THE GENUS PLEUROTUS AS AN AID FOR UNDERSTANDING THE CONCEPT OF SPECIES IN BASIDIOMYCETES

A. Bresinsky, O. Hilber*) and
H.P. Molitoris**)

1. Introduction

The species as basic unit of systematic diversity has been defined for the higher fungi by generations of mycologists on the basis of observations upon material collected in the field. In many cases, however, cultural experiments have been omitted, due to difficulties in obtaining fruit bodies. Careful observations and descriptions are important elements to recognize the evolutionary patterns, they are, however, not sufficient per se to understand the biology of species. Observations and descriptions, therefore, should be supplemented by experiments for delimitation and definability of species. In addition, questions about the evolutionary process of speciation must be solved experimentally.

Before dealing with the special suitability of the genus Pleurotus for the understanding of the species concept in higher fungi, we first would like to discuss some relevant questions and exemplary investigations.

Concerning constancy and stability of the observed taxonomic features against environmental conditions, Gäumann (1923) has made important contributions by a number of classic experiments. They are based on the consideration that phenetic differences may be modified by superposition of environmental influences which makes it difficult to recognize the differences due to genetic factors. In fact, Gäumann's experiments on Peronospora have shown that extreme fluctuations of temperature and humidity in connection with great differences in age result in deviations of maximal 11 microns in the diameter of the conidia. This equals after all half the diameter of a normal conidium

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*) Cultivation of carpophores, crossings and their evaluation by O. Hilber with technical assistance of Mrs. R. Maier.

**) Growth experiments and enzyme spectra by H.P. Molitoris with cooperation of A. Schärtl.

of Peronospora. In the field, however, character-shifting environmental conditions will hardly play together in that way that extreme deviations result from these.

It is an other question to what extent different substrates are to be made responsible for differences in the features of two compared fungi. The role of substrate-induced modifications and the extent of difficulties which arise by them for the delimitation of species is controversial. Concerning the powdery mildews (Erysiphales) and downy mildews (Peronospora) it was possible by crossinoculation and by observation on mutual hosts to exclude a hypothetically possible influence by different hosts (Blumer 1967, Gustavsson 1959). Contrary to that, other authors (Schweizer, 1919) observed substrate induced modifications of characters used as taxonomic criteria in other parasitic fungi (e.g. Bremia).

A profound understanding of the species as taxonomic unit is impossible without information about the evolutionary processes which result in speciation. Variation and selection are nowadays commonly accepted factors in evolution. Furthermore, for the taxonomist whose job is to look for discontinuities the i s o l a t i o n of species is another very important, almost predominant complex of factors. This, because due to the different isolating mechanisms developing divergencies are stabilized and therefore the distinction of species becomes possible.

In the fungi, inhibition or prevention of free mating by genetic isolation is the main isolating factor in the diversification of species. Ecological isolation, e.g. by specialization for a certain substrate or host, is in this context merely an additional factor. Only seldom, like in the case of yeasts, it seems to be an important one. Factors preventing mating become manifest in fungi partially already before a morphological or ecological differentiation may be observed (e.g. between strains of Podospora; heterogenic incompatibility, Esser, 1967). In this context the fungi differ from other organisms. Kemp (1975) even considers genetic isolation to be the first step in the evolution of new species of fungi and proposes the following sequence for the single steps of this process: Mutation of the genome - cytoplasmatic incompatibility - prevention of mating - morphological and ecological differentiation. It agrees well with the importance of

genetic isolation in fungi that interspecies hybrids - beside a few exceptions (Blastocladiales: Allomyces; Saccharomycetales; Sphaeriales: Neurospora; Ustilaginales) - have been never obtained. This fact is not only in favour of the efficiency of genetic isolation, but also supports the species concept of the taxonomist not being too narrow. How well the minutious and for the extern observer often suspicious way of the taxonomic work corresponds with the phenomenon of genetic isolation shall be shown by the studies of Lamour (1965 and 1972) on alpine species of Clitocybe (Fig.1).

V a r i a t i o n includes process and result of any change within the genome and leads to differences within a series or a population. Variation includes also the pattern of character distribution among the individuals of a population or a species. Changes may be caused by gene mutations. Within the context of speciation, however, the frequency of mutations during experimental work with fungi is often overestimated, since the process of natural selection in its eliminating and therefore stabilizing function tends to be underestimated. However it may be possible that changes by gene mutation (spontaneous rate of mutation in fungi 1×10^{-7} to 1×10^{-5} according to Esser and Kuenen, 1965) become decisive for evolution in the long run, since fungi show such a high number of nuclei and nucleic divisions before undergoing caryogamy. Unfortunately, changes in chromosome number, e.g. by polyploidization, in fungi can hardly be observed or indirectly via differences in nucleus volume. A change in the genome may also be accomplished by recombination during the sexual cycle according to the possibilities given by the respective mating system. (Esser, 1974). Finally, one has to keep in mind the changes by mitotic recombination following parasexual processes, especially since such processes have been shown to exist also in basidiomycetes (Coprinus: Prud'Homme, 1963; Swiezynski, 1963).

S e l e c t i o n may have as well a stabilizing (under fairly constant environmental conditions) as also a promoting function in the evolution of new species. Promoting factor for evolution means in this context that differences which occur in populations are enhanced by selection under variable and changing environmental conditions. The extended dicaryophase of higher basidiomycetes results in a specific mechanism of selection. According to our present knowledge in a dicaryon, not only the two nuclei within one dicaryotic hyphal com-

partment but also the pairs of nuclei in different hyphal compartments may differ genetically. A dicaryon therefore may represent a genotypic mosaic (Burnett and Partington, 1957). As indicated above, different parts of a mycelium may be genetically different and if reaching different substrates they are exposed to different environmental conditions. Forces of selection acting upon one single mycelium therefore may be heterogeneous and may lead to different results by virtue of the above mentioned mosaic structure of the mycelium. The diploid nuclei which are formed in the hymenium are already optimized by the selective processes before fusion of the nuclei takes place. Depending on different conditions of selection, genetically different fruit bodies may arise from one mycelium. The extension of the dicaryotic phase in the life cycle of basidiomycetes enhances the efficiency of selection and reduces the loss of individual mycelia.

In the formation, limitation and stabilization of species all three factors, variation, selection and isolation participate, often even in antagonistic interaction. Knowledge of the interaction of the various evolutionary factors should form the necessary guideline for the taxonomists work which often stresses more practical aspects. Gäumann (1923) during his work on the downy mildews has recognized the importance of experimental work for the solution of taxonomic problems. The results thus obtained for one taxonomic group, however, do not necessarily apply to others. It is therefore necessary to look for each group of fungi for the suitable representatives to work with. It will be shown here to what extent the genus Pleurotus is specially suited to reach an understanding of the species within the higher basidiomycetes.

The genus Pleurotus comprises mostly wood-decaying species, more seldom saprophytes on other plant material. Pleurotus species may be cultivated in the laboratory and fruit bodies may be obtained under certain conditions within 4 (3-6) weeks. Monocaryotic mycelia for mating experiments may be produced from spores. The genus Pleurotus includes several complex groups whose members occur in Europe under very different ecological conditions from Lapland in the north to Sicily in the south and which also show extensive variation of characteristics. Since fruit bodies may be obtained in the laboratory within a relatively short time one is able to study the influence of environmental conditions upon the expression of taxonomically rele-

vant characters. The prerequisites are given to study the presence or even the development of mating barriers in comparison to the various degrees of morphological and ecological divergency and to obtain insight into the process leading to speciation.

2. Survey of the Pleurotus - ostreatus - complex in Europe

A proper taxonomic treatment of the complex of P. ostreatus is not yet reached. On the one side attempts to divide the complex and to evaluate the rank of the units are still controversial, on the other side the total diversity has not yet been recorded completely. A great step forward in the taxonomy of this group was done by Romagnesi (1969) through consideration of a number of microscopical characters which had been neglected hitherto. The provisional key given at the end of this paper presents a survey of the species which can be distinguished at the moment.

Unlike the group of P. eryngii, the species complex around P. ostreatus does not show a narrow specificty for different substrates.

3. Realization and modification of taxonomic criteria under laboratory conditions

Our own investigations about the realization of characteristics under various environmental factors are only at an initial stage. Utmost care must be applied for interpretation. The preliminary results, reported here, show merely the tendency of further studies. Experiments and observations are discussed separately for some features which are used in the key for the distinction of species.

Color of cap: In our experiments different substrates did not effect the color of the cap. We used strain 2y, originating from fruit bodies grown an Salix. The strain was identified as P. ostreatus and produced in culture also fruit bodies on stumps of Picea and Betula. Under any circumstances the color of the cap was grey brown and was rated as 5F3 according to Methuen *). This color was

*) Kornerup and Wanscher 1967.

identical with that of the fruit bodies on Salix at the original site. Since this fungus, originally fruiting on Salix, produced fruit bodies also on Picea and Betula it becomes evident that it is doubtful to base P. salignus and P. ostreatus in the key only on different substrates.

