosis and candidiasis. Interestingly, the tests are capable of detecting antibodies in cases of systemic zygomycosis caused by species other than *Rhizopus arrhizus* and *Rhizomucor pusillus.* The EIA provides advantages over the ID test, which requires serum to be concentrated and preincubated for 3 h before the addition of antigen. The EIA has potentials, but obviously, additional studies are needed to improve its sensitivity and specificity (Kaufman et al. 1989). Toward this goal Kappe et al., (Kappe et al. 1994) are attempting to identify the immunodominant antigens in zygomycosis. They have reported that 24- and 32 kDa antigens found among several members of the class of Zygomycetes appear promising for serodiagnosing zygomycosis.

CONCLUSIONS

Studies to identify and characterize new antigens coupled with new technology are necessary to readily detect non-transient diagnostic antigens associated with systemic mycotic infections in neutropenic patients. Once these new methods are developed, extensively evaluated and standardized, their positive impact on diagnosis will be furthered by their being made commercially available and thus widely used.

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Paxillus involutus – a dangerous mushroom?

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Pohle W. (1995): Paxillus involutus – a dangerous mushroom? – Czech Mycol. 48: 31–38

The toxicological importance of Paxillus involutus is discussed controversially. Therefore it is necessary to give a critical review about this problem. In the mycological literature printed before 1970 Paxillus involutus was estimated as an edible mushroom of well taste. The only premise to avoid an toxication with gastrointestinal symptoms was the destruction of heatlabile toxins by heating the mushroom longer than 20 minutes.

Despite the mushroom were heated long enough, between 1940 and 1960 the number of severe intoxication caused by Paxillus involutus increased. The German mycologist J. Schäffer died after an ingestion of Paxillus involutus whereas all other participants of the meal did not show any symptoms of an intoxication. The mushroom intoxications recorded between 1961 and 1989 in the former GDR showed an increase of intoxications with Paxillus involutus till 1976. After an action of instructing people the number of intoxications decreased again.

Investigations of Deicher and Strangel (1977) and Winkelmann et al. (1986) showed that in the region of Hannover about half of the population of Paxillus involutus contained an unknown antigen, which causes a so called "immunehaemolytic anaemia" including following symptoms: Vomiting, abdominal pain, circulatory shock, icterus, haemolysis, anuria and pulmonary failure. A repeated contact induces the production of IgG which is liberated by a following ingestion, inducing haemolysis and the other above effects. The mushrooms containing the antigen and those without antigen can only be discriminated by specific immunological methods.

It is concluded that the use of Paxillus involutus as human food is dangerous because of the risk of a sensibilization followed by an immunehaemolytic anaemia.

The possible reasons of the increase of the "Paxillus syndrome" and the territorial distribution of the dangerous variant of Paxillus involutus are discussed.

Key words: Paxillus involutus, mushroom intoxication, immunehaemolysis, "Paxillus syndrome"

Pohle W. (1995): Paxillus involutus – nebezpečná houba? – Czech Mycol. 48: 31–38

Je podán kritický přehled o otravách čechratkou podvinutou (Paxillus involutus). Paxillus syndrom se vyznačuje jak gastrointestinálními symptomy (syrové či nedovařené plodnice) tak imunohemolytickou anémií po opakovaném požití vařené houby. Z těchto důvodů je třeba houbu považovat za jedovatou, i když ji starší příručky řadily mezi druhy jedlé.

The toxicity of *Paxillus involutus* is discussed controversially – therefore a critical review might be necessary.

The brown roll-rim – *Paxillus involutus* – is a common mushroom in Europe (Fig. 1). The cap of this mushroom is a medium dark dingy yellow brown, and the margin is inroled and market with short riblike lines for furrows. The edge of the cap may be slightly pubescent but is never bearded. The gills are close narrow, decurent, and yellow when young but stained brown if touched or hurt. Cross vains are often conspirous between the gills. Fruity bodies can be found from

June till September in coniferous forests but also under deciduous trees. If necessary (in unclear intoxications) *Paxillus involutus* can be identified in asservates by the typical spores (Kell 1961, Pohle and Wöllner-Siebert 1983), which are 8-10 x 5-6 μm in size, ellipsoid, dull brownish yellow, smooth and containing a large drop (Fig. 2).

In the mycological literature published before 1975 *Paxillus involutus* is designed as edible. Toxic compounds can be destroyed by heating like in some other mushrooms. Here some examples are given: Amann (1966): "Roh giftig, gut durchgekocht ein schmackhafter Speisepilz." Lange and Hora (1941): "Harmless if cooked, of little value; slightly poisonous to some people when raw." Michael and Hennig (1968): " – wird als ausgezeichneter Speisepilz gern gegessen" – "Längeres Braten (25 Min.) ist unbedingt erforderlich!" Moser (1967): " – Kann roh oder schlecht gekocht stark giftig wirken!" Pilat (1954): "Mushroom of inferior quality which can only be eaten boiled. Raw it causes indigestions in some persons." Ramsbottom (1923): " – taste mild" – "edible". Romagnesi (1977): "En France, cette espéce est consomé sans inconvéniment lorsqu'elle est bien cuite; mais crue ou insufisamente cuite, ell est extrémement toxique". Smith and Smith-Weber (1980): "In Poland a study was made over a period of 10 years. In 109 cases studied 93 were hospitalised and there were 3 fatalities. Apparently, the mushrooms were not cooked sufficiently. Never eat this species raw! In past it has been rated as an edible species – and it is true that an North America it has been used for the table fairly frequently."

In Germany intoxications caused by *Paxillus involutus* which were insufficiently cooked had been very rare. During and after the "second war" the intoxications increased drastically despite the fact that the fruity bodies of *Paxillus involutus* were heated well. Also the German mycologist J. Schäffer died after having eaten *Paxillus involutus* whereas his family remained without any symptoms.

Before 1961 we do not have exact informations about the number of intoxications, but between 1961 and 1989 in the eastern part of Germany (former GDR) mushroom intoxication had been registrated (Fig. 3). Between 1966 and 1976 the number of intoxications caused by *Paxillus involutus* further increased drastically (1968 – 56 and 1974 – 45 cases). – A large action of instructing people was started – and after some years the number of intoxication decreased again. Several cases were reported (Cochet 1974, Grzymala 1958, Herrmann 1961, Rauschert 1962, Sikorski, Marciniak and Gliniecka 1974, Straus 1949) but only Bschorr and Mallach (1963) and Kubička and Veselský (1975) suggested that also well heated fruity bodies of *Paxillus involutus* might be dangerous.

Between 1971 and 1986 in several publications the "Paxillus syndrome" was investigated and analysed (Albrecht 1983, Azema 1982, Bschorr and Mallach 1963, Cochet 1974, Deicher and Strangel 1977, Flammer 1985, Lagrande 1979, 1982, Lefévre 1982, Nieminen et al. 1977, Olesen 1991, Schmidt et al. 1971, Winkelmann

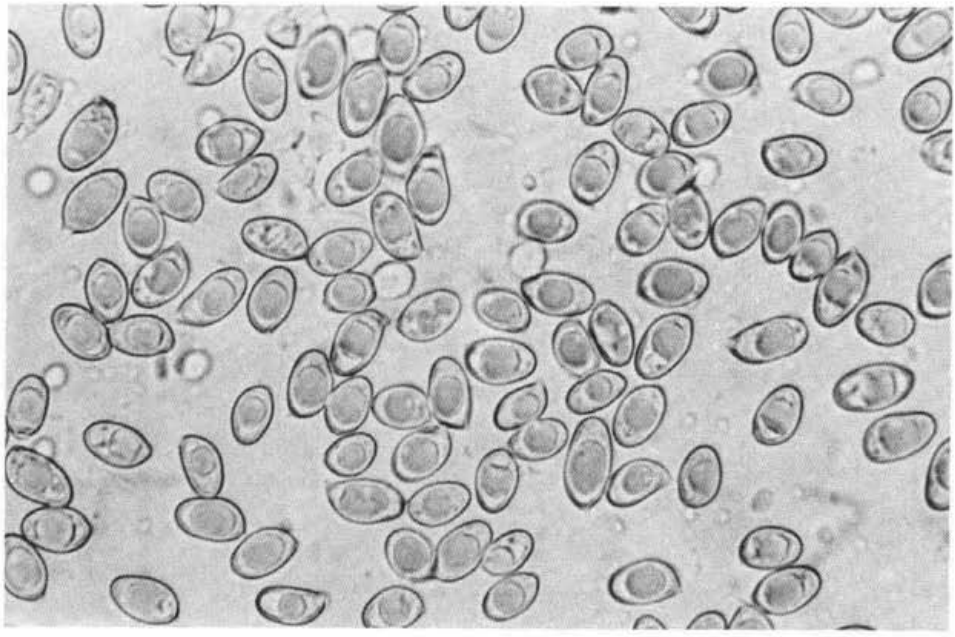

Fig. 1 The brown roll-rim – Paxillus involutus.

Fig. 2 Spores of Paxillus involutus.

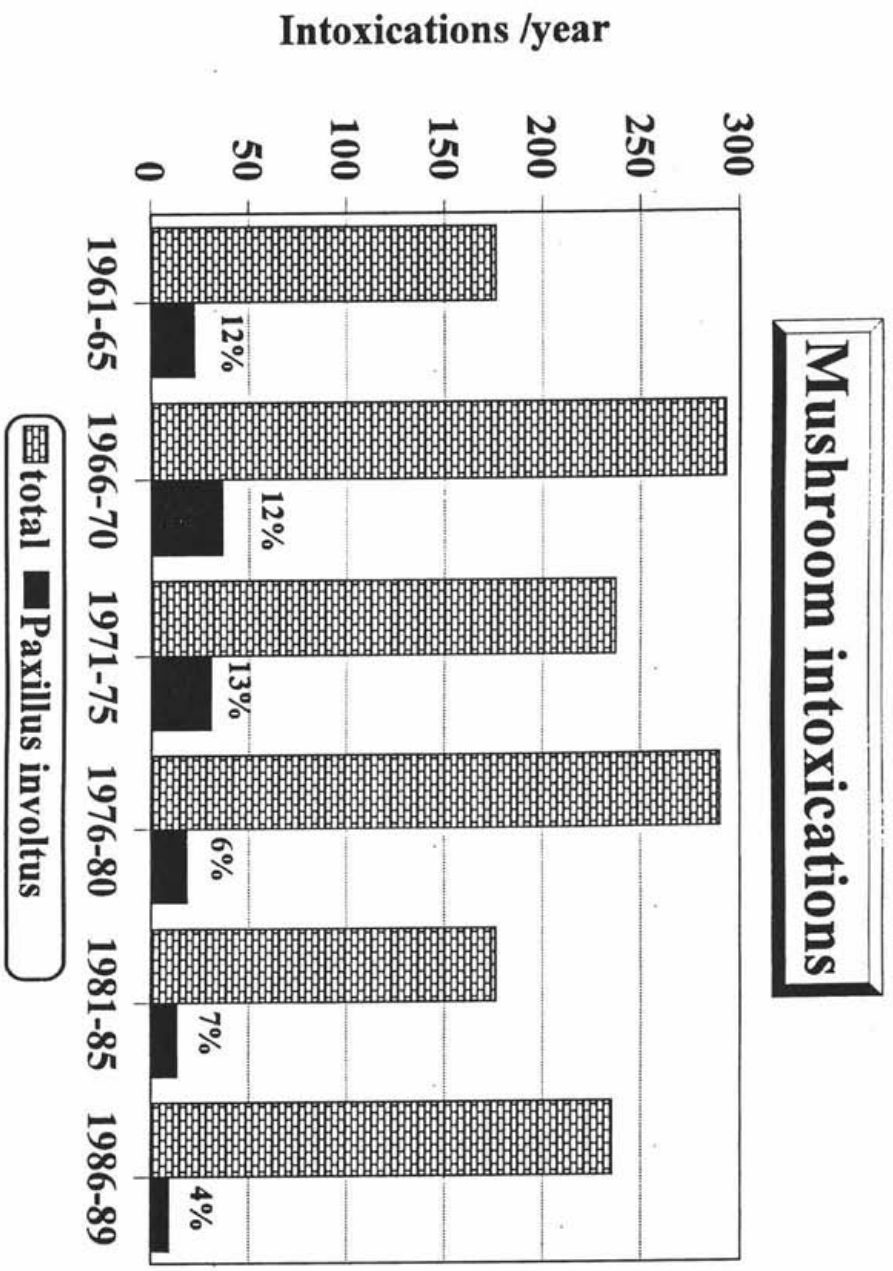

Fig. 3 Mushroom intoxication in the eastern part of Germany (former GDR). Always the mean of 5 years is shown as intoxication per year.

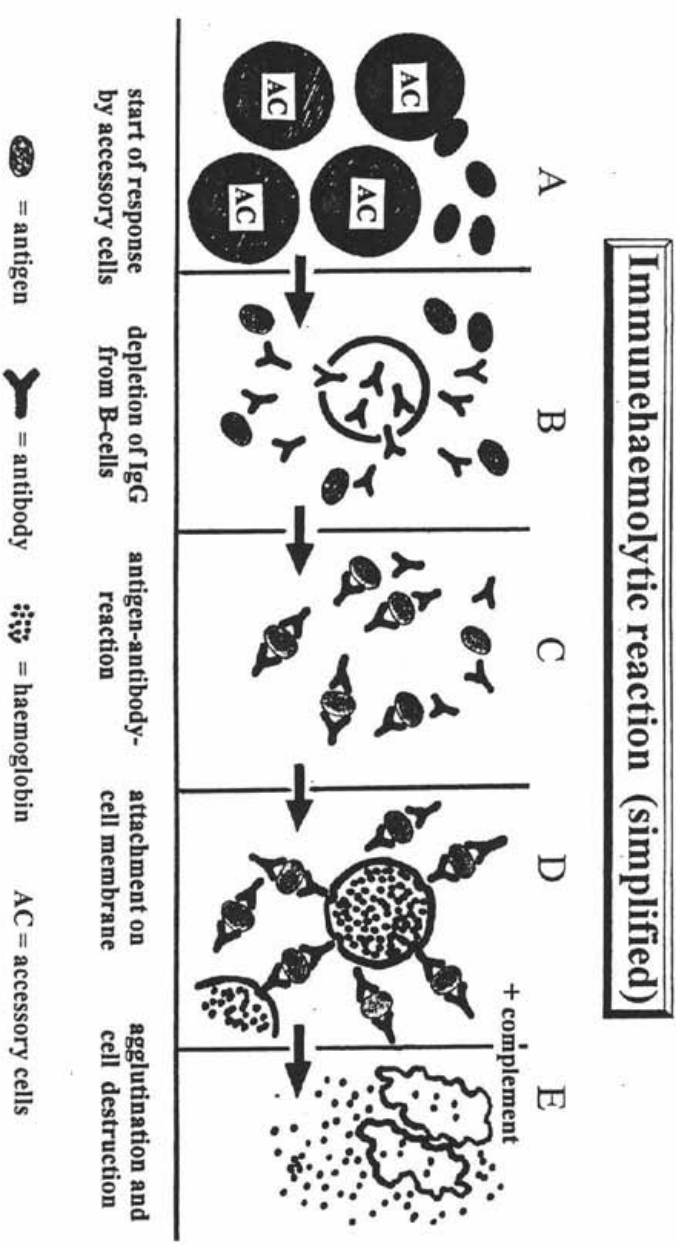

Fig. 4 Scheme of the immunehaemolytic reaction demonstrated in a simplifier manner.

et al. 1982, Winkelmann et al. 1986), and the results were also cited in the new mycological literature (Bresinsky and Besl 1989, 1990, Kell 1961, Michael et al. 1979).