If fruit bodies were produced in the laboratory under artificial light of relatively low intensity or on wheat straw or wheat grains, they showed paler colors. When fruit bodies were grown in open petridishes with wheat straw in a humid chamber, they had a yellowish grey color (Methuen 4B2), whereas the fruit bodies extending from the rim of Erlenmeyer-flasks filled with wheat grains were brownish grey (Methuen 5D4). This agrees with observations of Eger, Eden and Wissig (1976), who in addition determined the influence of temperature. They noted a darker color of the fruit bodies with lower temperatures and with higher light intensities.

The colors used in the key for distinction of species remain also under laboratory conditions fairly constant if fruiting occurs under constant and sufficient light (minimum 500 Lux). Strains of P. pulmonarius retain then their light, often almost whitish color and P. columbinus will always be distinguished from P. ostreatus by the color of its cap. If, however, fruit bodies of the different species were produced under different conditions the color may become similar. Changing environmental conditions therefore modify the color differences between the species. As long as the natural condition of fruiting is fairly well in concordance with the natural situation, there is only a shift, not a loss of the specific differences in color.

T h e s h a p e o f c a p may be different according to the culture method. By higher humidity, as is given within an Erlenmeyer-flask with substrate, fruit bodies are produced which are funnel-shaped and whose caps are more lobated, the margin being transparently striate and turned upward. The caps under these conditions are not seldom hygrophanous. Experiments where the fruit bodies are produced laterally on Erlenmeyer-flasks simulate most closely natural conditions. Here the shape of the fruit body, the central depression of the cap, the involution of the margin and other taxonomic criteria are not changed (e.g. shape of cap of P. cornucopiae).

L e n g t h o f s t i p e : Light intensity proved to be important for the length of stipe. Zadrazil and Schneidereit (1972) showed that fruit bodies grown under low light intensities produce long stipes and the fruit bodies finally assume the habitus of P. cornucopiae. Jablonsky (1975) could correlate length of stipe with light intensities. The fruit bodies show under defined light intensities a characteristic length of stipe for each strain, however, if this property is compared under variable light intensities, the overlapping of the length of stipes is rather large. From these and our own observations follows, that length of stipes can be used as taxonomic criterion only under defined and limited conditions. A genotypic differentiation is here - as it is often the case - obscured by modifications.

S c l e r i f i e d h y p h a e : Romagnesi (1969) paid special attention also to the presence of sclerified hyphae in characterizing P. pulmonarius and partially also P. cornucopiae. According to our own observations, however, this criterion is only of limited value, since it is rather easily modified. Depending on culture conditions sclerified hyphae in fruit bodies may be present or may be totally absent. This phenomenon was already demonstrated by investigation of one single strain of P. ostreatus (2 y), whose fruit bodies if grown on sawdust of Fagus produced many sclerified hyphae, whereas fruit bodies from wheat straw, stumps of Betula and Picea as well as the original collection from Salix did not contain thickwalled hyphae in the context.

In strain 1 w of P. ostreatus we found sclerified hyphae in fruit bodies from wheat straw, but not in those grown on wheat grains (Fig. 2). Possibly also other factors than substrate may be important for the occurrence of sclerified hyphae. Stankowicova (1973) e.g. states that especially older fruit bodies of P. ostreatus contain thickwalled hyphae (Fig. 2).

D i m e n s i o n o f s p o r e s : Until now dimension of spores has not been considered for distinguishing species within the Pleurotus-ostreatus-complex, although different maxima of spore length indicate that statistical treatment of spore measurements could result in relevant differences of spore size. To see whether spore size could be used for taxonomic purposes in Pleurotus, we also

looked for direct or indirect correlations between substrate and spore dimension, a question of general importance. Although at the moment only of preliminary nature, our data, obtained from a well suited organism, allow us to take up again the discussion of substrate modification of spores, a problem which is important especially in lower parasitic fungi.

Fruit bodies of one single strain of P. ostreatus (strain 2y) were grown on different substrates and the distribution of spore dimensions was determined. The length of spores showed greater variation than the diameter of spores and was therefore used for presentation of results (Fig. 3). 100 spores each for every experiment were measured. Greatest variation in spore length was found in the fruit bodies from P. ostreatus on Salix, the maximum of frequency being around 9,5 microns; the shoulder around 11 microns could represent a further, hidden maximum. With exception of the fruit bodies on stumps of Picea, in all other experiments variation of spore diameters was narrower. The average length of spores from wheat straw was smaller, the peak of frequency beeing around 8,5 microns. Clearly higher were the values for spores from fruit bodies on stumps of Picea where the maximum frequency was at 11,5 microns. Different substrates therefore have a strong influence on the average dimension of spores (8.5 - 10.95 microns) and the maxima of frequency may be in extreme cases as far apart as 3 microns.

A final interpretation of the results cannot be given at the moment. The different substrates either could modify directly or act indirectly on the dicaryons through selection, resulting in the observed picture.

From all this follows that taxonomic characters may exhibit considerable fluctation due to different environmental conditions. However, the average environmental situation which induces fruiting in the field limits the range of phenetic plasticity. Thus overlapping of features due to unequal shift of environmental conditions as possible in the laboratory should not be overemphasized in regard to the problem of finding proper phenetic differences for the delimitation of species.

4. Intersterility barriers between species of *Pleurotus*

In the following two chapters inability of gene exchange between different species is contrasted with internal fertility within species. Lack of external interbreeding combined with the presence of morphological differences between the units supports the validity of an adopted species concept. Regarding the "bona fide" - species of *Pleurotus* treated in the key this means necessity of validation by looking for the established mating barriers. This procedure was followed in some of the possible combinations.

a) *P. pulmonarius* x *P. columbinus*

For both species (*P. columbinus*, strain ln; *P.pulmonarius*, strain lr) the mating types were determined and the expected scheme of tetrapolar incompatibility has been confirmed. The monocaryotic mycelia were obtained from a single sporocarp of each of both species. For each species matings of the monocaryotic mycelia resulted in 25% formation of clamp connections (table la and lb). However, in no case clamp connections occurred in confrontations between monocaryotic mycelia of *P. pulmonarius* and *P. columbinus* (table lc). Using different strains of both species the crosses were repeated with the same result: in no case formation of clamp connections could be observed.

b) *P. pulmonarius* x *P. ostreatus*

Not any combination of monocaryons of these two species led to clamp forming mycelia (ld x lu and lr x ls).

c) *P. pulmonarius* x *P. cornucopiae*

Crosses of monocaryons of *P. pulmonarius* (strain lr) with monocaryons of *P. cornucopiae* (strain 4r) did not yield any clamp connections. From these observations follows that *P. pulmonarius* represents an independent species, which is genetically isolated from *P. columbinus* and *P. ostreatus* in spite of only minute differences in macroscopical and in microscopical features.

Further interspecies crosses are listed in table 2.

5. Internal fertility within the species of Pleurotus

According to multiple allelism of incompatibility genes the percentage of dicaryotization between strains of one species increases at a considerable rate if these strains are of different origin. This phenomenon is demonstrated with different strains of P. pulmonarius and P. ostreatus (table 3a-3d). Dicaryotization occurred between strains which showed clear deviation of several properties. Since recombination of varying characters is favored by multiple allelism of incompatibility genes this means increase of infraspecific variation. New species may predominantly originate from such units with a high degree of internal variation.

Crosses between monocaryons with throughout different A- and B-factors possess according to Eugenio and Anderson (1968) an increased chance to succeed, since the total weight of fruit bodies increases and by that the number of spores is also higher.

Some remarks on the results presented in table 3 are necessary in order to demonstrate exchange of differing features in intraspecies crosses. The fruit bodies of P. pulmonarius 4h are lighter (nearly white) than those of P. pulmonarius 1r (table 3b: 1r x 4h).

The so-called strain "Florida", originally obtained by Eger from Florida, became important for commercial cultivation, because it does not need a cold-phase for induction of fruiting. The fact that this strain "Florida" interbreeds with P. pulmonarius 1r from Austria shows that the former cannot been taken as an independant species (table 3c: 1r x 4b). Eger, Eden and Wissig (1976) describe the mating of a strain from Florida with some strains from America and Germany. The monocaryotic mycelia of these strains were in the crossings 100% compatible and largely fertile (regarding production of hybrid sporocarps and spores). Unfortunately it is impossible to identify these strains from Germany used by Eger et al. by the given general remarks about color changes due to different light intensities. The assumption of these authors, having crossed Pleurotus "strain Florida" with P. ostreatus, therefore cannot be verified. For our problem, however, it is important that Eger et al. were able to prove that interbreeding was linked with combination of different

properties, e.g. temperature optima of fruiting.

Monocaryotic isolates of P. ostreatus from Germany, Westfalen, are 100% compatible with two different strains of P. ostreatus from Japan (table 3d: 1s x 1w; 1s x 1u). Recombination takes place between strains which differ in the color of the cap. The colors of the fungi from Japan are lighter, especially in older stages, than those of the strain obtained from Westfalen. The F1-offspring of hybrid fruit bodies, which were fertile in regard to spore production, was split into darker and lighter colored individuals.

As a result of these observations we may state, that differences in features between two compared units are not per se reliable criteria to establish a species concept. In every case it is necessary to be sure that the differences are maintained by proper isolation mechanisms.

6. Enzyme spectra for characterization of species and strains of Pleurotus

Chemical analysis as tool of "biochemical systematics" (Heywood, 1973a) has been used successfully and increasingly within the last years in systematics and taxonomy of higher plants and fungi (Davis and Heywood, 1963; Hall, 1973; Vaughan, 1975; but see Heywood, 1973b for critical comments).

Proteins are - according to the one-gene-one-enzyme-hypotheses (Beadle and Tatum, 1941) - direct products of genes and therefore also indicative for the genetic material. For that reason proteins, together with pigments, have been analyzed for presence, quality and distribution by electrophoretical and in the case of proteins also serological methods. A number of good correlations with morphological and other data has been found (see Hall, 1969; Tyrell, 1969; Hall, 1973; Bucher, 1974).

General protein profiles are often obscured and unspecific by too many proteinbands. It therefore proved advantageous either only to investigate certain fractions of total protein or - even better - only specific enzymes, characteristic for a given organism. With due care

of interpretation such isoenzyme spectra appear to be useful for taxonomic and systematic purposes also for fungi (Nealson and Garber, 1967; Wang and Raper, 1969; Reddy and Threlkeld, 1971; Snyder and Kramer, 1974).

For these reasons it seemed to be worthwhile within the scope of our problem to investigate not only the morphologic criteria of the wood-rotting genus Pleurotus (white rot) but also the enzymes believed to be responsible for the decay of wood. We tested therefore the phenoloxidases tyrosinase and laccase and in addition peroxidase. It was hoped to find out, whether enzyme profiles in addition to the established morphological criteria could provide useful information for the delimitation of species and characterization of strains in fungi.

For this purpose several strains of Pleurotus (P. columbinus, P. ostreatus, P. pulmonarius) were tested. In order to see the effect of the nuclear status on growth and enzyme spectra, we furthermore analyzed of one species, P. ostreatus, monocaryotic strains (lw8, ls4, dicaryotic strains (lw, ls) and the dicaryotic hybrid of the cross lw x ls.

At first g r o w t h r a t e and y i e l d o f m y c e l i u m on different solid and liquid media at different temperatures were determined in order to characterize the strains and to obtain the optimal time of harvest for the enzyme determinations. Table 4 presents the results for growth on malt-agar and malt-broth at 27°C. The Pleurotus species and strains showed characteristic and constant growth rates on malt-agar, the growth rates being in the range of values given by Nobles (1965), Macaya - Lizano (1974-1975) and Blaich (1972). Only small differences in growth rate existed between the strains on solid media, whereas in liquid medium mycelial yield showed wider variation. It is striking that on solid medium the growth rate of the hybrid lw x ls is about twice as high as that of the monocaryotic crossing partners lw8 and ls4, whereas in liquid medium mycelial yield of the hybrid is only about one quarter of that of the monocaryotic strains.

Looking at the e n z y m e a c t i v i t i e s in solid and liquid medium, peroxidase could not be found in any strain with the methods used, neither on solid medium nor colorimetrically in culture

filtrates and mycelial extracts nor by staining of gels after electrophoresis. This confirms the results of Lyr (1958) for P. ostreatus.

After 2 weeks of incubation tyrosinase also was absent in malt-agar, in culture filtrates and in mycelial extracts of liquid culture. This enzyme appeared, however, in small amounts after 3 weeks on solid medium in cultures of P. columbinus and P. ostreatus. Only the monocaryotic strains and the dicaryotic hybrid of P. ostreatus did not show tyrosinase activity at this time. Presence of tyrosinase in P. columbinus and P. ostreatus agrees with the data of Hackl (1975). Our results show further the dependency of tyrosinase production from cultural age.

Laccase was always found in every strain tested in solid media with the drop-test (extracellular enzyme), in the culture filtrates (extracellular enzyme) and in mycelial extracts (intracellular enzyme) from liquid culture. This corresponds well with the data of Lyr (1958), Harkin et al. (1974), Blaich and Esser (1975), Hackl (1975) and Leonowicz and Trojanowski (1975a, 1975b).

The discrepancy between the semiquantitative data of the drop-test and the quantitative colorimetric determinations of laccase activity suggests that the "Bavendamm-test" (Lyr, 1958) should be used only for qualitative or preliminary determinations and shows that this test indicates only presence of extracellular enzymes.

In order to check also the presence of intracellular enzymes mycelial extracts in addition to the culture filtrates were investigated. From table 4 appears that laccase is here predominantly an extracellular enzyme. The monocaryons of P. ostreatus synthesize only about 1/3 of the laccase produced by the dicaryons and the hybrid. The inverse relationship between phenoloxidase- and mycelium production (see above) has been observed repeatedly (Bergmann, Molitoris, unpublished results).

As could be shown, a more specific and better reproducible picture of the enzymatic differences between the strains was obtained from the isoenzyme spectra of disc-electrophoresis and isoelectric focusing.

As figure 5 shows, in the culture filtrates generally fewer laccase bands were found, an indication for prevention or retardation of laccase secretion through the cell membrane into the medium (Molitoris and Esser, 1971).

In d i s c - e l e c t r o p h o r e s i s at pH 8.3 in all cases a laccase band remains in the spacer gel, indicating a laccase of high isoelectric point like the B-type laccases with an isoelectric point between pH 4 to 8 (Jonsson et al., 1968) or laccase II of Podospora anserina (Molitoris and Esser, 1971). In addition, all strains show a strong laccase band near the moving boundary, indicating a laccase of low molecular weight and/or low isoelectric point corresponding with the A-type laccases of Jonsson et al. (1968). Additional bands appeared in the mycelial extracts of P. columbinus and P. ostreatus and showed characteristic differences between the strains. In P. ostreatus such additional bands were found only in the mycelial extracts of the mono- and dicaryotic "s"-strains (ls4 and ls) but not in the mono- and dicaryotic "w"-strains (lw7 and lw) nor in the hybrid lw x ls. This hybrid strain therefore is not, as could be expected, intermediate in its laccase spectrum but is identical with one of the parental strains (lw8).

I s o e l e c t r i c f o c u s i n g gives considerably better separation than discelectrophoresis. It is therefore not surprising that this method produced more distinct laccase bands. The danger of artifacts, however, is in isoelectric focusing higher. Again, A- and B-type laccases are present and both are secreted into the culture medium. All species and strains of Pleurotus showed different laccase spectra. The similarity of laccase spectra in isoelectric focusing within a species was generally not higher than between species. An exception are the spectra of the culture filtrates of P. ostreatus. In the case of the spectra from isoelectric focusing interpretation of results should await further experimentation.

As a result of these preliminary investigations it can be stated already: The analyzed strains of Pleurotus all produce intra- and extracellular laccase and exhibit characteristic, reproducible isoenzyme spectra in electrophoresis. The spectra of the hybrid (lw x ls) of P. ostreatus show in disc-electrophoresis identity with one of the crossing partners (lw), in isoelectric focusing the spectra of the

culture filtrate are at least very similar to both monocaryotic crossing partners.

Before we finally can assess the suitability of these methods for characterization of strains and species of Pleurotus a number of additional enzymes should be tested as well as the influence on the enzyme spectra of factors like mycelial age, medium and environmental conditions like temperature and light.

7. Species concept

A species comprises all individuals with indentical or continously varying characters. Individuals belong to different species if the variation of characters shows discontinuities. Species are recognized and defined by such discontinuities (Davis and Heywood, 1963). A proper delimitation of species is not influenced by the question how many genes participate in an established discontinuity if stability of differences is warranted on one side, and continuity of variation is maintained within the defined species on the other side. Genetic isolation is the most important mechanism for establishing discontinuities in the variation of fungi.

In order to limitate taxonomic work with basidiomycetes on the species level the species concept should be based primarily on macro- and microscopic, macro- and microchemical and finally on ecological criteria. Physiological and biochemical characters would only be useful if the inbreeding units (i.e. the species) are characterized as a whole by such criteria. Otherwise these features would only serve to describe the internal variation and structure of species.

The species concept for the basidiomycetes seems to be comprehensible in an almost objective manner, although there are difficulties in application. In many groups genetic isolation cannot be assayed by mating experiments, and taxonomy of basidiomycetes is therefore based on a "bona-fide"-species concept. Environmental conditions may influence features to a high degree and this inhibits precise determination of specific differences.

Recognition of discontinuities and thereby of species is based on long experience, much patience and on the observation of often minute

differences. This demands aptitude for carrying out such observations, which is not given everyone. Finally, species are dynamic structures which can be altered by evolutionary processes. To recognize such changes is for the systematist - contrary to common view - not annoyance but object of his studies.