What has happened? – If *Paxillus involutus* was eaten repeatedly, suddenly, about 2 hours after a new ingestion this mushroom following symptoms could appear: weakness, nausea and vomiting, diarrhoea, colique-like abdominal pain, stupor, dyspnoe, circulatory shock, icterus, haemolysis, haemoglobinurea, anuria, ureamia, extrasystoly as well pulmonary and renal failure. Without medical help most patients must die within 2 or 3 days (Deicher and Strangel 1977, Flammer 1985, Lefévre 1982, Olesen 1991, Schmidt et al. 1971, Winkelmann et al. 1982, Winkelmann et al. 1986).

Post mortal examinations revealed signs of intravascular coagulations in lungs, kidneys, adrenals, myocardium, liver and spleen; – and also extensive fat embolism to the lung could be found (Deicher and Strangel 1977, Winkelmann et al. 1986). In the serum of the patients an antibody against *Paxillus involutus* was found (Deicher and Strangel 1977, Winkelmann et al. 1982, Winkelmann et al. 1986).

The "Paxillus syndrome" is no really intoxication, but a pathological immune reaction called "immuneheamolytic anaemia". This immune reaction is shown in a simplified manner in Fig. 4: During repeated confrontation with the till unknown antigen from *Paxillus involutus* antibodies were formed by the help of akzessory cells (Fig. 4, "A"), and if enough IgG-antibodies are synthesised a new contact with the antigen induces a more or less intense depletion of IgG from B-cells (Fig. 4, "B") (Roitt et al. 1987). The IgG-molecules react with the antigen in an antigen-antibody-reaction (Fig. 4, "C") (Winkelmann et al. 1982, Winkelmann et al. 1986). The former immune complexes now can be attached to the surface of the erythrocyte membrane (Fig. 4, "D"), and (by help of the complement chain) it is followed by the agglutination and destruction of this red blood cells (Fig. 4, "E"). – Haemoglobin emits from the damaged cells into the serum followed by haemoglobinnuria and other secondary effects as kidney damage and so on. The "Paxillus syndrome" is called to be an "immunehaemolytic anaemia". Immune reaction against other mushrooms are known (Albrecht 1983, Bruhn and Sonderberg 1991), but they must not be all as fatal as the immunehaemolysis caused by *Paxillus involutus*.

In addition to an adequate anti-shock treatment, elimination of the former immune compounds, liberation haemoglobin and erythrocyte debris by plasma separation and the compensation of renal failure by haemodialysis seem to be the therapy of choice (Winkelmann et al. 1986).

Not all fruity bodies of *Paxillus involutus* contain the dangerous antigen. Around Hannover mushrooms from several different populations of *Paxillus involutus* were investigated by a special immunological method using serum from patients who had been suffering from Paxillus disease. Deicher and Strangel (1977) and Winkelmann

et al. (1982, 1986) from the Medical School Hannover found that 7 out of 12 of the investigated population of *Paxillus involutus* contained the unknown dangerous antigen, and it can be suggested that the regional distribution of the dangerous and the harmless populations of the *Paxillus involutus* seems to be different. The harmless mushrooms and those containing the dangerous antigen do not differ in their morphology, and a differentiation is only possible by immunological methods.

In the Moscow region *Paxillus involutus* was the most used mushroom over long time, and no intoxication were published before 1991. Immunological investigation from this region are unknown.

As shown by the literature *Paxillus involutus* was eaten without any trouble in France and North America, but much intoxications were reported from Poland and Germany (Romagnesi 1977, Smith and Smith-Weber 1980). Also Kreisel (Michael et al. 1979) pointed out that territorial differences in the toxicity of *Paxillus involutus* may exist.

What about the sudden increase of the dangerous disease caused by *Paxillus involutus*? This question can only be answered hypothetically, and there are 3 possibilities:

1) Several years ago a population of *Paxillus involutus* was changed by a mutation to form the dangerous antigen. This new mutation extended more and more in Europe.
2) Both the harmless and the dangerous form did exist long ago. The immune-haemolytic anaemia was unknown, and for it in the most cases only one person out of a group was affected the association between *Paxillus involutus* and the illness was ignored. The cases were not registrated as intoxications.
3) Both variants with and without the dangerous antigen did exist long time ago, but due to the contamination of our environment by toxic agents people had become more sensitive against antigens. Therefore in general people may faster develop a sensibilization against antigens than years before.

To elucidate this open problem it will be of interest to investigate the territorial distribution of the dangerous variant of *Paxillus involutus* in a larger region than around Hannover. It will be helpful to investigate whether the territorial distribution of the dangerous variant of *Paxillus involutus* corresponds to the territorial distribution of the immunehaemolytic anaemia caused by this mushroom.

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Toxic terpenoids isolated from higher fungi

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Sterner O. and Anke H. (1995): Toxic terpenoids isolated from higher fungi.– Czech Mycol. 48: 39–52

A large number of toxic terpenoids have been isolated from cultures and fruit bodies of higher fungi. The chemistry, biological activity and possible natural functions of some of them are discussed in this paper. Especially interesting in this respect are natural defensive compounds that possess for example antibiotic and antifeedant activies and are likely to be toxic. The sesquiterpenoids of the pungent Lactarius species (e.g. L. necator, L. piperatus, L. rufus and L. vellereus) constitute an interesting example of this. In the fruit bodies of these species within seconds after an physical injury, an apparently inactive precursor is converted enzymatically into a range of pungent sesquiterpenes with an unsaturated dialdehyde functionality possessing potent antimicrobial and cytotoxic activities. The injury brings the precursor, which is present as an emulsion in the latex of specialised hyphae of the fruit bodies, in contact with the enzyme systems that are kept apart in the intact fruit body. Fruit bodies of non-pungent and edible Lactarius species (e.g. L. deliciosus and L. flavidulus) contain precursors with completely different chemical structures that also are converted as a response to injury, although to products with less striking biological activities and with uncertain function.

Key words: Terpenoids, toxicity, biological activity, higher fungi, Basidiomycotina

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Z kultur a plodnic vyšších hub bylo izolováno mnoho toxických terpenoidů. Je diskutována jejich chemie, biologická aktivita a u některých i možný jejich význam v přírodě. Zvláště zajímavé z tohoto pohledu jsou přírodní obranné sloučeniny antibioticky aktivní, které jsou pravděpodobně toxické. Sesquiterpenoidy palčivých druhů rodu Lactarius (např. L. necator, L. piperatus. L. rufus, L. vellereus) tvoří zajímavé příklady. V plodnicích těchto druhů jsou krátce po poškození inaktivní prekursory konvertovány enzymaticky do skupiny palčivých sesquiterpenů s nenasyceným dialdehydem, které působí silně antimikrobiálně a cytotoxicky. Při poškození plodnice jsou původně intaktní enzymatické systémy spuštěny a prekurzor emulgovaný v latexu mléčnic je aktivován. Plodnice nepalčivých a jedlých druhů rodu Lactarius (např. L. deliciosus, L. flavidulus) obsahují prekursory zcela jiného chemického složení, které jsou také konvertovány jako odpověď na poškození plodnice, avšak na produkty s méně nápadnými biologickými aktivitami a s nejistou funkcí.

INTRODUCTION

Although the terpenoids form the largest group of natural products (Connolly and Hill 1991) and are widespread in the kingdom of Fungi, it is conspicuous that only few of the classical mushroom poisons belong to the terpenoids. However, many of the terpenoids isolated from species belonging to the Basidiomycotina

subdivision of Fungi possess potent toxic activities (Bresinsky and Besl 1985; Anke and Steglich 1988), and some of these are shown in Figure 1.

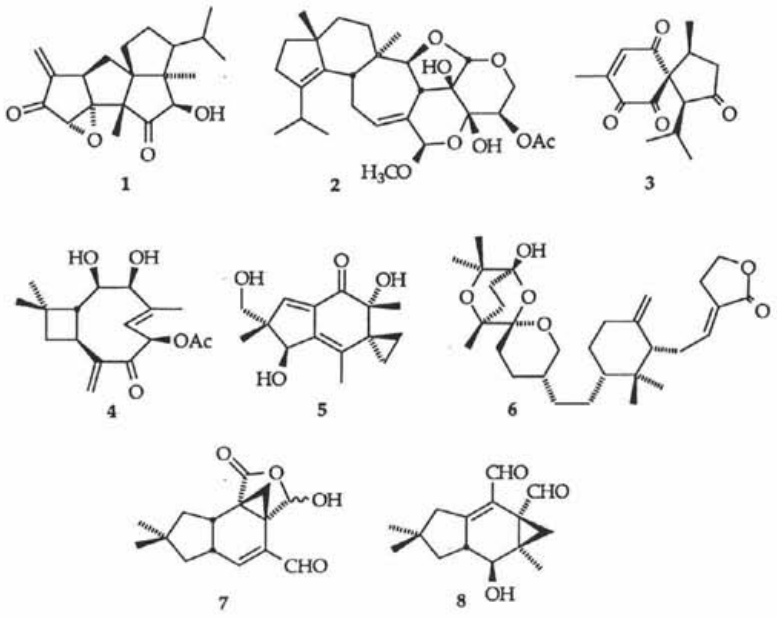

Figure 1

The crinipellins (e.g. crinipellin A [1]), are cytotoxic diterpenoids isolated from cultures of *Crinipellis stipitaria* (Anke et al. 1985), while the striatins (e.g. striatin A [2]) are produced by both cultures (Hecht et al. 1978) and fruit bodies (Rabe 1989) of *Cyathus striatus* as well as by other species. The LD_{50} value for striatin A (2) is 60-110 mg/kg (i.p. administration to tumour-bearing mice) (Douros and Anke 1994). The acorane hemimycin (3) was isolated from cultures of *Hemimycena cucullata* and *H. candida*, and possess potent cytotoxic activity (Bäuerle et al.1986). The fruit bodies of *Hypholoma fasciculare* have yielded a series of toxic triterpenoids, the fasciculols (Suzuki et al. 1983), and from cultures of several *Hypholoma* species the cytotoxic caryophyllane naematolon (4) was obtained (Backens et al. 1984). However, the toxicity of the latter toward mammals appear to be limited, as the LD_{50} value is well above 225 mg/kg (i.p. administration to tumour-bearing mice) (Douros and Anke 1994). Fruit bodies and cultures of *Omphalotus olearius* produce the toxic but also antineoplastic illudane illudin S (5) (McMorris and Anchel 1963). Several cytotoxic triterpenes, the saponaceolides, have been isolated from *Tricholoma* species, e.g. *T. saponaceum* (De Bernardi et al.

1988; 1991), and although their cytotoxic activity is remarkable (for saponaceolide B (6) ID_{50} on the LoVo cell line is 0.16 μg/ml, and LD_{50} on brine shrimps is 40 ng/ml) they possess no antimicrobial activity (De Bernardi et al. 1991). Two further examples of toxic sesquiterpenes are the mutagenic (Anke and Sterner 1991) marasmic acid (7) and merulidial (8), originally isolated from *Marasmius conigenus* (Kavanagh et al. 1949) and *Merulius tremellosus* (Giannetti et al. 1986), respectively. The LD_{50} value for marasmic acid (7) is 15-30 mg/kg (i.p. administration to tumour-bearing mice) (Dourns and Anke 1994).

The sesquiterpenes of the Russulaceae species

The function of the terpenoids in the fungi, if any, is not clear. However, in the fruit bodies of the pungent species belonging to the genus *Lactarius* (family Russulaceae of the Basidomycotina subdivision) biologically active terpenoids are formed as a response to physical injury, in what appears to be a chemical defence system that protects the fruit bodies against parasites and infections (Camazine et al. 1983; Sterner et al. 1985a). In this, pungent metabolites with antifeedant and antimicrobial activity are formed when an inactive precursor is brought in contact with enzymatic systems by the injury, not unlike a binary weapon system. The precursor is present as an emulsion in the latex of the fruit bodies, this latex is characteristic for the *Lactarius* species and can be observed if a fruit body is cut or broken. The colour and taste of the latex, as well of the flesh, vary between different species, such characters areimportant taxonomic markers for mycologists and the chemistry related to these differences has been clarified for several species. Interestingly, there seems to a general pattern within the *Lactarius* genus, also in the non-pungent species, in that the metabolites responsible for the characteristic differences in taste and colour are formed enzymatically from fatty acid ester precursors as a response to injury to the fruit bodies. Depending upon the precursor originally present in the fruit bodies, the *Lactarius* species and the metabolites may be divided into three major groups:

1. The largest is made up by species belonging to the *Albati* and *Lactarius sections*, for example *L. vellereus, L. piperatus* and L. *scrobiculatus.* They generally have white latex containing large amounts of a biologically inactive fatty acid ester of a marasmane sesquiterpene which rapidly (in seconds) is converted enzymatically to bioactive marasmane, lactarane and seco-lactarane sesquiterpenes as a response to injury (vide infra).
2. In the species belonging to the Dapetes section of *Lactarius* (e.g. *L. deliciosus, L. deterrimus*, and *L. sanguifluus*) the latex is initially carrot-coloured or wine-coloured but slowly turns green. This has been shown to be due to the presence of stearic acid esters of guaiane sesquiterpenes in the intact fruit bodies which

are converted mainly by ester hydrolysis and oxidation to guaiane alcohols and aldehydes in the injured fruit bodies (vide infra).