8. Material and methods

Isolates, pure cultures and monocaryotic mycelia were obtained and propagated using conventional methods.

Fruit bodies, very similar to those grown in nature, were produced by filling Erlenmeyer-flasks (300 ml, wide mouth) halfway with wheat grain. After addition of distilled water the grain swelled and filled the flasks up to the rim. The flasks then were inoculated, closed with cotton plugs, covered with aluminium foil and incubated at 23°C. After 14 days the flasks were transferred to 11°C and 500 - 600 lux constant light. The foil was removed on one side to allow lateral growth of the fungus, simulating natural conditions. Fruit bodies were produced after 4 (3 - 6) weeks, depending on strain and species. This method was superior to fruit body production inside flasks and petri-dishes.

Flasks: The flasks were filled with 30 g of substrate: a) straw of wheat; sawdust of b) Fagus, c) Picea, d) Abies, e) Betula. The substrate was covered by a thin film of the following composition: Inositol 50 mg; KH_2PO_4 0,5 g; $MgSO_4$ 0,5 g; $ZnSO_4$ 0,001g, $FeCl_3$ 0,01 g, $CaCl_2$ 0,055 g, $MnSO_4$ 0,005 g, maltose 20 g, glucose 10 g, agar-agar 20 g, asparagine 1.2 g, alanine 0,8 g, dest.water ad 1000 ml.

The flasks were inoculated and incubated for 14 days in the dark at 23°C, then transferred to 11°C and 500 - 600 lux light intensity; removal of the plug, 80% relative air humidity. Fruit bodies were produced after 8 (6 - 10) weeks.

Petri-dishes: 20 g of substrate, covered with a film of medium as above. The petri-dishes were kept closed in the first incubation period. During the 11°C-period they were opened and kept under a tent of polyethylene foil. CO_2, inhibiting fruiting body production,

was removed by absorption by a solution of $Ca(OH)_2$.

Mycelial growth: Growth in connection with the enzyme determinations was observed on malt-broth (1,5% malt), solidified if needed by 1,5% agar. The liquid cultures (surface culture, 250 ml Erlenmeyer-flasks containing 90 ml of medium) were inoculated with 10 ml of a homogenate of a 14 day old liquid pre-culture. After 14 days of incubation at 27°C and diffuse light, the mycelium was harvested and separated from the culture filtrate by suction through filterpaper on a Buchner-funnel. Wet weight, dry weigth (80°C) and pH were determined for each experiment.

Cultural filtrate and mycelial extracts. 5 g mycelium (wet weight) were homogenized for 3 min in a precooled mortar with 10 g of washed seasand. After addition of 30 ml 0,05 M phosphate buffer, pH 6.0, the mycelium was ground for another 5 min. Mycelial fragments were separated from the mycelial extract by centrifugation (20 min, 4°C, 10,000 x g). If necessary, mycelial extracts and cultural filtrates were concentrated for the enzyme assays in disc-electrophoresis and isoelectric focusing by ultrafiltration.

Electrophoretic separation of proteins: Disc-electrophoresis was conducted according to Ornstein and Davis (1964) at pH 8,3 in 7,5% polyacrylamide gel, isoelectric focusing in a pH-gradient from pH 3,5 to 10 in 7,5% polyacrylamide gel according to Wrigley (1968).

Enzyme assays: Presence of peroxidase on malt-agar was determined after Lyr (drop-test, 1958) with a solution of 0.1% benzidine in extracts and cultural filtrates peroxidase was assayed for by a colorimetric test at 436 nm with guajacol and H_2O_2 according to Pütter (1974a,b).

Tyrosinase on malt-agar was determined following Lyr (1958), using an aqueous solution of 0,01 M p-cresol containing 0,05 glycine. Tyrosinase in cultural filtrates and mycelial extracts was tested colorimetrically at 436 mm with 0,02 M L-tyrosine (Esser, 1963).

L a c c a s e on malt-agar was assayed for according to Lyr (1958) with an aqueous solution of 0,005% α-naphthol or 0,005% guaiacol. Presence of laccase in cultured filtrates or mycelial extracts was investigated colorimetrically at 436 nm with 0,65mM 2,6-dimethoxyphenol in 0,1M Na-citrate/NaOH buffer, pH 5,0 according to Prillinger (1976). Laccase in the electrophoretic experiments was determined by incubation of the gels in 0.02 M guaiacol in 0.05 M phosphate buffer, pH 6.0. The gels after isoelectric focusing were equilibrated in buffer for 30 min before addition of the substrate.

1 unit of laccase-activity (1E) corresponds with a difference in absorption of 0.2/min at 436 nm and 1 cm light path.

The specifity of the enzyme assays was tested with pure enzymes, single and in mixtures. Mycelial extracts and cultural filtrates were prepared and concentrated at 4°C, electrophoreses and enzyme assays were conducted at 25°C.

All determinations on petri-dishes were done in triplicate, for the assays in liquid culture always 10 parallel flasks were used.

H e r b a r i u m s p e c i m e n s of species and strains treated in this paper are deposited in the Botanische Staatssammlung München (M) together with notes on the fruit bodies.

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1

2

3

4

1 *Pleurotus cornucopiae* Paul. ex Fr. ss. Romagn.: strain 4r. 2 *Pleurotus "cornucopiae"*: strain 2v. 3 *Pleurotus columbinus* Quél. apud Bres.: strain 1n. 4 *Pleurotus columbinus* Quél. apud Bres.: strain 1n.

5

6

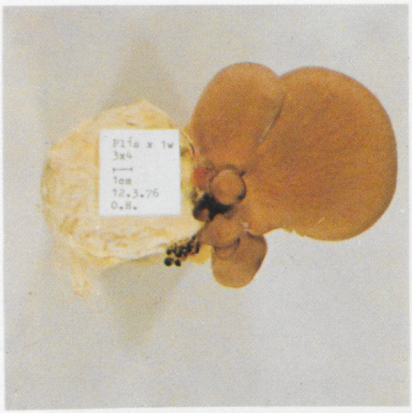
7

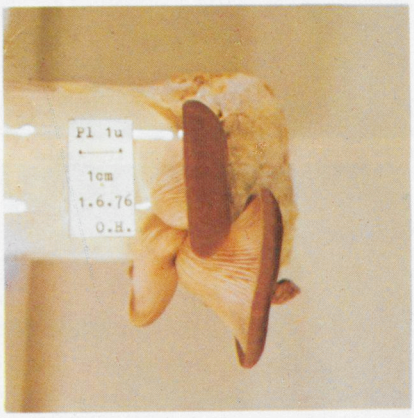
8

5 *Pleurotus pulmonarius* Fr.: strain 1r (f_1-fruit bodies). 6 *Pleurotus pulmonarius* Fr. (''Florida''): strain 4b. 7 *Pleurotus pulmonarius* Fr. (''Florida''): strain 4b. 8 *Pleurotus ostreatus* (Jacqu. ex Fr.) Kummer: strain 1w (left), strain 1s (right).

9

10





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12

9 *Pleurotus ostreatus* (Jacqu. ex Fr.) Kummer: dark fruit bodies, f_1-generation from 1s × 1w. 10 *Pleurotus ostreatus* (Jacqu. ex Fr.) Kummer: light fruit bodies, f_1-generation from 1s × 1w. 11 *Pleurotus ostreatus* (Jacqu. ex Fr.) Kummer: strain 1u; young fruit body. 12 *Pleurotus ostreatus* (Jacqu. ex Fr.) Kummer: strain 1u; older fruit body.

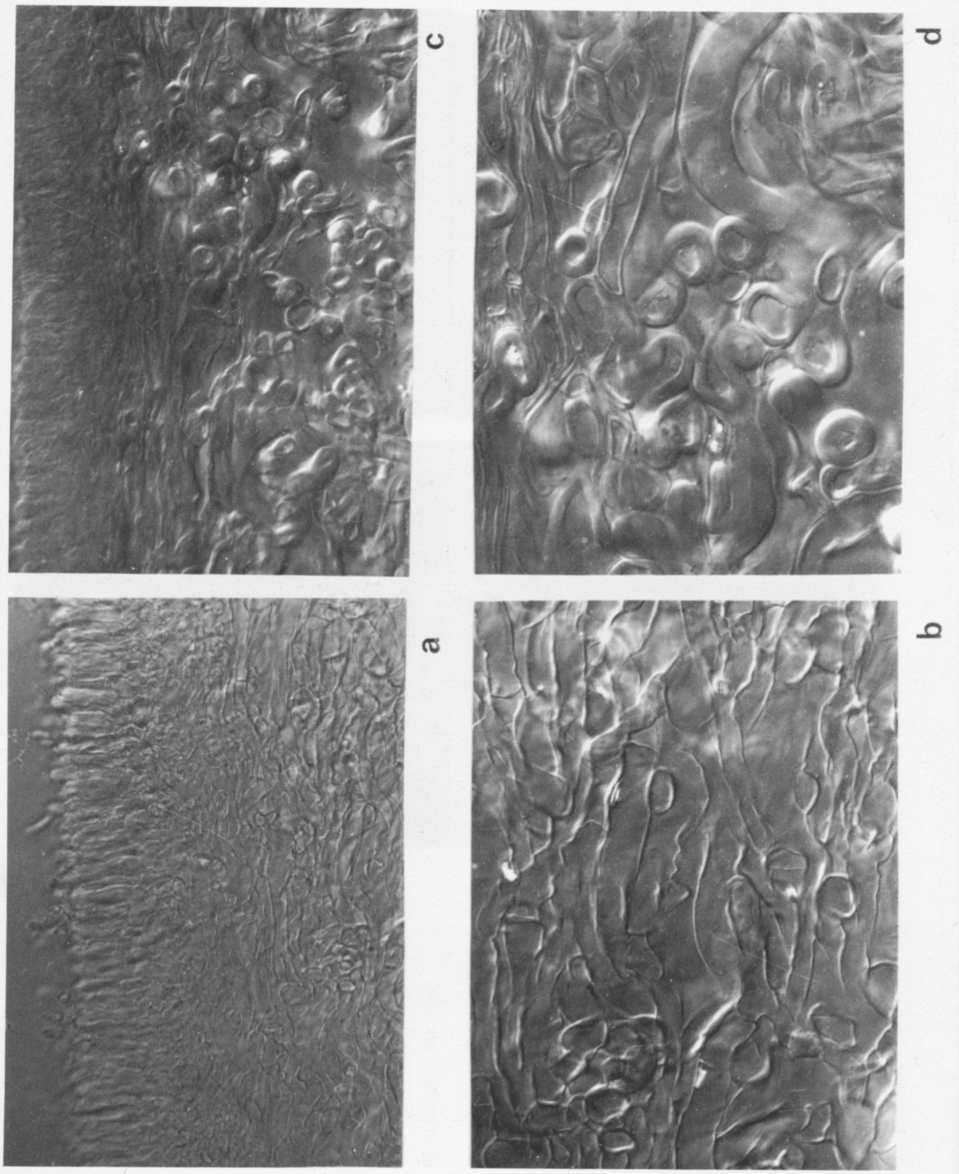


Fig. 2: P. ostreatus (1w): Trama of gills from fruit bodies cultivated on wheat grains (a-b) and on wheat straw (c-d). Note sclerified hyphae in c and d.-b and d magnified sections of a and c.

2. Survey of the Pleurotus-ostreatus-complex in Europe

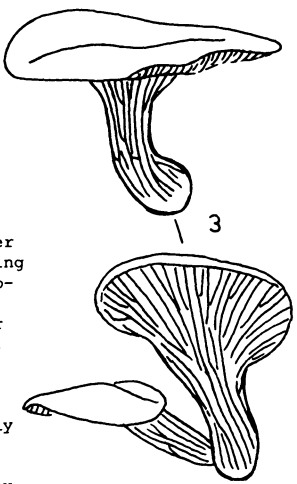
K e y

1 (2) Stipe dark brown in contrast with the cap which is much lighter. In the Mediterranean: Sicily
P. nigripes Inzenga

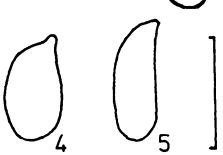
2 (1) Stipe not dark brown; equalling in color to the cap or even lighter

3 (6) Gills decurrent on long, ± centric to excentric stipes and cap at the same time ± infundibiliform

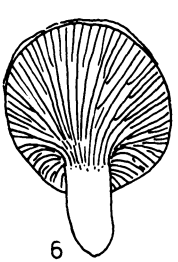
4 (5) Spores elliptical at the inner side being slightly convex. - Cap infundibiliform, with ± light or deeper brownish colors. Gills running down the stipe reaching the base, being there repeatedly connected by anastomoses; first white then changing color to cream and becoming yellowish if dried. Stipe nearly centric or excentric, often fairly long, 3- 6.5 x 0.8 - 2.3 cm. Spore deposit pale and dingy greyish lilac. Odor specific, pleasant sweetish to nearly disagreeable, at the base of the stipe also farinaceous. Hyphae mostly with thin walls, partly also with increasingly thick walls (so called false skeletal hyphae). Fructification partly early in the season
P. cornucopiae Pahl.ex Fr. ss. Romagn.



5 (4) Spores cylindric at the inner side being concave or straight. Cap ± umbilicate according to the age; light brownish-grey colored; covered by whitish scales, within the depressed center also with white felt. Gills running down the long stipe (5 - 6.5 x 0.5 - 1 - 2 cm) to the base, connected by crossveins and forked at least near the top of the stipe; of the same color like the cap, getting orange - ochraceous in older specimens. Mycelium with strong anislike odor.
"P. cornucopiae ?"



6 (3) Gills not strongly decurrent and cap not at the same time infundibiliform. Stipes often laterally attached. Spores at the inner side being concave or straight



7 (8) Cap with light colors even when young: whitish, pale brownish or pale grey. Trama partly with skeletal hyphae, the walls of which are 1 - 3.5 um thick. Gills strongly decurrent, without anastomoses at the top of the stipe; first cream colored, later pale lemon yellowish tinged.

Stipe often very short, 1 - 1.5 x 0.3 - 0.8 cm, rarely longer. Spore deposit greyish white, sometimes getting lilac grey when dried. Odor comparable with that of 4 and 5, sweetish, however not so strong. Fructification starts early in the season (May, July), partly also later (e.g. November)

P. pulmonarius Fr.

On Opuntia in the Mediterranean: *P. opuntiae*

8 (7) Cap with deeper colors and trama (mostly?) lacking skeletal hyphae. Fructification late in the season

9 (10) Cap if young dark steelblue to light blue; when older more and more ochraceous starting from the center and leaving the margin of the cap greyish blue, blue greenish or dove-colored. Gills whitish with a weak lilac tinge, getting yellow patches and finaly somewhat ochraceous; partly connected by anastomoses near the top of the stipe. Stipe laterally attached, claviform to fusiform, lighter in color than the cap, however with comparable colors; strigose at the base, 1 - 2 x 0.5 cm. Spore deposit white.

P. columbinus Quél.ap.Bres.

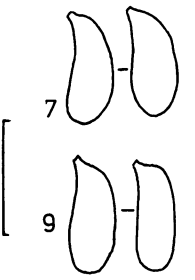
10 (9) Cap not remaining blue at the margin while the center changes color to ochraceous; uniformly colored, mostly brownish to greybrown

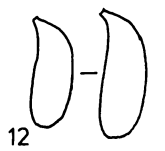
11 (12) On Salix and Populus; separating features against P. ostreatus still unknown

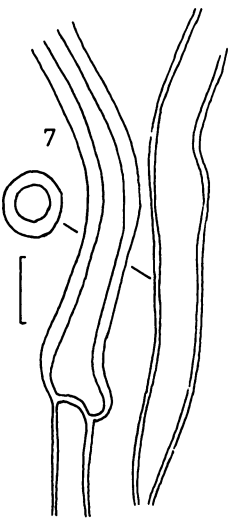
"P. salignus"(Pers.ex Fr.)

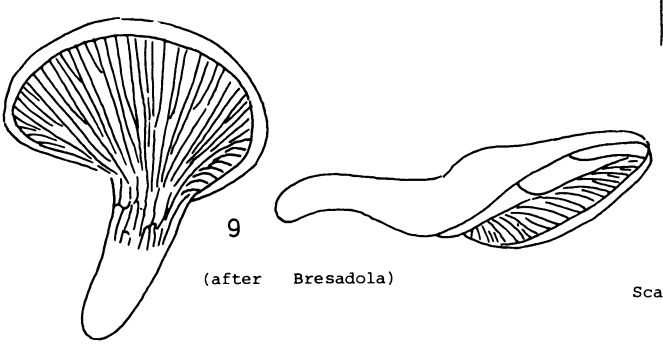
12 (11) On different kinds of wood including that of conifers and frondose trees (incl. Salix). - Cap deep brown, greyish brown, black brown; partially also rather pale in older stages of development. Gills whitish to slightly brownish or grey, decurrent and covering the upper 1/3 of the stipe. Stipe 1.2 - 9 x 0.7 - 2.7 cm, whitish or with slight brownish tinge, sometimes strigose at the base, mostly distinct excentric or even lateral. Spore deposit whitish to cream colored whith slight incarnate tinge. Context with strong odor resembling that of some species of Polyporus.