3. The latex and flesh of the fruit bodies of species belonging to the *Plinthogali* section (e.g. *L fuliginosus* and *L. picinus*) is originally white and sweet, but due to the enzymatic hydrolysis of the stearic acid ester 9 to the free phenol 10 (a potent fungicid) and its enzymatic oxidation to a number of derivatives (e.g. the dimers 11 and 12) as a response to injury, the latex and flesh turn reddish and bitterly acrid (De Bernardi et al. 1992). Similar mixed phenolic/terpenoid metabolites, e.g. flavidulol A (13) and flavidulol C (14), possessing antimicrobial activity have been isolated from the fruit bodies of *L. flavidulus* (Takahashi et al. 1988; 1993; Fujimoto et al. 1993), a species not known in Europe.

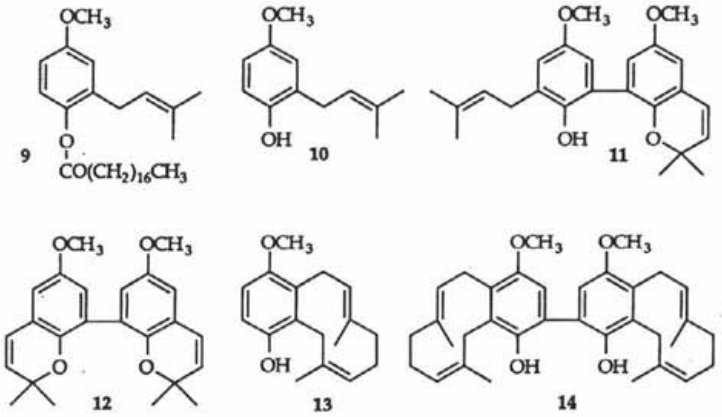

Figure 2

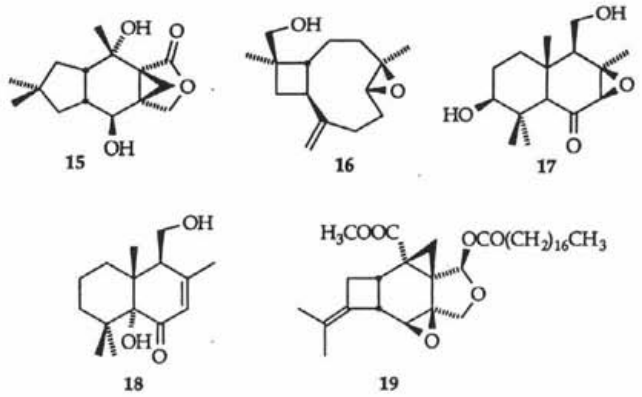

Figure 3

The isolactarane isolactarorufin (15) has in some investigations been isolated from extracts of *L. rufus* and *L. vellereus* (Daniewski et al. 1976a; 1988), species that mainly make marasmane and lactarane sesquiterpenes (vide infra). In addition, from fruit bodies of species belonging to other sections of *Lactarius*, sesquiterpenes with caryophyllane (compound 16 isolated from *L. camphoratus* [Daniewski et al. 1981]), drimane (e.g. uvidin A (17) and uvidin E (18) isolated from *L. uvidus* [De Bernardi el al. 1980; 1983]) and glutinopallal (e.g. stearoylglutinopallal (19) isolated from fruit bodies *of L. glutinopallens* [Fabre-Bonvin and Gluchoff-Fiasson 1988]) skeletons have also been identified.

A CHEMICAL DEFENCE SYSTEM IN THE PUNGENT LACTARIUS SPECIES

Isovelleral (20) and velleral (23) possess such striking biological activities that at least part of their function in for instance *L. vellereus* must be considered to be in a chemical defence system (Sterner et al. 1985a; Anke and Sterner 1991). In *L vellereus*, the two compounds are formed immediately after injury of a fruit body, and both compounds constitute 6 % of a hexane extract made 10 seconds after injury by grinding a fruit body in a meat grinder (Sterner et al. 1985a). The dialdehydes are subsequently reduced to isovellerol (21) and vellerol (24), and after several hours it was observed that small amounts of vellerol (24) had been further reduced to the corresponding diol (Sterner et al. 1985a). The reduced derivatives have lost the pungency and most of the biological activities of the unsaturated dialdehydes (Sterner et al. 1985b; 1987). No further conversion of isovellerol (21) was observed in this investigation, although the marasmane lactone 22, a possible oxidation product of isovellerol (21) (vide infra), has later been isolated from extracts of *L. vellereus* prepared in a different way (Daniewski et al. 1992a). In fruit bodies of *L. bertillonii*, only velleral (23) is formed from the precursor stearoylvelutinal (38b), velleral (23) is then reduced to vellerol (24) which subsequently is oxidised to vellerolactone (25) by the injured mushroom tissue (Hansson et al. 1994). Piperdial (26), piperalol (27) and blennin A (28) have been isolated from fruit bodies of for example *L. torminosus* (Seppä and Widen 1980; Sterner et al. 1985b), while the epimers epi-piperdial (29), epi-piperalol (30) and lactarorufin N (31) were isolated from the fruit bodies of *L. necator* (Daniewski et al. 1976b; Sterner 1989). Chrysorrhedial (32), chrysorrheal (also called scrobicalol) (33) and lactaroscrobiculide A (34) have been isolated from fruit bodies of *L. scrobiculatus* (Pang et al. 1992; De Bernardi et al. 1993) and *L. chrysorrheus* (De Bernardi et al. 1993). Lactardial (35) has been isolated from several species (Sterner et al. 1985b), for example *L. necator*, and its status as a natural product is somewhat questionable as it under certain circumstances may be formed as an artefact by chemical transformation of the velutinal esters (vide infra). However, it contains the unsaturated dialdehyde functionality, although in

disguise, and it is pungent even if its antimicrobial activities and cytotoxicity are relatively weak compared with for instance isovelleral (20) and velleral (23) (Anke and Sterner 1991). The corresponding reduced form has been isolated in small amounts from *L. necator* in the form of lactarol (36) (reduction of the free aldehyde of lactardial (35) destabilises the dihydrohydroxyfuran functionality which spontaneously eliminates water to form the furan). Lactaronecatorin A (also called blennin C) (37) (Daniewski et al. 1975; structure revision Vidari et al. 1976) was obtained from the same species, and also from *L. blennius*.

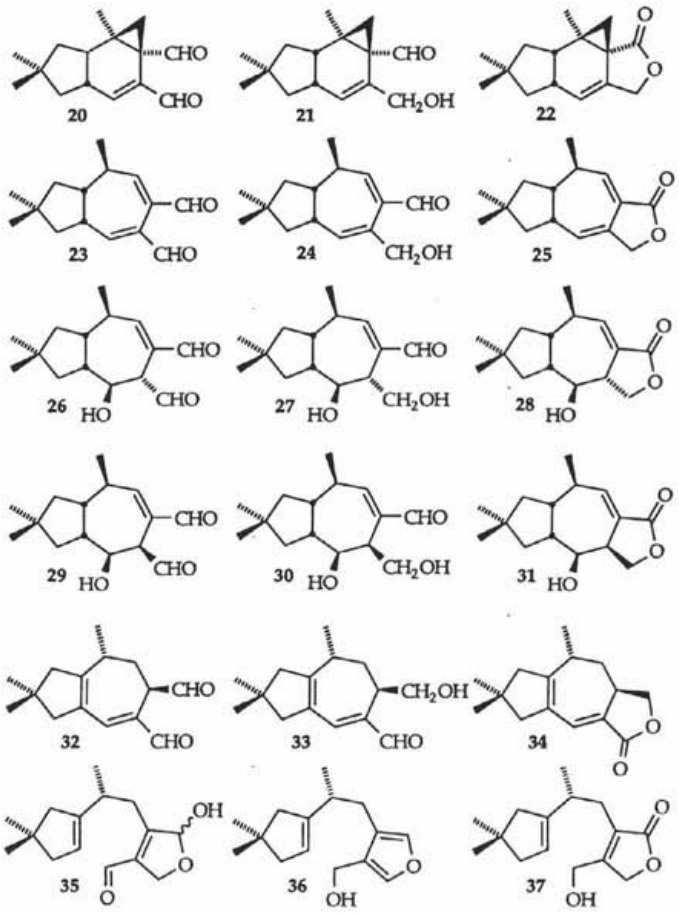

Figure 4

Besides the potent antimicrobial, cytotoxic and, in some cases, mutagenic activities of the unsaturated dialdehydes isolated from *Lactarius* fruit bodies (Anke and Sterner 1991), features that are shared with unsaturated dialdehydes isolated from other natural sources such as insects, molluscs and plants, an additional important quality is their intense pungent taste which actually make them excellent as human antifeedants. It has also been shown that mammals that normally feed on mushrooms will avoid edible specimens that have been treated with isovelleral (20) (Camazine et al. 1983). However, their pungency probably limits the hazard to consumers of wild mushrooms, as it would be difficult to consume significant amounts of the dialdehydes. 1 μg of for example isovelleral (20) (adsorbed on a filter paper disc) distinctly stimulates the taste-buds of the human tongue, and as 1 g of injured *L. vellereus* tissue may contain more than 1000 μg isovelleral (20) and velleral (23) only small amounts of the raw mushrooms can in practice be consumed by a normal individual. No toxicity tests with mammals have been performed, but the in vitro data suggest that the unsaturated dialdehydes would be highly toxic to mammals. The lactones, which appear to be the end-products in several species, also possess cytotoxic activity (De Bernardi et al. 1993).

The precursor of all the sesquiterpenes shown in Figure 4 is velutinal (38a) (Favre-Bonvin et al. 1982; Sterner et al. 1983), present as an emulsion (the latex) in specialised hyphae (Gluchoff-Fiasson and Kühner 1982) in the intact fruit bodies as various biologically apparently inactive (Sterner et al. 1985a) fatty acid esters (e.g. stearoylvelutinal (38b) in *L. vellereus* and *L. bertillonii* and 6-ketostearoylvelutinal (38c) in *L. necator* and *L. chrysorrheus*). The enzymatic conversions of stearoylvelutinal (38b) to form isovelleral (20), velleral (23), piperdial (26) and epi-piperdial (29) have been studied, and the biosynthetic pathways shown in Figure 5 have been proposed (Hansson et al. 1991; 1993). The formation of lactardial (35) during the enzymatic processes is probably similar to its formation from acid catalysed chemical transformation of the velutinal esters (vide infra), while the formation of chrysorrhedial (32) remains to be clarified.

In addition, quite a number of lactarane furans have been reported from the species yielding the compounds shown in Figure 4, although the majority of the furans are believed to be artefacts formed by chemical transformations of the labile velutinal esters (Sterner et al. 1985c). Traces of acid (present in for instance undistilled solvent or in chromatography gels) will rapidly transform any velutinal derivative to a number of dihydro-hydroxy-(acyloxy)-furans that easily eliminate water to form furans. As shown in Figure 6, some intermediates are carbocations that undergo additions, eliminations or rearrangements, and the product mixture obtained is rather complex. However, a few furans are actually formed as true natural products only in the enzymatic processes, e.g. the dihydroxyfuran 39 (see

Figure 6) (Sterner et al. 1988). Interestingly, some of the lactones and furans have been reported to possess antifeedant activity against storage pests (Daniewski et al. 1992b).

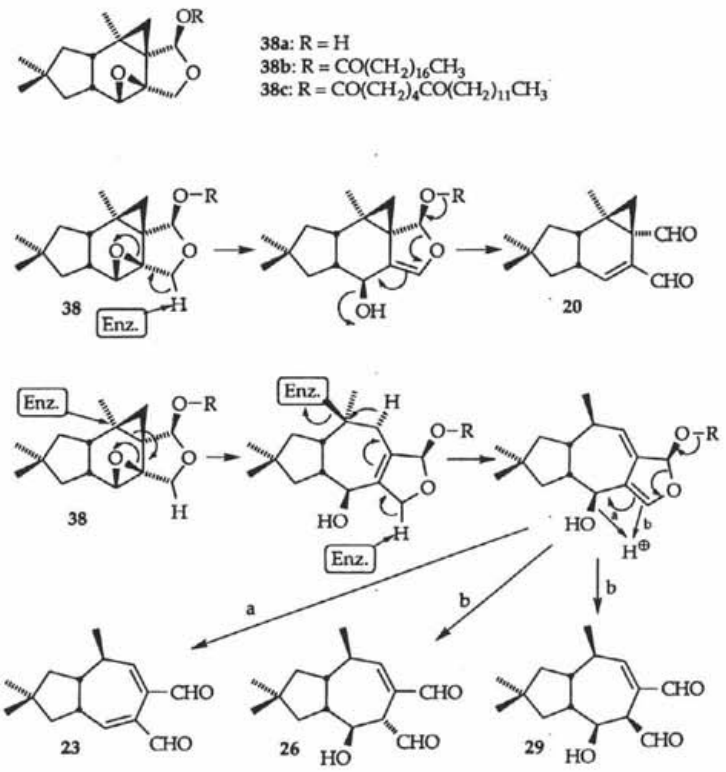

Figure 5

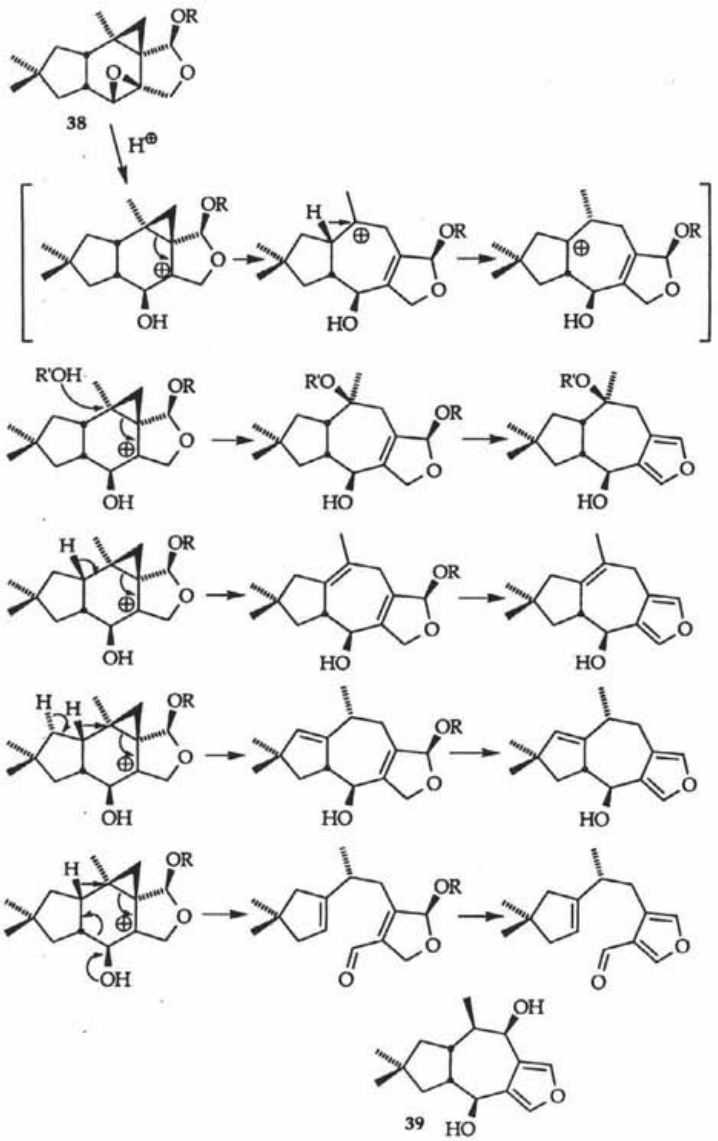

Figure 6

CONVERSIONS OF SESQUITERPENOIDS IN THE SAFFRON MILK CAPS (SECTION DAPETES)