P. ostreatus (Jacqu.ex Fr.) Kummer









(after Bresadola)

Scale: 10 µm

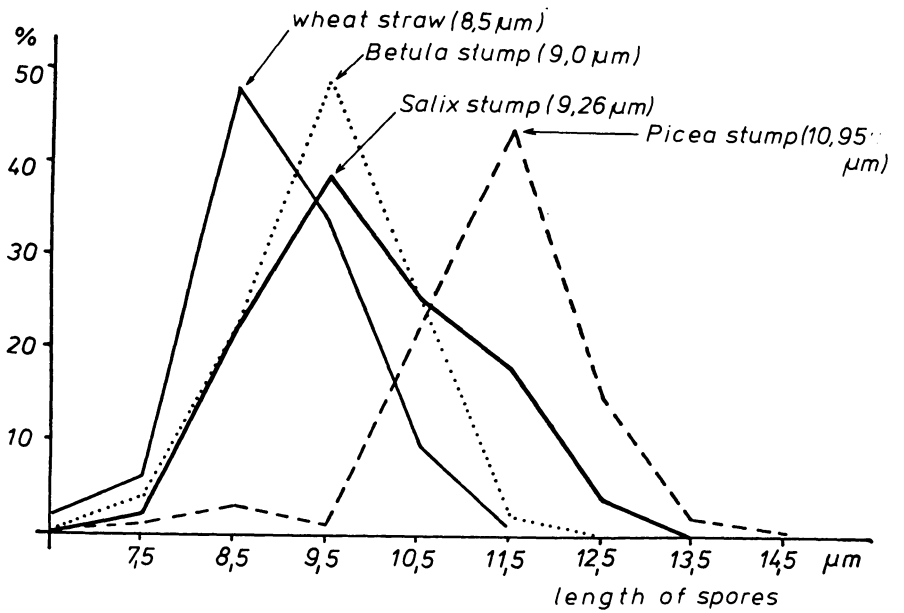
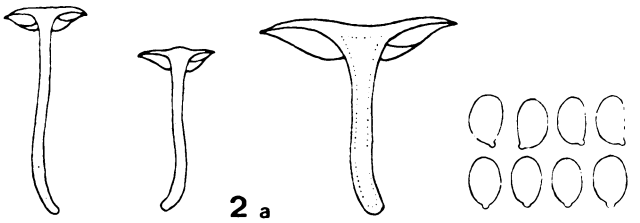


Fig. 3: Frequency of spore length in Pleurotus ostreatus, strain 2y, on various substrates. The values in brackets indicate average spore length.

Merkmalsdivergenz und Intersterilität bei Clitocybe

zusammengestellt nach Ergebnissen von D. Lamoure, 1965 und 1972

	Interfertilität	
1a C. pausiaca Herkunft: Schwedisch Lappland	+	1b C. pausiaca Herkunft: Französischer Jura
2a C. candicans Sporen: 5 x 3,5 µm Vorkommen: mit versch. Bäumen der Waldstufe und im subalp. Bereich, z.T. mit Helianthemum p-Kresol: mittlere Reakt.	+	2b C. candicans var. dryadicola Sporen 5-5,5 x 3,5 - 4 µm Vorkommen: in der alpinen Stufe mit Dryas p-Kresol: starke Reakt.
3a C. gracilipes Lamellen weniger gedrängt, bald beigefarben. Sporen: 4,5-5,5 x 3,5-4,5 µm	-	3b C. candicans var. dryadicola Lamellen sehr gedrängt, rein weiß, später gelblich Sporen: 4-5 x 3-3,5 µm Stämme aus Lappland und den Alpen interfertil
4a C. festiva Hut 1,2 - 3,5 cm Frk einzeln wachsend Stieloberfläche nicht faserig, hell Geruch: kampferartig Vorkommen: mit Dryas Sporen: 5-6 x 3-4 µm	-	4b C. festivoides Hut 1,1 - 5,1 cm Frk in Gruppen und Ringen Stieloberfläche faserig, dunkeler gefärbt Geruch: sehr unangenehm Vorkommen: mit Salix reticulata und retusa Sporen: 5,5 x 3-4 µm
5a C. gibba Sporen: 5,5-6,5 x 3,5-4,5 µm	-	5b C. catinus Sporen: 7-8 x 5-6 µm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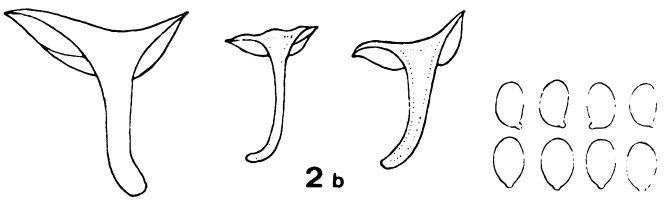
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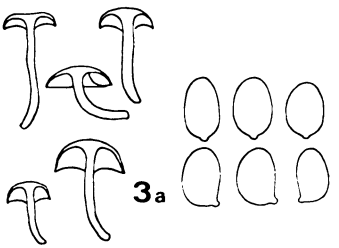
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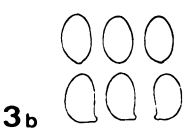
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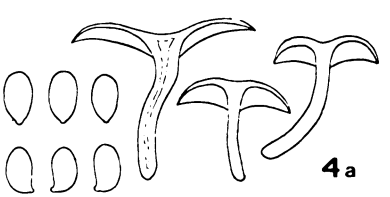
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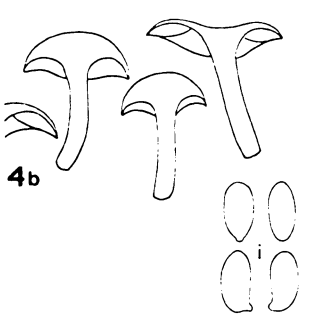
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5a

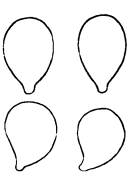

5b

Table 1

a) P.pulmonarius (1r)

Confrontation of monocaryotic mycelia isolated from a single sporocarp

		A_1 B_1					A_2 B_2				A_1 B_2			A_2B_1
		3	8	11	12	14	2	18	7	10	15	9	1	5
A_1B_1	3	-	-	-	-	-	+	+	+	+	-	-	-	-
	8	-	-	-	-	-	+	+	+	+	-	-	-	+
	11	-	-	-	-	-	+	+	+	+	-	+	+	-
	12	-	-	-	-	-	+	+	+	+	-	-	-	-
	14	-	-	-	-	-	+	+	+	+	-	-	-	-
A_2B_2	2	+	+	+	+	+	-	-	-	-	-	-	-	-
	18	+	+	+	+	+	-	-	-	-	-	-	-	-
	7	+	+	+	+	+	-	-	-	-	-	-	-	-
	10	+	+	+	+	+	-	-	-	-	-	-	-	+
A_1B_2	15	-	-	-	-	-	-	-	-	-	-	-	-	+
	9	-	-	+	-	-	-	-	-	-	-	-	-	+
	1	-	-	+	-	-	-	-	-	-	-	-	-	+
A_2B_1	5	-	+	-	-	-	-	-	-	+	+	+	+	-

b) P. columbinus (1n)

Confrontation of monocaryotic mycelia isolated from a single sporocarp

		A_1B_1			A_2B_2			A_1B_2			A_2B_1	
		4	7	11	3	6	10	5	9	12	1	8
A_1B_1	4	-	-	-	+	+	+	-	-	-	-	-
	7	-	-	-	+	+	+	-	-	-	-	-
	11	-	-	-	+	+	+	-	-	-	-	-
A_2B_2	3	+	+	+	-	-	-	-	-	-	-	-
	6	+	+	+	-	-	-	-	-	-	-	-
	10	+	+	+	-	-	-	-	-	-	-	-
A_1B_2	5	-	-	-	-	-	-	-	-	-	+	+
	9	-	-	-	-	-	-	-	-	-	-	+
	12	-	-	-	-	-	-	-	-	-	-	+
A_2B_1	1	-	-	-	-	-	-	+	-	-	-	-
	8	-	-	-	-	-	-	+	+	+	-	-

c) P. pulmonarius (1r) x P. columbinus(1n)

		A_1B_2		A_2B_1		A_1B_1		A_2B_2		
		9	12	8	1	7	11	3	6	10 ← 1n
A_2B_2	2	-'	-'	-'	-'	-'	-'	-'	-'	-'
	7	-'	-'	-'	-'	-'	-'	-'	-'	-'
	10	-'	-	-'	-'	-'	-'	-'	-'	-'
A_1B_1	3	-'	-'	-'	-'	-'	-'	-'	-'	-'
	8	-'	-'	-'	-'	-'	-'	-'	-'	-'
A_2B_1	5	-'	-'	-'	-'	-'	-'	-'	-'	-'
	9	-'	-'	-'	-'	-'	-'	-'	-'	-'
A_1B_2	1	-'	-'	-'	-'	-'	-'	-'	-'	-'

↑ 1r

d) P.pulmonarius x P.ostreatus:

	1	2	3	4	5	6	7	8	9	10	11 ← 1u (ostreatus)
1	-'	-'	-'	-'	-'	-'	-	-'	-'	-'	-'
2	-'	-'	-'	-'	o	-'	-	-	-	-	-
3	o	-	-	-	-	-'	-'	-	o	-'	-'

↑ 1d (pulmonarius)

	3	5	8	11	21	22	23	24	30 ← 1r (pulmonarius)
1	-'	-'	-'	-'	-'	-'	-'	-'	-'
2	-'	-'	-'	-'	-'	-'	-'	-'	-'
4	-'	-'	-'	-'	-'	-'	-'	-'	-'