Contrary to the pungent *Lactarius* fruit bodies which only are consumed in exceptional cases, the saffron milk caps are considered to be among the most desirable by consumers of wild mushrooms. They are characterised by strong and with time changing colours of the latex as well as by an agreeable peppery taste, but also by their lack of resistance to parasites compared to the pungent species. The colours of the latex are caused by the presence and formation of azulene and hydroazulene sesquiterpenoids with a guaiane skeleton, and the enzymatic conversions that take place as a response to injury resembles those of the pungent species in that the intact fruit bodies contain fatty acid (mainly stearic acid) esters of sesquiterpenes which are converted to sesquiterpenoic alcohols and aldehydes. The major differences are that the amounts of sesquitepenoids in the saffron milk caps are much smaller, that the enzymatic conversions are less rapid, and that the chemical functionalities present in the guaiane sesquiterpenes make them considerably less biologically active. In intact fruit bodies of *L. deliciosus* and *L. deterrimus*, only the orange ester 40 could be detected (together with minor amounts of the corresponding linolic acid ester) (Vokáč et al. 1970; Bergendorff and Sterner 1988). As a response to injury, 40 is slowly (minutes) converted by ester hydrolysis and oxidations to the dihydroazulenes alcohol 41 and delicial (42), as well as the azulenes deterrol (47) and lactaroviolin (48) (Bergendorff and Sterner 1988). The reduced azulene lactarazulene 49) was also detected in extracts of injured specimens, and it is believed that all these sesquiterpenes are formed enzymatically as they never could be observed as transformation products during work-up and isolation. Several of the compounds have been isolated in previous investigations of *L. deliciosus* and *L. deterrimus*, i.e. alcohol 41 (Vokáč et al. 1970), lactaroviolin (48) (Heilbronner and Schmid 1953), and lactarazulene (49) (Šorm et al. 1954). From Californian specimens of *L. deliciosus* lactarofulvene (50) was obtained (Bertelli and Crabtree 1968) while Indian specimens of *L. deterrimus* yielded the aldehyde 51 (Koul et al. 1985), although no traces of the latter two were seen in a recent investigation (Bergendorff and Sterner 1988), The green colour of the injured mushroom tissue emerges from a mixture of orange-yellow (40, 41 and 42) and violet-blue (47 and 48) compounds. The fruit bodies of *L, sanguifluus* originally contain a mixture of the orange 40 and the red 43, predominantly the stearic acid esters, which gives the latex its deep red colour (Sterner et al. 1989). Fruit bodies that had been injured (by grinding them in a meat grinder) for 30 minutes prior to extraction, yielded only sangol (44) (Sterner et al. 1989), although the aldehydes 45 and 51 previously have been isolated from *L. sanguifluus* (De Rosa and De Stefano 1981). The blue ester 46 has only been isolated from fruit bodies of *L. indigo*, which also yielded lactaroviolin (48) (Harmon et al. 1980).

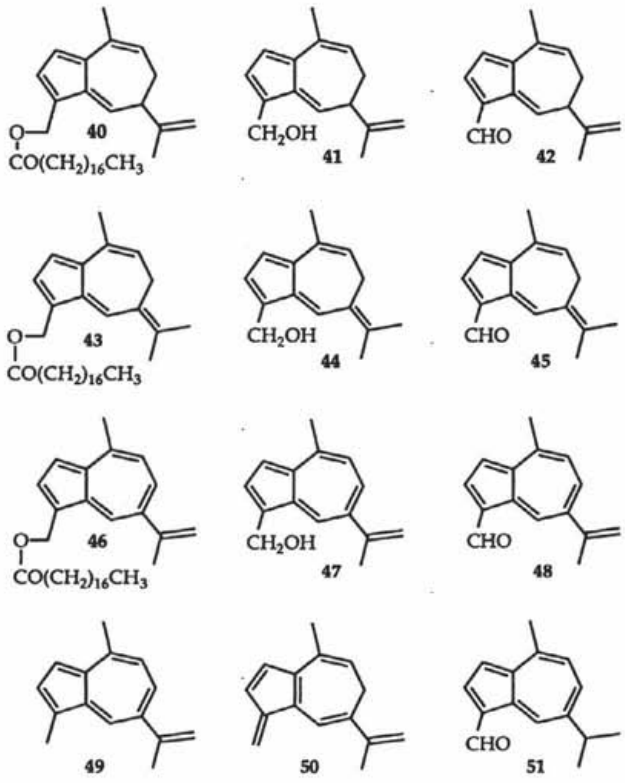

Figure 7

Although the bacteriostatic activity of lactaroviolin (48) was reported already in the 1940-ies (Willstaedt and Zetterberg 1946), the guaiane sesquiterpenoids formed in the saffron milk caps do not appear to affect humans otherwise than by colouring the urine. However, in vitro assays performed with compounds 40, 41 and 42 (which all are reasonable stable), showed that especially deterrol (41) possesses moderate cytotoxic activity (10 μg/ml inhibits the growth of ECA cells 50 %) and weak mutagenic activity towards Ames tester strains TA98 and TA100 (2.4 revertants/μg/plate) in the presence of rat liver extract (Anke et al. 1989).

CONCLUSIONS

The secondary metabolism of higher fungi has evolved during millions of years in order to increase the competitiveness of fungi, and produces a large number of biologically active and toxic compounds of which a substantial part is terpenoids.

Although the natural function of natural products in general is unknown, many have never the less been suggested to play roles for example as pheromones, as feedants, antifeedants or repellants, as regulators of the development of organisms as well as social behaviours, and in chemical defence systems. All such functions demand of a compound that it possesses biological activities, and the higher the activity is the more efficiently would it normally be able to perform its duties. The likelihood for such compounds to be toxic is therefore not negligible. In view of the fact that mushrooms have a low nutritional value and should be regarded more as a spice than as food in modern cuisine, one way to limit the risks to consumers is simply to avoid consuming excessive amounts of wild mushrooms.

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Fungi in biotechnology. Past, present, future

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From multiple uses of fungi in early historic times, "early biotechnological processes" developed. Fungi were used here already e.g. for production of cheese, bread, wine, beer and other foodstuff.

Based on these processes, fungi today have become one of the most important group of organisms in modern technology, where food, fodder and various metabolites such as antibiotics, enzymes, steroids etc. are produced on an industrial scale.

An equally important role of fungi in biotechnology is also to be expected for the future where – in addition to existing uses – fungi will be increasingly used employing modern methods such as genetic engineering and will also new applications as in pollution control, biological control of pests, microbial leaching and even biotechnology.

Key words: Fungi, biotechnology, past, present, future

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Z četných použití hub v ranných historických dobách se vyvinuly „ranné biotechnologické metody". Houby byly při nich už používány při výrobě sýrů, chleba, vína, piva a dalších potravin. Houby se dnes staly, na základě těchto metod, jednou z nejdůležitějších skupin organismů v moderních biotechnologiích, kdy potraviny, krmiva a různé metabolity jako antibiotika, enzymy, steroidy atd. se vyrábějí na průmyslovém základě. Rovnocenná role hub v biotechnologiích je očekávána i v budoucnu, kdy – kromě řady dosavadních použití – budou houby využívány za účasti moderních metod, jako je genetické inženýrství a naleznou využití při zvládání znečištění prostředí, biologickém potlačování škůdců, mikrobiálním vyluhování a možná i v biotechnologiích v kosmickém prostoru.

INTRODUCTION AND DEFINITION

Fungi are becoming more and more important in our life. Therefore, as a start for the symposium on FILAMENTOUS FUNGI IN BIOTECHNOLOGY a short survey of the role of fungi in biotechnology in the past, in the present and in the foreseeable future shall be presented (see also Molitoris 1989, 1990a).

Biotechnology is a relatively new term meaning "the use of organisms for the production of biomass or metabolites on an industrial scale". BIOTECHNOLOGY involves and interconnects the fields of BIOLOGY (the organisms involved), ECONOMICS (costs), KINETICS (physical/chemical influences on biotechnological processes) and PROCESS MANAGEMENT (see Lafferty 1981).

FUNGI AND MAN

In their relation to man, fungi may be considered as FOE or as FRIEND (Table 1).

Tab. 1 Fungi and Man

FOE	FRIEND
Spoilage	Mineralization
Pathogen	Mykorriza
Allergen	Food and Fodder
Toxin production	Medicine
	Religious/mystical ceremonies
	Science and Research
	Biotechnology
	Biological control
	Pollution control

Examples for the negative action of fungi are their activities in causing SPOILAGE of natural substrates (dry-rot of wood in buildings by *Serpula lacrymans*) and man-made substrates (plastics by *Penicillium simplicissimum*), their action as PATHOGENS FOR PLANTS (corn-smut of maize, *Ustilago maydis*), for animals and man in the latter case not only as ALLERGEN but also causing often severe diseases (blastomycosis by *Blastomyces dermatitis*) or INTOXICATIONS (poisoning by consumption of *Amanita phalloides* poisoning).

In many respects, however, fungi have important and positive values in nature and for man: They are part of the microbiota in soil causing MINERALIZATION by degradation of organic material and as MYCORRHIZA they are important symbionts for many higher plants, especially trees. Coming to the topic of this review, they are used in the production of FOOD and FODDER; since ancient times they are used in MEDICINE (Molitoris 1978, 1994) and more recently they have became important tools in SCIENCE and RESEARCH and are used increasingly to fight increasing problems of o ur modern society in the BIOLOGICAL CONTROL OF PATHOGENS and in POLLUTION CONTROL.

FUNGI IN BIOTECHNOLOGY IN THE PAST

In the history of mankind, fungi have been used in various ways for a variety of purposes (Table 2).

Tab. 2 Fungi in biotechnology in the past

EARLY GENERAL USES OF FUNGI	EARLY BIOTECHNOLOGICAL PROCESSES
Food	Cheese
Poison	Bread
Medicine	Beer
Mystical/religious ceremonies	Wine
	Cultivated edible mushrooms

Early general uses of fungi

Food

Since earliest times, fungi have been widely used as FOOD as symbolized in the medieval fresco of the chapel of Plaincorault in France (1291 a.C.) where Adam and Eve in the famous temptation scene are seen eating from a "fungus-tree" (Wasson 1968).

Poison

Fungi were important also as POISON, either consumed accidentally or administered purposely like in the alleged poisoning of the Roman emperor Claudius by Agrippina (Ramsbottom 1972). They were also used for causing artificial abortion by the "wise women" in the middle ages which gave for this purpose the alkaloid-containing sclerotia of ergot (*Claviceps purpurea*), a fungal parasite of grasses.

Medicine

Ergot by the way, was used also as MEDICINE already in the middle ages in gynaecology and other areas, as also many other fungi have been used as medicinal plants (Molitoris 1978, 1994).

Mystical/religious ceremonies

Some fungi, in particular hallucinogenic ones, such as *Psilocybe cubensins* or *Amanita muscaria* have been used by many ethnic groups in mystical, long-forgotten religious or shamanic ceremonies, which often date back thousands of years as documented by the so-called "fungus stones" from Guatemala in Middle America (Heim, 1963).

"Early biotechnological processes" involving fungi

The use of fungi by early man led in some cases o processes which could well be termed "early biotechnological" processes by procedure importance, amount and value of the products.

Cheese

One of the earliest examples would be the production of milk products such as CHEESE. When man turned from gatherer and hunter to be nomad and later farmer, herds of domesticated cattle gave meat, milk and other goods. Milk is one of the most nourishing but also perishable natural goods, which by action of microorganisms either can be destroyed but also can become a stable, nutritious and delicious food like cheese. One of the earliest documents for the use of milk is an almost 4000 year-old relief inthe sumeric town of Ur showing the whole procedure from getting the milk until making butter and/or chease.

Bread

Another early example would be the preparation of BREAD from ground cereals where yeasts under appropriate conditions (water, temperature) are responsible for the leavening of the dough by their carbon dioxide production. Early bread-making has been shown in an old Egyptian relief dating back about 4500 years.

Already in ancient times BEER and WINE, two alcoholic beverages, were produced in large amounts and played an important role as recreational drinks, but also as necessary food. Here fungi (yeasts) convert the sugar of cereals and grapes into carbon dioxide and alcohol.

Wine

For making WINE particularly the Greeks, Jews and Romans were known in ancient times. This is often documented in sculptures, paintings and written documents as in the Holy Bible (the miracle of the wedding of Kanaan).

Beer

Also the history of beer-making goes back a very long time as is illustrated by the famous relief from ancient Babylon, showing two persons drinking beer with straws from an amphora. The explanation for this unusual way of drinking beer is of course that filtration of beer in ancient times was an unknown technique. Therefore the fresh beer was cleared first by sedimentation of the particles and then the supernatant beer was drunk with straws. It might be also interesting to know that the old Babylonians knew already about 20 different kinds of beer.

BEER and WINE became more and more important as food and even as medicine (every Roman soldier had to drink daily 2 litters of wine for his health – happy times!). No wonder that some of the oldest food laws were concerned with these liquids. The German "purity law for beer" of 1516 exactly regulated ingredients, production and marketing of beer and punished those not obeying it. This law is still in effect in Germany.

Cultivated edible mushrooms

Cultivation of edible mushrooms most probably started more than thousand years ago in the Far East with mushrooms such as *Lentinus edodes* ("Shii-take").

In middle Europe the first reports are from the Middle ages about cultivation of the edible mushroom *Agaricus bisporus* in caves in France.

FUNGI IN BIOTECHNOLOGY IN THE PRESENT

From the impressive list given in table 3, just a few items will be discussed.

Tab. 3 Fungi in biotechnology in the present

FOOD	PRODUCTION OF FOOD AND FODDER
	Edible Asiatic fermentation products
Mushrooms	Alcoholic beverages
Fungal biomass	Milk products

METABOLIC PRODUCTS		
Alcohol	Organic acids	Vitamins
Steroids	Amino Acids	Hormones
Antibiotics	Nucleic acids	Ergot alkaloid
Enzymes		

RESEARCH	OTHER
Genetics	Biological control
Metabolism	Pollution control
	Recycling

Food

Fungi as food presently play an important role in our economy by amount and value.

Edible mushrooms

Edible fungi for human consumption are cultivated nowadays in highly mechanized, automatized and even computerized enterprises world-wide. This production is constantly increasing by rising demand, more efficient procedures, but with decreasing prices. Advantages of the cultivation of edible mushrooms are cheap substrates – even waste products such as straw – no need to use arable land, excellent flavour, high vitamin and mineral content, and in some cases even positive health effects such as in *Lentinus edodes*.

World-wide, the production of cultivated edible mushrooms reaches now more than 4 million tons per year, with the cultivated mushroom, *Agaricus bisporus*, leading, followed by the oyster mushroom, *Pleurotus ostreatus*, the Shii-take mushroom, *Lentinus edodes*, and others as shown in table 4.

Tab. 4 World production of cultivated mushrooms in 1991 (from Chang 1993)

Species	(common name)	Production (tons x 1000)
Agaricus bisporus and other spp.	(cultivated mushroom)	1,590
Pleurotus ostreatus and other spp.	(oyster mushroom)	917
Lentinus edodes	(Shii-take)	526
Auricularia auricula-judae	(jew's ear mushroom)	465
Volvariella volvacea	(straw mushroom, Chinese mushroom)	253
Others		531
Total		3,742

Biomass

In contrast to this, fungal biomass production from submerged cultivation on originally cheap hydrocarbons (single cell protein = SCP's), which was intended for fighting hunger in third-world-countries, did not fulfil the expectations because of rising costs of the substrates and for food preferences in those countries for which the product originally was meant for.

The only exception is QUORN, a British fermentation product from the Deuteromycete *Fusarium graminearum* which fulfils strict foodstuff requirements, has good nutritional values and seems to be produced at compatible prices.

Production of food and fodder

Presently about 25% of our food is already produced by biotechnological processes and fungi play an important part in it.

Alcoholic beverages

In the western world WINE and BEER are leading in importance and also this country is famous for these products. So one of the great typical beers, the "Pilsener" with its typical taste, originates from the Czech town of Pilsen, where it was originally brewed.

The production of WINE, the other major alcoholic drink, is still increasing. By new and improved methods and new breeds of grapes, new areas such as California and Chile in America, South Africa and Australia, in recent decades started to grow and sell quality wine with increasing success.

Milk products

CHEESE is still one of the most important fungal products from milk. A number of these cheeses are quite typical for certain areas and are even named for them, e.g. "Roquefort" (caves of Roquefort, France), "Edamer" (town in Holland), "Emmentaler" (valley in Switzerland), "Danish Blue" or "Bavarian Blue" and last not least the "Camembert" from witch the producing fungus, *Penicillium*

camemberti, got its name. More recently, also other fungal products from milk, such as Kefir, Yoghurt and others, gain more and more importance.

Metabolic products

One of the main areas where fungi are used today in biotechnology, is in the production of certain metabolites for various purposes as given in table 3.

Alcohol

Alcohol is still a fungal product of major importance. It has changed, however, insofar as it is not only used in beverages, but is used increasingly as a component of medicines and for preparative and synthetic processes in industry. More recently, however, the amount of alcohol produced from prokaryotes, is increasing.

Steroids

Steroids became very important since the advent of the anti-baby pill and also for other medical purposes. Fungi such as several *Penicillium* as *Aspergillus* strains by virtue of their ability to catalyse very specific one- or few-step transformations in steroids became essential in the production of these drugs.

Antibiotics

Since Alexander Fleming in 1928 discovered the production of the first antibiotic, Penicillin, from the imperfect fungus *Penicillium notatum*, everyone knows about the importance of these metabolites in medicine. About 6000 antibiotics are known today, at least by their formula, but only about 400 of them, for various reasons, are used in human medicine. Fungi produce about a quarter of them. Constantly new antibiotics are looked for and found, a necessity by the fact that pathogenic microorganisms constantly acquire resistance against the known and administered antibiotics.

Enzymes

Quite a number of fungal enzymes are produced commercially and have become indispensable tools in industry and research (Table 5). As the table shows, they are used for a wide variety of applications from biochemical analysis, over medical diagnosis to food processing.

Organic acids, amino acids, nucleic acids

Among the organic acids of fungal origin, citric acid is leading with an annual production of over 600.000 tons. It is mainly used in the food and beverage industry. But also amino acids and nucleic acids are commercially produced from fungi and find wide application.

Ergot alkaloids

Fungal alkaloids, mainly originating from ergot, *Claviceps purpurea*, have been mentioned earlier mainly as poison, an abortivum and occasionally as medicinal drug. The latter use nowadays is their main importance. These alkaloids are

produced now in increasing amount by saprophytic culture or even by biosynthesis using certain – even genetically engineered – strains. These alkaloids find now wide medicinal application e.g. in gynaecology, against migraine and in the treatment of heart and circulatory diseases.

Tab. 5 Important industrial enzymes from fungi (Molitoris 1991)

Enzyme	Use
OXIDOREDUCTASES (EC 1.)	
Glucose oxidase	Taste intensifier
Catalase	Removal of hydrogenperoxide
HYDROLASES (EC 3.)	
Esterases (Ec 3.1)	
Lipase	Aroma development, aid for extraction
Ribonuclease	Taste intensifier
Glucosidas (EC 3.2)	
α-Amylase	Baking and brewing industry
β-Amylase	Starch hydrolysis
Dextranase	Cosmetic industry (tooth paste)
Glucoamylase	Starch hydrolysis, brewing industry
Hemicellulase	Food industry
Invertase	Production and processing of sweets
Lactase	Milk- and baking industry
Melibiase	Sugar production from sugar beets
Naringinase	Beverage industry (removal of bitter taste)
Pectinase	Beverage industry (fruit juices)
Xylanase	Food industry
Cellulase	Food-, cellulose-, paper-industry, SCP production
Peptide hydrolases (EC 3.4)	
Protease	Food industry, medicine (peptic preparation, digestion)
Rennin	Milk industry (cheese production)

Research

Genetics

Because fungi possess a relatively low level of organisation and are easy to handle in the laboratory, fungi represent increasingly important tools in modern research, especially in genetics. The different types of life cycles, the different ways of genetic inheritance and e.g. the possibility to identify by tetratede analysis in certain

ascomycetes the stepwise production of the meiosis products, are advantages of this group of microorganisms. Successes in this area of research are documented e.g. by the first Nobel Prise given for fungal research in 1958 for physiology and medicine to BEADLE and TATUM for their work with the red bread mould *Neurospora crassa.*

Metabolism

Fungal metabolites are indispensable substances in modern research. Fungal antibiotics e.g. already mentioned as highly efficient medicaments, are used also in research. The antibiotics Penicillin, whose action against many pathogenic bacteria is based on its effect on the bacterial cell-wall synthesis, is used to analyse and identify specific steps in this important morphogenetic process.

Other

Biological control of pathogens

To reduce the need for chemicals in control of pests and pathogens, recently much work has been done to find biological control measures. These methods would reduce the need for chemicals, which are expensive, relatively unspecific and also cause environmental problems because of their toxicity and recalcitrance. Again, in this quite modern aspect of biotechnology, fungi are involved, particularly because they show a high specificity of action (Burge 1988).

Nematodes, a deadly pest for mushroom culture, can efficiently be controlled by certain fungal preparations (Stirling 1988), such as from *Arthrobotrys robusta* ("Royal 300").

Some of the enthomopathogenic fungi, fungi pathogenic against insects, can be used for control of insect pest, such as "Mycotal" and "Vertalec", preparations from *Verticilium lecanii,* which is used in the green houses industry (Quinlan 1988).

And finally, fungi may be used to fight fungal diseases as the Deuteromycete *Trichoderma* can be used against a number of pathogenic fungi, in particular if used as "integrated control" in connection with other control measures (Papavizas and Lewis 1988).

Pollution control

As already indicated above, biological control measures using fungi in turn help in reducing the need for chemicals in pest and pathogen control. They therefore are one new and important aspects in the involvement of fungi in the ever more necessary measures for the protection of our environment.

Recycling

Recycling in other word, making use of materials which otherwise would contribute to pollution or are at least useless, can consider as another aspect of environment protection. Two methods from mushroom cultivation might serve here as example.

First, the oyster mushroom, *Pleurotus ostreatus*, can be grown on straw as sole substrate. It has been found in this country that spent *Pleurotus* compost, being very nutritious and having a good smell, can be used as an addition to the fodder for cattle. In this way straw, an almost costless agricultural surplus product is totally recycled, partially giving rise to fruitbodies (ca 10% of substrate weight), partially replacing peat in gardening, and partially being used as fodder.

Secondly, experiments in Germany have shown that edible mushrooms can be successfully grown on composted household garbage. The reason, however, why this method has not been introduced into practise is, that unfortunately the average content of heavy metals in ordinary household is too high and results in too high contents of these substances in the fruitbodies produced on this substrate.

FUNGI IN BIOTECHNOLOGY IN THE FUTURE

In the future many of the biotechnological uses of fungi are expected to continue, even at an increased scale, and new application will be added. Some of these products and areas are listed in table 6.

Tab. 6 Fungi in biotechnology in the future

Food
Metabolic products
Genetic engineering
Microbial leaching
Biological control of pests and pathogens
Environments protection
Research
Basic research
Space biology

Food

Besides using other fungi for the production of new types of food such as QUORN, constantly new strains and species of edible fungi are investigated for mushroom cultivation. An interesting example is the cultivation of the edible morel, *Morchella conica,* based on an US patent, which is reported to enter now an economically feasible stage (Coombs 1994).

Metabolic products

Constantly new, better and more metabolites will be produced in the future by fungal species, some of them modified by genetic engineering. This is true for work on the production of "Hirudin" (HOECHST AG, Germany), for human "Insulin" (NOVO, Denmark) and for new types of brewing yeasts. In all cases yeasts are used

because of the relative ease and efficiency of their genetic manipulation, cultivation and metabolic production.

Genetic engineering

Genetic engineering usually includes in principle the following steps. First: Isolation of the genetic information for the production of an important metabolite from a naturally producing organism (often a higher plant or an animal, usually slow growing and producing). Second: Transfer of that genetic information into another organism (usually a fast growing and fast producing microorganism). Third: Integration of this information into its genetic material. Finally these microorganisms, in our case, fungi, often yeasts, produce with modern fermentation technology the desired metabolites faster, in higher quantities and at much lower costs because of their simple growth requirements and modern fermentation technology.

Microbial leaching

Bacterial leaching of ores by now is a well established method to obtain economically valuable metals from ores too low in metal content for conventional mining. The principle is that the metal-containing ores are percolated with microorganism-containing liquids, the metals are solubilized by the bacterial metabolism and may be later extracted or precipitated. Recently, methods are being developed to employ also fungi (*Saccharomyces, Rhizopus*), for specific extraction of such valuable metals as manganese, uranium, gold and platinum (Gröger et al. 1987).

Biological control

A number of promising projects involving fungi are being investigated, e.g. control of the deadly chestnut blight or the Dutch elm disease, using hypovirulent strains of the respective fungal pathogens (Adams 1988).

Environment protection

A number of projects are concerned with the use of fungi for degradation of waste or toxic materials. Of particular interest are here the often highly toxic xenobiotics, such as polycyclic aromatic hydrocarbons, where research at the Czechoslovak Academy of Sciences and in our own laboratory has shown that fungi, particularly ligninolytic fungi, are able to degrade a high proportion of these substances in a relatively short time (Vyas et al. 1994a, Vyas et al. 1994b).

Other problems are caused by our indispensable plastic materials, because they are usually produced from the rapidly diminishing fossil resources and because of their lacking biological degradability (originally considered to be an advantage). As a possible solution new microbial biosynthetic thermoplasts from renewable resources (e.g. BIOPOL, ICI, England) have been developed. However, in order to use them generally and in large quantities, their complete bio-degradability (without toxic residues) has to be established first. Research concerning the ability

of fungi to degrade these materials is being conducted in our laboratory and the results look quite promising.

Research

Also in the future fungi will be involved in research. Among these projects are the elucidation of basic principles like the effect of gravity on living systems. Because of their low level of organisation and good handability, fungi again are investigated in the laboratory to solve these questions and because of new and interesting approaches a new aspects of the use of fungi in space biology research are presented.

The recent developments in space flight and the possibility to conduct research in orbit, recently provided the chance to investigate living systems under space conditions such as (near) weightlessness (microgravity), cosmic irradiation and low pressure. A few examples for the involvement of fungi in this type of research shall be presented, which in some cases may lead to new biotechnological processes (Molitoris 1990b).