↑ 1s (ostreatus)

\+ = clamp connections formed
\- = no clamp connections formed
' = with barrage phenomenon (see fig.4)
o = outfall of confrontation

equivalence of mating factors in interspecies crosses could not be determined

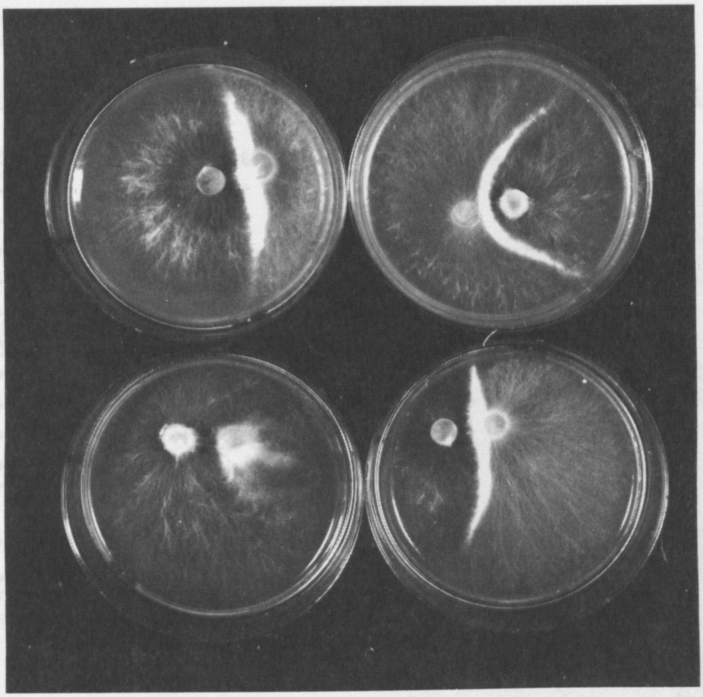


Fig. 4: *P.columbinus* (1n) x *P.pulmonarius* (1r)

Barrage phenomen in interspecies crosses:

Upper row from left to right: 11 x 13; 6 x 8

Row below from left to right: 12 x 10; 6 x 7

Table 2: Survey of non fertile crosses of Pleurotus:

cornucopiae [1]	x	*pulmonarius*	(4r x 4j)
	x	*columbinus*	(4r x 1n)
	x	*ostreatus*	(4r x 1u)
pulmonarius	x	*cornucopiae* [1]	(4j x 4r)
	x	*columbinus*	(1r x 2w)
	x	*ostreatus*	(1r x 1u)
pulmonarius "Stamm Florida"	x	*ostreatus*	(4b x 1a, 1u)
columbinus	x	*cornucopiae* [1]	(1n x 4r)
	x	*pulmonarius*	(1n x 1r)
	x	*ostreatus*	(1n x 1w)
ostreatus	x	*cornucopiae*	(1u x 4r)
	x	*pulmonarius*	(1u x 1r)
			(1s x 4x)
	x	*columbinus*	(1w x 1n)

1) ss.Romagn.

non fertile = no clamp connections formed

Table 3

a) P. pulmonarius:

	4i ↓											
1	o	+	+	o	-'	+	+	-	+	-	o	-
2	+	o	+	+	-'	+	+	-	+	-'	+	+
3	+	+	+	+	+	+	o	-	+	+	+	+
4	+	+	+	+	-'	+	+	-'	+	-	+	-
	1	2	3	4	5	6	7	8	9	10	11	12 ← 4h
1	+	+	+	+	o	+	+	+	+	+	+	+
2	o	o	+	+	+	+	+	+	+	+	+	o
3	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	-	+	+	-'	+	-'	+	+
5	+	+	+	+	-	+	+	-	+	-'	+	-'
	↑ 4j											

Origin: Bayerischer Wald; Zwieseler Waldhaus; 4i und 4j growing 1 km appart from 4h

b) P. pulmonarius:

	3	8	7	1	18	19	20	24	29	30 ← 1r
1	+	+	+	+	+	+	+	+	(+)	+
3	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	(+)	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	(+)	+	+
9	+	+	+	+	+	+	+	+	+	+
11	-	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+
	↑ 4h									

Origin: 4h: Bayerischer Wald, Zwieseler Waldhaus;
1r: Österreich, Almsee near Scharnstein

c) P. pulmonarius:

	10	11	12	13	14	15	16	17	18	19 ← 4b
1	+	+	+'	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+
8	+	+	+'	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+
10	+	+	+'	+	+	+	+	+'	+	+
11	+	+	+	o	+	+	+	+'	o	+
16	+	+	+	+	+	+	+	+'	+	+
	↑ 1r									

Origin: 1r: Österreich, Almsee near Scharnstein;
4b: North America; "strain Florida"

d) P. ostreatus:

	1w ↓					
1	+	+	+	+	+	
2	+	+	+	+	+	
3	+'	+'	+'	+'	+	
4	+	+	+	+	+	
5	+'	+'	+'	+'	+	
7	+	+	+'	+	+'	
8	+	+	+	+	+	
9	+	+	+	+	+	
	1	2	3	4	6 ← 1s	
1	+	+	+	+	+	
2	+	+	+	+	+	
3	+	+	+	+	+	
4	+	+	+	+	+	
5	+	+	+	+	+	
6	+	+	+	+	+	
7	+	+	+	+	+	
8	+	+	+	+	+	
9	+	+	+	+	+	
10	+	+	+	+	+	
	↑ 1u					

Origin:
1s: Westfalen, BRD
1w: Japan
1u: Japan

Pleurotus species Isolate[a]	pulmonarius 1 r (D)	columbinus 1 n (D)	ostreatus 1 w (D)	ostreatus 1 w 8 (M)	ostreatus x ostreatus 1 w x 1 s (D)	ostreatus 1 s 4 (M)	ostreatus 1 s (D)
Growth							
Malt-agar (mm/day)[b]	4.7	4.2	4.2	3.9	6.6	3.2	6.1
Liquid culture[c] (g wet weight/l medium)	17.0	9.7	12.2	19.5	4.0	14.1	13.7
Enzyme activity							
Peroxidase							
Drop test[d]	-	-	-	-	-	-	-
Mycelial extract (E/ml)[e]	0	0	0	0	0	0	0
Culture filtrate (E/ml)[e]	0	0	0	0	0	0	0
Tyrosinase							
Drop test[d]	-	(+)	(+)	-	-	-	(+)
Mycelial extract (E/ml)[e]	0	0	0	0	0	0	0
Culture filtrate (E/ml)[e]	0	0	0	0	0	0	0
Laccase							
Drop test[d]	+++	+++	++	+++	++	++	++
Mycelial extract (E/ml)[e]	1.6	0.2	8.4	1.4	4.6	1.2	7.2
total act. (E/l medium)[f]	79.5	7.7	436.8	152.8	64.6	76.5	390.8
Culture filtrate (E/ml)[e]	4.0	12.3	11.7	4.6	13.7	5.6	15.5
total act. (E/l medium)	3400	10886	10296	4094	12467	4928	13950

Table 4 Comparison of growth and enzyme production on solid and liquid medium between different dicaryotic (D) and monocaryotic (M) mycelia of Pleurotus and of a cross between two monocaryotic strains of P. ostreatus.
a) D = dicaryon, M = monocaryon. b) Malt-agar, petri-dishes. c) Malt-broth, surface culture, 14 days.
d) Malt-agar, petri-dishes, 21 days. Enzyme activity: - = none, (+) = trace, + = small, ++ = strong, +++ = very strong. e)Malt-broth, surface culture, 14 days. E/ml = units of activity/ml sample. f) Total activity in the mycelial extract from the mycelium of 10 flasks (= 1 l medium). All incubations at 27°C and diffuse light.