Genetic engineering often involves optimisation of hybrid formation. Electrofusion is such a method, where cells are brought into contact in an electric field, form chains and are hybridised by electric pulses. Experiments using yeast cells under simulated and actual space conditions (US/German space mission D-1, 1985) have shown a much higher yield of hybrid cells than on the ground since thermoconvection and sedimentation do not interfere.

Finally let us came back to a product where fungi were essential in biotechnology already in the past and still are important in the present and certainly will be also in the future, let us speak about beer: In the last US/German spacemission (D-2, 1994), space experiments with brewers yeast were conducted. Whether they were successful, the forthcoming publications will show.

Summarising the role of fungi in biotechnology in the past, in the present and in the future, we can state:

Fungi in biotechnology are important and their future certainly looks bright,
but it depends on us, on the scientists and the people who use our results,
whether our positive expectations may be fulfilled,
so that modern biotechnology with fungi
does not destroy but protects and improves
the blue planet earth on which we live.

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Book review

D. H. GRIFFIN: FUNGAL PHYSIOLOGY

2nd Edition, Wiley-Liss, New York 1994, ix + 458 pp

Twelve years after the first edition, the Griffins book on fungal physiology appears in a completely rewritten, modernized form. The autor had to cope with the great progress in experimental methods and achievements of the last decade without writing a book on fungal biochemistry and molecular genetics. In my opinion, he has mastered the task. The book is written for advanced students and replaces neither introductory texts nor specialized treatises. A background knowledge in mycology and also in biochemistry and physics is assumed but the text is clear and logical so that consulting of further books will seldom be necessary. The author omits generalities and stresses the aspects of physiology peculiar to fungi. The experimental results are use to derive current physiological concepts and the text never becomes a tangle of hardly generalizable data. Typical is an experimental approach, explaining the basic methods and illustrating the experiments that have lead to important conclusion. The book is written by one person and that gave it and uniform style and terminology and well-balanced contents of individual chapters.

"Fungal Physiology" consists of fourteen chapters. After an introduction to the world of fungi the author describes the most important chemical components of fungal cells and the "molecular architecture", in fact the cytology of fungi. Four chapters deal with various aspects of individual cells and whole thalli, including acquisition, digestion and transport of nutrients and physical factors influencing the growth. Following parts give account on the primary and secondary metabolism of fungal mycelia and its regulation. Sexual reproduction including meiosis and physiology of spore development, dormancy and germination are subjects of three chapters. The last chapter covers antifungal antibiotic and synthetic fungicides. An ecologically oriented mycologist may miss a chapter on physiological aspects of fungal ecology, nevertheless, many facts pertinent to this area can be found dispersed throughout the book.

The "Fungal Physiology" fills an important gap in available texts and is an excellent source of knowledge not only to advanced students but also to anybody who is engaged in experimental mycology.

Jiří Kunert

CZECH MYCOL. 48 (1), 1995

Protective action against Amanita poisoning by iridoid glucoside, aucubin

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Yamaura Y. and Chang I.-M. (1995): Protective action against Amanita poisoning by iridoid gluoside, aucubin. – Czech Mycol. 48: 67–72

Aucubin, an iridoid glucoside, exhibits significant protective activities against Amanita poisoning in beagle dogs. The post-injection of aucubin helps beagles survive from lethal poisoning caused by Amanita virosa. Protective activities of aucubin result from primarily preventing hepatic injury caused by Amanita poisoning, and is partly due to a protective affect of aucubin on the depression of m-RNA biosynthesis in the liver caused by α-amanitin intoxication.

Key words: Aucubin, antidote, Amanita poisoning, hepatic injury, beagle dog.

Yamaura Y. a Chang I.-M. (1995): Protektivní působení iridoidního glukosidu aukubinu při otravě muchomůrkou (Amanita). – Czech Mycol. 48: 67–72

Aukubin, iridoidní glukosid působí významně protektivně při otravě Amanita u laboratorních psů (beagle). Injekční aplikace aukubinu podaná laboratorním psům po otravě letální dávkou Amanita virosa způsobila jejich přežití. Protektivní účinek aukubinu vyplývá z jeho primární prevence jaterního poškození a částečně i chrání před poklesem biosyntézy m-RNA v játrech, kterou způsobuje otrava alfa amanitinem.

INTRODUCTION

Over the past decades, many efforts have been made to search for an antidote for deadly *Amanita* poisoning. Various compounds such as cytochrome C, pencillin G, thioctic acid and silibinin (Vögel et al. 1984) have been reported to exhibit some degree of potency for the treatment of *Amanita* poisoning (Floersheim 1987), but their clinical efficacies have been controversial (Piqueras 1989). Previously, we reported that an iridoid glucoside, aucubin, markedly increased survival rate in mice intoxicated with α-amanitin (Chang 1984). based on this finding, the present study aims to evaluate the protective potency of aucubin as an antidote in beagle dog that had ingested aqueous extract of *Amanita virosa.*

MATERIALS AND METHODS

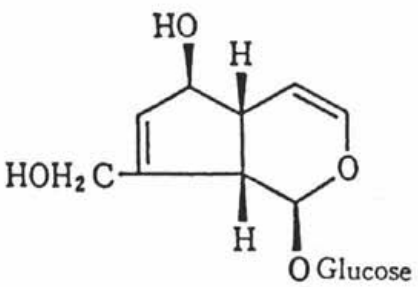
Isolation of aucubin

Aucubin (Fig.1) was isolated from fresh leaves of *Aucuba japonica* Thunb. (*Cornaceae*) (Trim and Hill 1952).

Preparation of aqueous extract of *A. virosa.*

An aqueous extract of the mushroom was prepared and contents of toxins were analysed by the method reported previously (Yamaura et al. 1981). One gram of fresh mushroom contained 1.06 mg of α-amanitin and 0.64 mg of phalloidin.

1



Aucubin $C_{15}H_{22}O_9$
mol. wt. 346.33

Fig. 1. The structural formula of aucubin (C15H22O9, mol. wt. 346.33) isolated from *Aucuba japonica* Thunb.

Administration routes of aqueous extract of the mushroom and aucubin

Animals and treatment

Each of two male beagle dogs (9.8 – 1.6 kg) were used as a survival study. Two groups received orally a lethal dose (0.4 g fresh mushroom/kg body weight) of aqueous extract of the mushroom. Treated group was administrated intravenously with a single dose (100 mg/kg) of aucubin 30 min, 2 hrs and 4 hrs after the administration of *A. virosa* extract (aucubin treated dog). The other dogs served as the control and received only aucubin (100 mg/kg).

Assay of blood and urinary parameters

Contents of serum glucose and glutamic-oxaloacetic transaminase (SGOT) activity was assayed by the method using commercial kit. Other serum enzymes were carried out to measure the extent of glucose and protein excretion on 1st, 2nd and 3rd day using Urinary Analyzer (Ames Co. USA).

Measurement of the incorporation rates of ratio-labelled adenosine into polyadenylated sequence of m-RNA precursor in mice liver

Male ICR mice (20-22 g) were used. Each specified time comprised four groups; control (saline), aucubin only-treated, α-amanitin only-treated, and α-amanitin plus aucubin-treated group in which each mice received a single dose of aucubin (80 mg/kg) 1 hr after α-amanitin intoxication (0.1 mg/kg) except the mice of the saline-control group. At each specified time interval, each mouse in the four group (3 mice/group) was injected intraperitoneally with [8-^{14}C]-adenosine (1 μCi/mouse; specific activity, 55 mCi/mmole) and radio-labelling was allowed for 1 hr. the isolation of liver polyribosomes (Lee and Brawerman 1971a), the extraction of RNA portion from polyribosomes (Perry et al. 1972), and the adsorption of m-RNA on nitrocellulose filters (Millipore) (Lee et al. 1971b). The radio activities on nitrocellulose filter discs were measured in a liquid scintillation spectrometer (Tricarb 1000, Packard Co., USA). The radioactivities per mg of extracted RNA were expressed as a percentage of the control values.

RESULTS AND DISCUSSION

After about 20 hrs, the *Amanita* poisoning and aucubin treated dogs started vomiting and discharging stools covered with mucus. At 48 hrs, the *Amanita* poisoning dogs discharged stools with blood stains and the final outcome was death in the end of 72 hrs. However, we did not observe such toxic symptoms and death in aucubin treated dogs.

As shown in Fig. 2, a rapid depletion of serum glucose was observed during a 3 days period following intoxication in the *Amanita* poisoning dogs, but in the aucubin treated dogs, however, the level of glucose decreased slightly and then recovered to the normal range after 3 days.

Time course change of SGOT in the indicators if liver damage was shown in Fig. 3. SGOT increased markedly from 24 hrs, and a continuous increase of SGOT was observed during a 3 days period and it's level was about 250 times of the normal range at 3rd day. In the aucubin treated dogs, the level of SGOT was only about 40 times of the normal range after 3 days, and decreased gradually and returned to nearly normal range at 14th day. The other parameters indicate the extent of liver damage were shown in Table 1. In the aucubin treated dogs, their values were lower than that of the *Amanita* poisoning dogs.

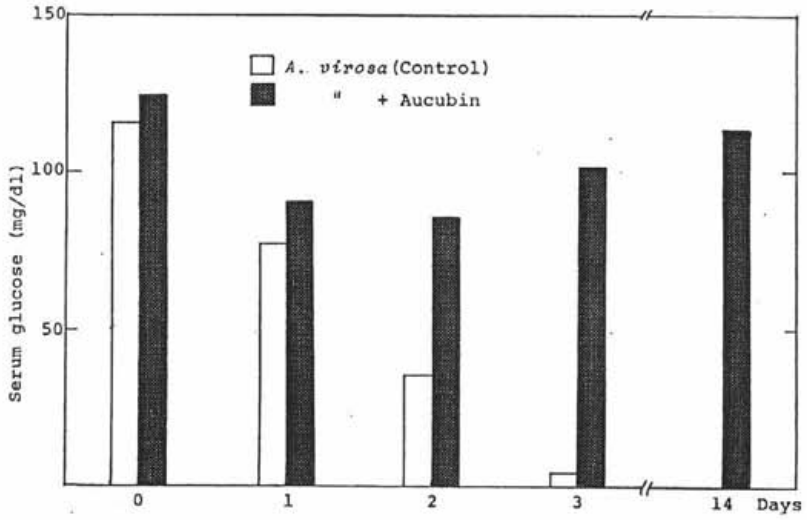


Fig. 2. Effect of aucubin of glucose in dogs after intoxication. Control was administered orally 4.0 g/kg of *A. virosa*. Aucubin was i.v. injected with a single dose (100 mg/kg) at 30 min, 3 hrs and 6 hrs after administration of the mushroom.

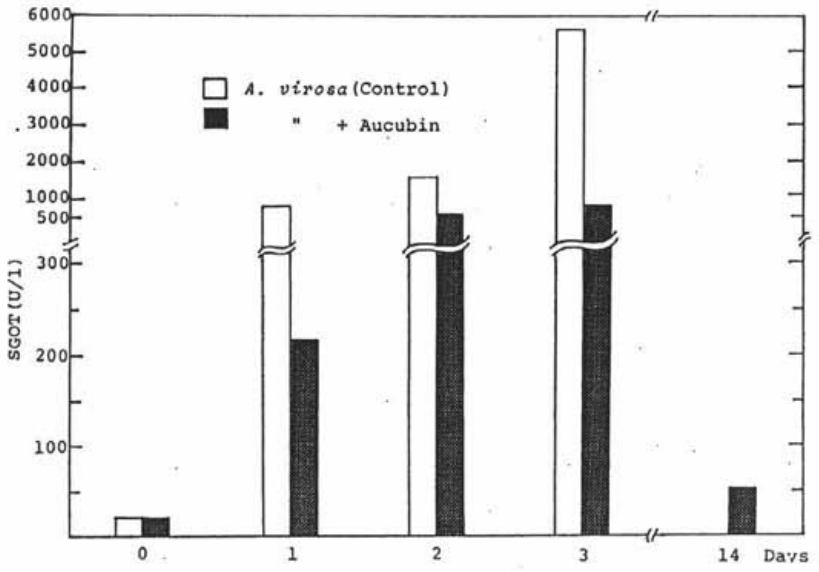


Fig. 3. Effect of aucubin of SGOT in dogs after intoxication. Control was administered orally 4.0 g/kg of *A. virosa*. Aucubin was i.v. injected with a single dose (100 mg/kg) at 30 min, 3 hrs and 6 hrs after administration of the mushroom.

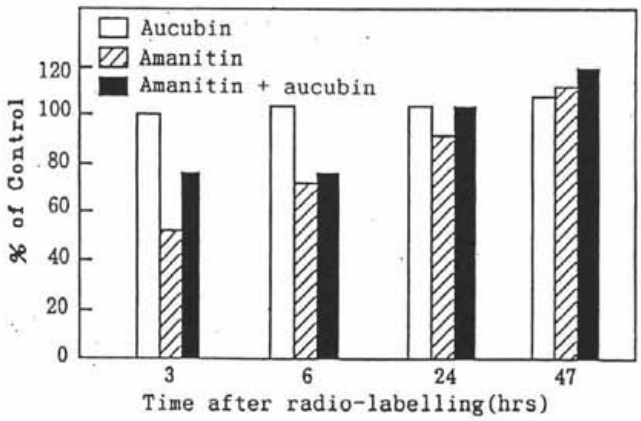


Fig. 4. Effect of aucubin on the incorporation rate of radio-labelled adenosine into polyadenylated sequence of m-RNA precursor in mouse liver.

Tab. 1 Effects of aucubin on components and enzymes in blood.

	GPT (u/l)	γ-GTP (u/l)	LDH (u/l)	BUN (u/l)	CRN (mg/dl)
A. virosa	8800	60	3700	62	0.8
A. virosa + acubin	110	11	752	38	0.5

Tab. 2 Excretion of glucose and protein into urine. Urine was collected for 24 hrs.

	Glucose (mg/dl)		Protein (mg/dl)	
	A. virosa		A. virosa	
Days	Control	+Acubin	Control	+Acubin
1	60	3700	62	0.8
2	11	752	38	0.5
3				

Excretion of glucose and protein into urine was shown in Table 2. Their levels were lower in the aucubin treated dogs than that of the *Amanita* poisoning dogs.

On the other hand, the results of histological examination also support biochemical effect. Massive liver cell necrosis was observed in the liver of *Amanita* poisoning dogs, but we can't see it in the liver of the aucubin treated dogs.

On the glycogen granules in the liver specimens, no glycogen granules were found in the liver of the *Amanita* poisoning dogs. In contrast, we could see glycogen granules in the liver of the aucubin treated dog.

It's well known that α-amanitin inhibit eucaryotic RNA polymerase II. Consequently, the formation of m-RNA precursors is blocked and cellular functions are impaired. We study how the aucubin influenced the liver's m-RNA biosynthesis in mice. As a measure of m-RNA biosynthesis, we used the degree of incorporation rates of radioactively labelled adenosine into the polyadenylated sequence of m-RNA precursors (Fig. 4). The incorporation rate was reduced to 54% of the control level after 3 hrs in the α-amanitin group. In the aucubin treated group, the incorporation rate was 75% of the control level after 3 hrs, and then it reached the control level at 24 hrs. These results suggested that the aucubin treatment prevents the depression of m-RNA biosynthesis in the liver by α-amanitin.

Such biochemical activity of aucubin may account in art of the protective activities against hepatic injury caused by *Amanita* poisoning.

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CZECH MYCOL. 48 (1), 1995

Heat-resistant fungi

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Jesenská, Z. and Piecková, E.(1995): Heat-resistant fungi. – Czech Mycol. 48: 73–76

The survival of fungi from soil samples has been investigated after temperature of 60, 70, 80 and 90 °C in Sabouraud agar. The number of isolated propagules and species had significantly different quantities. The heat-resistant fungi are an economically and scientifically important group of fungi and represent a matter for further investigation.

Key words: Heat resistance, fungi,

Jesenská, Z. a Piecková, E. (1995): Termorezistentné huby. – Czech Mycol. 48: 73–76

Študovalo sa prežívanie húb zo vzoriek zeminy v Sabouraudovom agare pri teplotách 60, 70, 80, and 90 °C. Počet izolovaných zárodkov i druhov sa významne líšil. Termorezistentné huby sú ekonomicky a vedecky významnou skupinou húb a predstavujú objekt pre ďalší výzkum.

Heat-resistant species, which survive the heat treatments used in fruit and juice canning processes are an important group of fungi.

The spoilage of heat processed fruit is caused mainly by the species *Byssochlamys nivea, Neosartorya fischeri, Talaromyces flavus.*

Their ascospores survive temperatures of 70, 80, and 90 °C in most cases for a significant time.

In addition of these fungi *Talaromyces bacillisporus, Talaromyces trachyspermus, Eupenicillium brefeldianum* and *Eupenicillium lapidosum* have been isolated as spoilage organisms for canned foods, and *Eupenicillium levitum, Eupenicillium ehrlichii, Aspergillus quadricintus, Talaromyces striatus, Talaromyces emersonii, Talaromyces wortmanii, Byssochlamys verrucosa, Eleutherascus tuberculatus, Neosartorya aurata, Neosartorya aureola, Neosartorya hiratsukae, Neosartorya fennelliae, Neosartorya fischeri* var. *fischeri, Neosartorya fischeri* var. *glabra, Neosartorya fischeri* var. *spinosa, Neosartorya primulina, Neosartorya pseudofischeri, Neosartorya quadricincta, Neosartorya stramenia*, along with *Eurotium herbariorum* and *Eurotium chevalieri* may also be added to the list of these heat-resistant species.

Gilmaniella humicola was isolated from heat-treated soil from hothouses.

The role of the majority of these fungi in spoilage of foods has not been studied.

Searching for recourses of processed fruit contamination, we focused our attention on questions of occurrence of the heat-resistant fungi in the soil (Jesenská and

Piecková 1993, 1994, Jesenská et al. 1991, 1992 a,b, 1993, 1994, Piecková et al. 1994). The aim was, briefly said, to clarify certain questions of ecology of heat-resistant micromycetes. That might contribute to better protection of certain kind of foodstufs against undesirable activity of these fungi. It was necessary to find out to what degree poor sanitation in food industry and lack of working discipline on the one hand, and to what degree other, not quite known, objective factors are to blame for decay of a considerable portion of food supplies for people.

We examined samples of Slovak soil for fungi whose propagules are able to survive increased temperatures in Sabouraud agar with Bengal Rose for certain time.

Ten grams of soil yielded on average 178 and 102 colony forming propagules, those were able to survive in Sabouraud agar 70 and 80 °C/60 minutes and 32 colony forming propagules those were able to survive 90 °C/30 minutes.

In 32 soil samples taken from various localities of the Slovak republic and exposed to 70 °C for 60 min, *Eupenicillium baarnense* occurred most frequently – in 93 %, along with *Neosartorya fischeri* – in 90 % and *Talaromyces avellaneus* – in 68 % of samples. *Byssochlamys nivea* and *Gilmaniella humicola* were isolated from 34 %, *Talaromyces flavus* from 25% of samples.

The incidence of *Dichotomomyces cejpii, Talaromyces trachyspermus, Talaromyces bacillisporus* and *Nodulisporium* sp. was sporadic.

The survival of fungi from soil samples has been investigated after exposure to temperatures of 60, 70, 80 and 90 °C in Sabouraud agar in 10 minutes intervals.

I. *Aspergillus niger* group, *Chaetomium* sp., *Penicillium* sp. and *Scytalidium lignicola* were the least heat-resistant fungi – propagules survived 60 °C/60 min., did not survive 70 °C/10 min,
II. next group's *Aspergillus glaucus* group, *Byssochlamys nivea, Dichotomomyces cejpii, Gelasinospora* sp., *Rhizoctonium* sp. and *Talaromyces flavus* – propagules survived 70 °C/60 min., did not survive 80 °C/10 min,
III. *Aspergillus fumigatus, Aspergillus nidulans, Eupenicillium baarnense* and *Ulocladium* sp. – propagules survived 80 °C/60 min., did not survived 90 °C/10 min,
IV. *Acremonium sclerotigenum, Aspergillus ochraceus, Botryotrichum piluliferum, Byssochlamys fulva, Gilmaniella humicola, Neosartorya fischeri* – propagules survived 90 °C/10 min,
V. the most heat-resistant were *Nodulisporium* sp. and *Talaromyces avellaneus.* The propagules survived 90 °C/60 min.

Some of those fungi are not "really heat-resistant" species, but species capable of forming sclerotia, thick-walled cells, etc.

The isolates of *Nodulisporium* sp. did not form structures of the teleomorphic stage on our media used. However it is obvious that the species must exist in soil in

a form capable of resisting the effect of a temperature of as high as 90 °C for over 60 min.

The numbers of surviving propagules had significantly different quantities.

The survival ability of the heat-resistant fungi is usually determined by two means:

a) determination of the thermal death time (TDT), or of the final point and
b) multipoint methods – determination of the decimal reduction time (D) and z values.

Each of the methods have their own advantages and drawbacks.

We determined TDT for new heat-resistant species of fungi, namely for the strains of *Dichotomomyces cejpii, Gilmaniella humicola, Talaromyces avellaneus* and *Talaromyces bacillisporus.*

Their TDT values were compared under the same conditions in vitro with the TDT values of known heat-resistant species *Byssochlamys nivea, Neosartorya fischeri* and *Talaromyces flavus.*

All of the new species showed considerable resistance against higher temperatures in the Sabouraud medium. Their propagules withstood the exposure to the temperature of 70 °C/95 – 300 minutes.

The most resistant strain *Talaromyces avellaneus* withstood the temperature of 80 °C for up to 120 minutes, 90 °C for 10 min.

TDT values were in vitro affected by the number of propagules in the inoculum suspension.

The significance of new species of heat-resistant fungi for the canning industry is not sufficiently estimated till now.

We assume that their occurrence in mouldy canned fruits and juices can be overlooked and the isolated strain remain undeterminated.

Conclusion:

The group of the heat-resistant fungi represents a matter for scientific investigation: The heat-resistance mechanisms, the influence of different factor in heat-resistance, the germicidal control, physiological properties, secondary metabolites and others.

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Micromycetes in archives and book depositories in the Czech Republic

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Fassatiová O. (1995): Micromycetes in archives and book depositories in the Czech Republic.– Czech Mycol. 48: 77–86

Species representation of micromycetes and their frequency were studied in the period 1981-1988 in four archives in Prague and near Prague and in one depository in West Bohemia. Representants of genera Penicillium, Cladosporium, Alternaria, Aspergillus, Mucor and Rhizopus isolated by sediment plate method and from the surfaces of the archive depots were the most frequent in all observed spaces. The higher number of the most frequent species was always found in older and for the archive purposes less convenient buildings, while inside the new building the number of the most frequent species was very low. In suitable conditions only a limited number of species for which the given specific conditions are convenient, act as destructive. They are mostly penicillia which form coherent growing covers on the backs of the books and cartons. Spores of these fungi released by their growth contaminate the atmosphere of these spaces and can cause allergies in sensitive persons.

Key words: Micromycetes, archives, allergies, Czech Republic

Fassatiová O. (1995): Mikromycety v archivech a knižních depotech v České republice. – Czech Mycol. 48: 77–86

V letech 1981 – 1988 bylo sledováno druhové zastoupení mikromycetů i jejich frekvence ve 4 archivních budovách v Praze a v okolí Prahy a v knihovním depositáři v záp. Čechách. Ve všech sledovaných prostorách byly ze spadu i ze stěru archiválií nejčastěji izolováni zástupci r. Penicillium, Cladosporium, Alternaria, Aspergillus, Mucor a Rhizopus. Ve starších a pro archivní účely méně vhodných prostorách byl pravidelně zjištěn vyšší počet nejfrekventovanějších druhů, zatímco v prostorách nové budovy byly počty nejfrekventovanějších druhů velmi nízké. Za vhodných podmínek se destruktivně projeví jen omezený počet druhů, jimž dané podmínky vyhovují. Jsou to ponejvíce penicilia, která tvoří souvislé porosty na hřbetech knih a kartonů. Spóry těchto hub uvolňované z porostů na archiváliích zamořují ovzduší těchto prostor a mohou způsobovat alergie u citlivých jedinců.

INTRODUCTION

All over the world, a great attention is paid to studies on indoor fungi, especially in connection with mould allergies in household, as well as in some work places. This paper, presenting results from a less explored field, is devoted to moulds contaminating archives and book depositories where they cause biodegradation of archive material and in addition they can affect the staff health.

A certain tradition in studying archive fungi exists at the State Central Archive in Prague. This was due also to efforts of the physician and hygienist B. Skorkovský who elaborated a precise hygienic regime for archives and archive keepers published

later in the manual: Microorganisms as resources of degradation of archive material (1981) (in Czech).

The aim of my study was to establish micromycetes species representation in some archive rooms and books depositories, their extent and range of activities in connection with conditions of the examined spaces. Moulds isolation and determination were done in collaboration with my colleagues mycologists of the Department of Botany. Our first results have been published in 1987 (Fassatiová et al. 1987).

Robledo and Moretti (1986) report about 60 genera of fungi from archive material, the most frequent were *Penicillium, Aspergillus, Chaetomium* and *Phoma.* Kowalik (1980a, 1980b) studying microbiodeterioration in book material in Poland and examined various fungicides against micromycetes. The most resistant were: *Chaetomium globosum, Trichoderma viride, Fusarium* sp. and *Penicillium* sp. Samson (1985) briefly reports about the present strong mould contamination of some libraries and archives in the Netherlands. Gandjar et al. (1989) mention 32 species of micromycetes found in atmosphere and in archive material in Jakarta in Indonesia. The most frequent genera were *Aspergillus* and *Penicillium.*

MATERIAL AND METHODS

In the period 1981 – 1989 filamentous fungi were studied in three buildings of the State Central Archive, in the building of the archive of the city of Prague and in one depository of the National Library in West Bohemia. The only building constructed directly for archive purpose is the State Central Archive in Prague 6, the other ones are old historical objects inconvenient for archives. The Tab. 1 gives a survey of the observed buildings with their characteristics and abbreviations.

The sedimentation method marked as fall-out and the method wipping-off with cotton swab were used for isolation of micromycetes. The quality of species, their number, number of colonies as well as the most frequent species were analysed. Following media were used for isolation: soil agar with Bengal red and streptomycin, Sabouraud agar and malt-extract agar normal and with 40% saccharose (for osmophilic species). Special diagnostic media for identification were used.

Tab. 1 – Survey of the observed archives and book depositories

Abbreviation	Name	Locality	Time of construction	Purpose of building	Climatisation	Maintenance
SAP	State central archive	Prague 6	1930	archive	yes	very good
SAL	State central archive "Loreta"	Prague 1	16th-17th century	municipal administrative building, later prison, then college, since 1945 archive	none	bad
SAM	State central archive Mníšek	Mníšek near Prague	17th-18th century	castle, since 1943 archive	none	bad
AP	Archive of the city of Prague	Prague 1	18th century	palace, since 1945 archive	none	bad
DK	State library depository	Postoloprty (west Bohemia)	19th century	castle, since 1988 depository	partial	partial

CONCLUSIONS

Isolated species in different archive buildings with total numbers and the numbers obtained with both isolation methods are given in Tab. 2.– 8.

Following facts are important for evaluation of internal conditions of archives and book depositories from point of view of micromycetes occurence: 1. Total number of found species, 2. Average number of colonies obtained from both isolation methods, 3. Number of the most frequent species. The difference in number of species obtained by individual isolation method was not significant in individual localities, also the quality of species differed only slightly. The higher number of the most frequent species was always found in older and for the archive purposes less convenient buildings, while inside the new building or in the building with the equipment for stable R.H., the number of the most frequent species was very low (Tab. 7., 8.). It was found out, that in suitable conditions only a limited number of present species of micromycetes act as destructive. In the old and very badly maintened building: archive of the city of Prague, moulds were growing on the backs of the archive materials following the orientation of air draughts from window or humidity source towards the door. They are mostly penicillia which form coherent growing covers on the back of the books and cartons. The most frequent species of all archive buildings studied were *Penicillium aurantiogriseum, Alternaria alternata, Cladosporium herbarum, Cl. cladosporioides, Aspergillus versicolor* and *Aspergillus*

of the group *glaucus*. The predominance of the genera *Penicillium* and *Aspergillus* corresponds also with the results of other authors. Spores of these fungi present in a large extent in the atmosphere of that rooms may cause allergies of the archive staff and the visitors. After two hours of presence in the depository of one library, I was affected, as well as my colleague, by bronchitis lasting several days. Relative humidity of 68% does not prevent in these spaces growing of osmophilic species of *Aspergillus glaucus* group on the archive surfaces. For that reason, it is necessary within the regular maintenance of the archive regime, to apply a desinfectory means to book backs. A good effect in that sense was found when using Lastanox Q in slight alcohol solution (produced by Lachema in Czech Republic).

Tab. 2 – State central archive (SAP) in Prague 6

Building constructed for archive purposes, very well maintained archive spaces, regulary cleaning up, equipment for maintaining stable R.H. below 65 %, archive materials mostly on metal shelves.

Isolated species		Alternaria alternata Aspergillus ustus Cladosporium cladosporioides C. herbarum C. sphaerospermum Mucor plumbeus M. racemosus Penicillium aurantiogriseum P. brevicompactum P. chrysogenum P. citrinum P. corylophilum P. glabrum P. griseofulvum P. roquefortii
Total no. of species		15
No. of species	– fall-out	9
	– wipping out	10
Average no. of colonies		5

Tab. 3 – State central archive "Loreta" (SAL) in Prague 1

Old building unsuitable for archive, moist walls in the ground-floor, without equipment for maintaining reduced humidity, R.H. 70 – 75%. Archive materials on wooden shelves.

Isolated	Acremonium butyri	Paecilomyces sp.
species	Acremonium sp.	Penicillium aurantiogriseum
	Alternaria alternata	P. brevicompactum
	Alternaria sp.	P. chrysogenum
	Arthrinium phaeospermum	P. commune
	Aspergillus fumigatus	P. expansum
	Aspergillus gr. glaucus	P. glabrum
	A. niger	P. griseofulvum
	A. versicolor	P. luteum
	Aureobasidium pullulans	P. roquefortii
	Botrytis cinerea	P. variabile
	Cladosporium cladosporioides	P. viridicatum
	C. herbarum	Phoma sp.
	C. sphaerospermum	Rhizopus arrhizus
	Epicoccum purpurascens	R. stolonifer
	Fusarium sp.	Scytalidium sp.
	Chaetomium globosum	Trichoderma viride
Total no. of species		34
No. of species	– fall-out	30
	– wipping off	32
Average no. of colonies		35

Tab. 4 – State central archive in Mníšek near Prague (SAM)

Old building unsuitable for archive purposes, ground floor with moist walls, space insufficiently maintained. Wooden shelves. R.H. 70-75%.

Isolated	Acremonium strictum	Drechslera sativa
species	Alternaria alternata	Epicoccum purpurascens
	A. tenuissima	Humicola fuscoatra
	Arthrinium phaeospermum	Mucor plumbeus
	Aspergillus flavus	Oidiodendron sp.
	A. fumigatus	Penicillium aurantiogriseum
	Aspergillus gr. glaucus	P. brevicompactum
	A. niger	P. chermesinum
	A. ochraceus	P. citrinum
	A. ustus	P. corylophilum
	A. versicolor	P. glabrum
	Aureobasidium pullulans	P. griseofulvum
	Chaetomium globosum	P. janthinellum
	Chrysosporium pannorum	P. lanosum
	Cladosporium cladosporioides	P. variabile
	C. herbarum	Phoma sp.
	C. macrocarpum	Rhizopus arrhizus
	C. sphaerospermum	Ulocladium botrytis
	Cunninghamella elegans	
Total no. of species		37
No. of species	– fall-out	24
	– wipping off	24
Average no. of colonies		36

Tab. 5 – Archive of the city of Prague (AP), Prague 1

Old building unsuitable for archive, in ground-floor moist walls, in some places water is leaking into the rooms when raining, insufficient ventilation, in ground-floor archive materials partly stocked on piles, in the first floor partly tidied, archive materials mostly on wooden shelves. R.H. 72-86%.

Isolated species	Acremonium sp.	Mucor circinelloides
	Alternaria alternata	M. plumbeus
	Alternaria sp.	M. racemosus
	Aspergillus candidus	Paecilomyces fumosoroseus
	A. clavatus	Penicillium aurantiogriseum
	A. flavus	P. brevicompactum
	Aspergillus gr. glaucus	P. caseicolum
	A. fumigatus	P. chermesinum
	A. nidulans	P. chrysogenum
	A. versicolor	P. commune
	Aureobasidium sp.	P. corylophilum
	Beauveria bassiana	P. expansum
	Botryotrichum piluliferum	P. glabrum
	Botrytis cinerea	P. griseofulvum
	Chrysosporium pannorum	P. lanosum
	Ch. pruinosum	P. roquefortii
	Cladosporium cladosporioides	P. roseopurpureum
	C. herbarum	P. variabile
	Chaetomium globosum	Phoma sp.
	Epicoccum purpurascens	Rhizopus arrhizus
	Geotrichum candidum	Scopulariopsis brevicaulis
	Monodictys sp.	Trichoderma viride
	Mortierella sp.	Ulocladium botrytis
Total no. of species		46
No. of species	– fall-out	32
	– wipping off	37
Average no. of colonies		40

Tab. 6 – State library depository (DK), Postoloprty (West Bohemia)

Old building inconvenient for book deposits, rooms tidied up, equipment for humidity control, books on wooden shelves. R.H. 65 – 68%.

Isolated species		Alternaria alternata A. tenuissima Alternaria sp. A. corymbifera Aspergillus flavus Aspergillus gr. glaucus A. niger A. versicolor Aureobasidium pullulans Botrytis cinerea Chaetomium globosum Cladosporium cladosporioides C. herbarum C. sphaerospermum Penicillium aurantiogriseum P. chrysogenum
Total no. of species		16
No. of species	– fall-out	15
	– wipping off	11
Average no. of colonies		4

Tab. 7 – The most frequent species in individual archives and book depositories

SAP	Cladosporium cladosporioides
	C. herbarum
	Penicillium aurantiogriseum
SAL	Alternaria alternata
	Aspergillus niger
	A.versicolor
	Botrytis cinerea
	Cladosporium cladosporioides
	C. herbarum
	Penicillium aurantiogriseum
SAM	Alternaria alternata
	Aspergillus versicolor
	Chrysosporium pannorum
	Cladosporium cladosporioides
	C. herbarum
	Mucor plumbeus
	Penicillium aurantiogriseum
AP	Aspergillus gr. glaucus
	A. versicolor
	Cladosporium cladosporioides
	Mucor plumbeus
	Penicillium aurantiogriseum
	P. chrysogenum
	P. griseofulvum
	Rhizopus arrhizus
	Trichoderma viride
DK	Aspergillus gr. glaucus
	Chaetomium globosum
	Cladosporium cladosporioides
	Penicillium aurantiogriseum

Tab. 8 – Comparison of internal technical equipment of individual archives with total number of isolated species and number of the most frequent species

Archive	Year of study	No. of observed rooms	Maintenance	Climatisation	R.H.	No. of isolated species	No. of the most frequent species
SAP	1985	7	very good	yes	under 64%	15	3
SAL	1985	6	bad	no	72-78%	34	7
SAM	1985	7	bad	no	70-75%	36	7
AP	1981	8	bad	no	72-86%	46	9
DK	1988	7	partial	yes	60-67%	16	4

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Book review

KÁLMÁN G. VÁNKY: EUROPEAN SMUT FUNGI.

– 570 p., 1003 figs., Gustav Fischer Verlag, Stuttgart, 1994. – 398 DM (the book is in the library of the Society).

Smut fungi (Ustilaginales) are important plant parasites. This book comprises, for the first time, the knowledge about 500 known smut fungi from Europe. For these species and for further 70, detailed descriptions are given. 82 doubtful or excluded taxa are also presented. The descriptions are completed by 220 drawings of diseased plants and by more than 770 microphotographs of the spores seen in LM, SEM or TEM. Keys to genera and species, a host plant / smut fungus index, make an easy identification of these plant pathogens possible.

In 1985 has been published by K. Vánky a valuable monograph of smut fungi of the Carpathian basin: Carpathian Ustilaginales (see review by Urban in Čes. Mykol. 40: 59-60, 1986). Vánky's interest in smut fungi was aroused beginning the fifties on phytopathological lectures given by academician Tr. Săvulescu in Bucharest (Romania). since the time Vánky is engaged in collection and study of smuts and in edition and distribution of smut fungus exsiccata which has now reached 950 numbers. In the fifties he worked in the present day Institutul de Biologie "Tr. Săvulescu" in Bucharest. In 1970 he left for Sweden where he worked first as a physician in a small town Gagnef. Later on, supported by scholarships of some Swedish institutions, he ended the above mentioned doctoral dissertation and since 1986 he is working at the Institute of Biology I, University of Tübingen.

A short preface is written by Professor H. Scholtz, Botanical Museum Berlin-Dahlem, the co-author of a voluminous work on German smuts (1988). In the preface is stressed that fungal taxonomy and especially of smut fungi in Europe has not yet come to its end. In the introduction Vánky writes: ... "there are numerous undescribed species and unsolved problems regarding smut fungus classification, ... biology, ... host-parasite relationship, phylogeny. ... At the same time, increasing population and expansion of cities, industries, traffic ways and cultivated areas are rapidly destroying the natural floras all over the world. Smut fungi as yet undescribed are destroyed with their hosts. Heavy application of agricultural fungicides, combined with pollution from cities and industry, are also reducing or eradicating populations of the smuts (and other groups of fungi) and, because they are part of the unique ecosystem of the world, it is high time to take measure to preserve them for the future (Vánky et Harada 1989/1990: 445)."

Five pages of thickly printed literature quotations embrace as sources for inventary of Europeanm smuts as well as extra-European smut floras and papers dealing with general or special problems. All quotation are to be found in the chapter "Literature cited" which presents ca 60 pages printed in two columns.

The chapter "Materials and methods" begin with a short note concerning the applied species concept to the smut fungi. It is a fairly broad morphological concept defining species as groups of organisms recognizable by consistently distinct morphology. In many cases host specifity must be considered in definitions, due to the simple morphology of the parasite. More details elucidating the author's species concept to the smut fungi are dealt on p. 17 and 153. Vánky writes: "I am conservative regarding modifications based on (often one-sided) knowledge of ultrastructure (septal pore, haustoria, spindle pole bodies), biochemistry (enzymes, sugar pattern of the cell walls, extracellular amyloid components), ribosomal ribonucleic acid sequence (5 S r RNA) data and others known for relatively few taxa. E.g. under *Microbotryum* I am treating anther smuts of Caryophyllaceae only (see also comments in the introductory part of the chapter *Microbotryum*)." On p. 153, after repeating the new characterization of the genus *Microbotryum* proposed by Deml et Prillinger (in Prillinger et al. 1991: 9), Vánky writes:" This is a completely new interpretation and characterization of this genus, a characterization which certainly will lead to the recombinations of perhaps one hundred or more Ustilago species into Microbotryum. ... In my opinion it is too early to change the systematic of the Ustilaginales radically on the basis of the knowledge of some biochemical parameters analyzed for only relatively few species, even if we know that other characters (e.g. ultrastructure of the septal pore; partly unpublished) are supporting such tendences. One of the weaknesses of this kind genus-delimitation is the fact that too many characters are used. The absence of one or several of these characters will, on the other hand, automatically exclude many, closely related species, ... on the other hand,will bring together

a heterogenous assamblage of species regarding other characters. Application of biochemical methods used by yeasts specialists in delimitation of smut genera is another inconvenience. These methods ... are difficult to repeat for checking previously published data." According to me, Vánky is right in evaluating with care all biochemical, molecular and ultrastructural characters by delemitation not only of genera but also smut species.

The genera are arranged alphabetically and the species appear alphabetically under each genus. The species (and genera) are numbered. For species (and genera) that would be expected to occur in Europe, but have not yet been found there, the numbers are given in parentheses. The names of species and genera for which no description is given in this book, appear in square brackets in the keys.

Following are noted abbreviations of the generic names of Ustilaginales, countries and territories, the collections of exsiccata and for consulted herbaria. Separated are listed consulted private herbaria and herbaria not mentioned in the Index herbariorum.

A short chapter deals partly on nomenclature which has been followed according to the latest issue of the International Code of Botanical Nomenclature (Berlin 1987), partly on species concept in smut fungi, delimitation of species and classification of the Ustilaginales.

The key of the European smut genera is constructed first of all according to morphologic characters but indices on host families are also exploited. All other specific keys are based on host taxonomy.

The valid name of every smut species is followed by synonymy and quoting the type specimen. In many cases lectotypes or neotypes had to be selected.

Some genera as e.g. *Nannfeldtiomycetes, Neovossia, Orphanomyces* etc. are very poor what about species or it is noted that the species are known from sporadic findings only. In such cases it would be useful to bring the quotations in full.

The genus Ustilago contains most species (over 400) and Vánky anticipates that it will be split into several smaller, more uniform (but it is not sure that simultaneously more natural) genera when sufficient additional characters are examined (e.g. ontogenical, ultrastructural etc.).

The economically relevant species are named by correct scientific names what is important for plant pathologists. It is remarkable that the name *Ustilago hordei* (Pers.) Lagerh. is uncorrect and should be by replaced by the valid name *U. jensenii* Rostr. Vánky prefers to retain the well known binomial *U. hordei* which he proposed for conservation. Very useful is the review of the differences between *U. tritici* and *U. nuda* (after J. Nielsen in litt.). For economically relevant smut diseases short control measures are also given.

In the chapter dealing with doubtful and excluded taxa it is interesting that Nagler et al. (1989), based on germination and ultrastructure studies, showed that the genus *Schroeteria* (six species on *Veronica* sp. div.) is an ascomycete.

Vánky's excellent book will prove an invaluable reference source and standard work for all mycologists of various aspects of study but also for plant pathologists and botanists who are engaged in problems of the preservation of our environment and organismal biodiversity as vascular plants as smut fungi.

Zdeněk Urban

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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. – Oslo, 507 pp. (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, pp. 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).

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