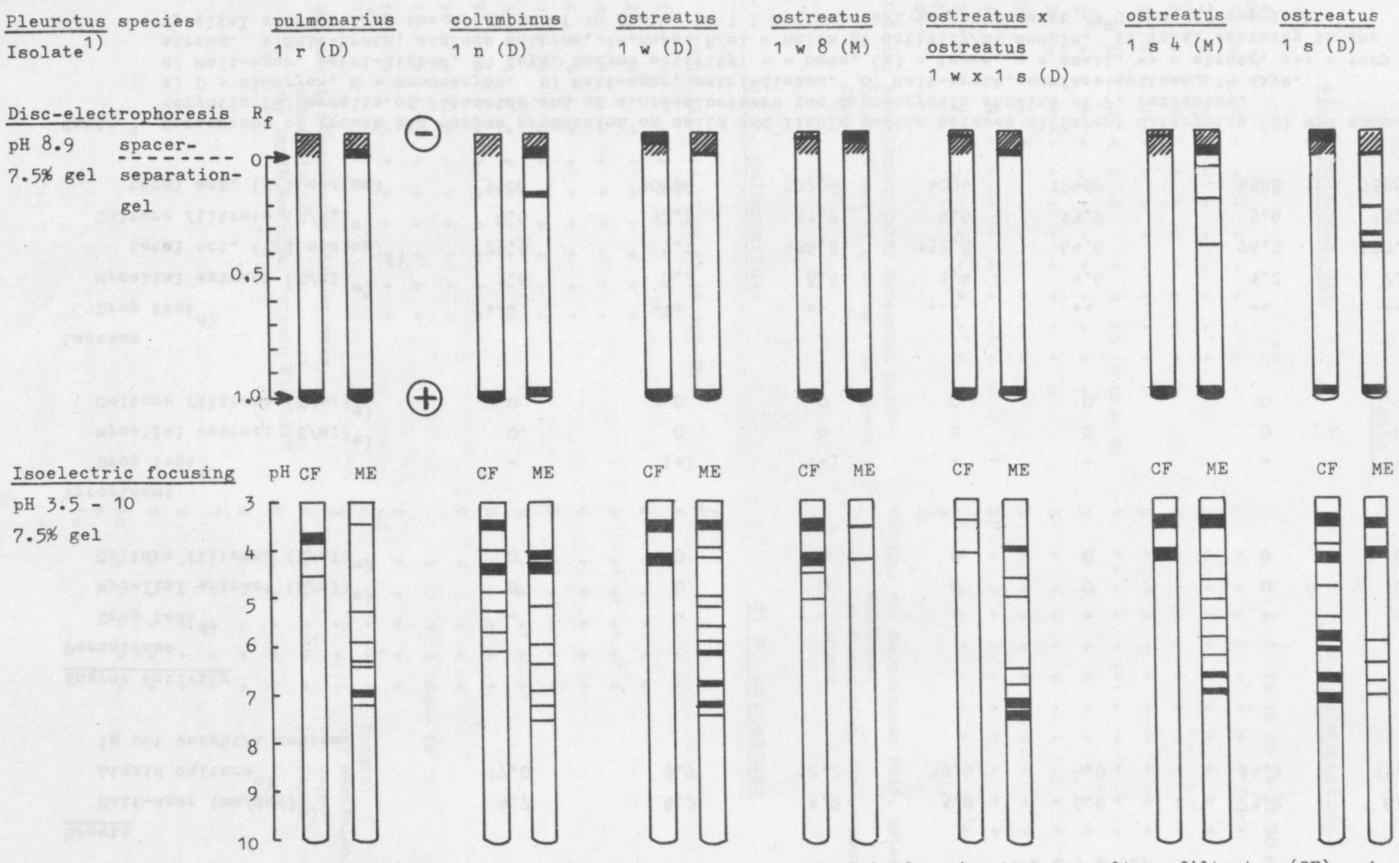


Fig. 5 Comparison of laccase spectra after disc-electrophoresis and isoelectric focusing between culture filtrates (CF) and mycelial extracts (ME) of different dicaryotic (D) and monocaryotic (M) mycelia of Pleurotus and of a cross between two monocaryotic strains of P. ostreatus.
1) D = Dicaryon, M = Monocaryon. Guaiacol was used as substrate for laccase.

DISCUSSION FOLLOWING DRS. BRESINSKY, HILBER AND MOLITORIS' PAPER

CLEMENCON: I just wanted to add a small observation of my own. You showed us that the substrate, Picea or Salix, influences the spore length. I was playing around with Hypholoma (or Naematoloma) fasciculare collected from nature, and I found consistently that the spores collected from fruiting bodies growing on conifers were longer than spores from fruiting bodies growing on Fagus. I was so much troubled that I was asking myself, are there two different species or not, but in view of what you are telling us I think we have not two different species. The differences were relatively small but statistically significant.

BAS: I have the impression that in the Pleurotus that I always used to call P. ostreatus, but I think now that it has to be called P. columbinus, at the end of the year, october, november, spores are getting longer. I collect these fungi along the coast, and I always thought the spores are getting longer because of higher humidity, but this is just an impression. Another thing I noticed: when you keep them too long in the refrigerator, the spores are getting longer also.

WATLING: I have measured literally thousands of spores in various members of the Bolbitiaceae to see the sort of effect one gets with different substrates etc.; I think in the Bolbitiaceae changes may be extreme, because the fruitbodies have very thin flesh and whatever happens to the environment is immediately expressed in the spores. In fact, in Bolbitiaceae we also see a slight change in shape. If you measure the Q-Value for the spore deposit you find there too you get not only a shortening of the spores or a lengthening of the spores, but also a change in shape; this change in shape may also be correlated with age of the fruiting body. These are not isolated caps, this is a fruiting body which is still growing.

CLEMENCON: I did this with many fungi, also with fleshy fungi, like Hebeloma leucosarx that is growing in my backyard, and I noticed one very interesting thing. Usually the length of the spore and the width, that means also the volume, are increasing; then decreasing. Once I had a strong north wind that somewhat dried the mushrooms. And even

in the fleshy fruit bodies the spore volume, the length and width, dropped sharply and came back again when normal conditions were restored.

WATLING: Yes, you can alter the spores with the environmental conditions.

CLEMENCON: And even in fleshy fungi, not only in thin things.

WATLING: That is very interesting, because I always thought that in species like Tricholoma or other fleshy fruiting bodies this would not be so much the case.

BRESINSKY: To what extend do you have those differences? How many microns?

WATLING: Oh! 3 microns!

SMITH: What about the color of the spore deposit?

BRESINSKY: I did not include this character here. Of course we noted the color of spore print, but we did not include it in any experimental setup so far. Certainly it will be a very important feature, and I know that you in the States have different species, P. sapidus and P. ostreatus, that are pretty well distinguished by different colors of the spore print. This does apparently not work in Europe.

SMITH: Does your dark Pleurotus ostreatus have a distinctly colored spore deposit?

BRESINSKY: Mostly it starts with a color whitish and then we get more and more lilac colors.

SMITH: Yes, you have not worked on this whitish ostreatus-like fungus that we have from our Biological Station in Northern Michigan?

BRESINSKY: No, I have not. I would like to have spores.

BLAICH: You told us about segregation of the cap color. What type of segregation? One to one?

BRESINSKY: So far as we could make calculations, on a limited material only, it is one to one.

BLAICH: So here is certainly the possibility of a monogenic difference between the two strains. And this would be a difference which is clearly not a specific difference.

ESSER: I was very pleased to see that you have observed about the same phenomena in your Westfalen strain, where does it come from?

BRESINSKY: Münster, Westfalen, leg.A.Runge 13.12.1973; isolated by O. Hilber 17.12.1973.

ESSER: You use the word "intersterility". And I have a tendency to look carefully at intersterility, because intersterility might be true sterility, but it might be due to other mechanisms which have nothing to do with sterility, like heterogenic incompatibility. At the moment I would say failure of interfertility, I would not say intersterility.

WATLING: I do not know whether our last speaker suffered from the restless nights trying to put together his experimental work and his field work as I have because in the Bolbitiaceae we have similar variabilities of spore size on different substrates. When you go into the field you do not find the same degree of variability. In the field I really never reached the extremes so that in fact nature seems to put a buffering effect on variation. You did mention selection. There is a strain of Psilocybe merdaria which was found in 1968; at the base of the fruiting body small gastroid fruiting bodies were observed. They did not have all the normal characteristics, and by selection of these fruiting bodies, and not the agaricoid fruiting bodies, after three generations a culture was produced which was basically gastroid.

BRESINSKY: A published paper?

WATLING: Yes, New Phytologist 70: 307-326, 1971. The problem, of course, is whether it is cytoplasmically inherited, and at the moment we are investigating this possibility; is it a virus particle which

in fact we are selcting for each time we select these "aberrant" fruiting bodies? It so we finish up with a culture which gets more and more of this viral "compound"; it is a hypothesis, but we have yet to find the virus particles.

SINGER: I understand that McKnight, who first produced such gastroid fruiting bodies in Psilocybe, considers this now as a mutation triggered by the conditions.

WATLING: From comparing McKnight's experimental work it certainly does not look to be the same thing. My material has been passed over to a geneticist who is much more competent than I to work out the problem. Professor Burnett now has cultures of all the different strains which we have been able to select out. Hopefully in the future we will have some evidence in the Basidiomycetes of selection of this type.

ROMAGNESI: Monsieur Bresinsky, vous avez pu faire ce que je n'ai pas pu faire à mon grand regret. Je voudrais surtout vous demander quelques précisions en me placant surtout du point de vue du systématicien. Je dois vous donner mon accord complet en ce qui concerne la présence d'hyphes à paroi épaissie dans la trame du Pleurotus pulmonarius. J'ai toujours été préoccupé de savoir s'il s'agissait d'un caractère constant. Parce que je récoltais souvent dans la nature des carpophores, ou des basidiomes, si vous préférez, qui avaient tous les caractères extérieurs de pulmonarius sans en avoir les hyphes épaissies. Vous savez peut-être, qu'au Laboratoire de Cryptogamie du Muséum de Paris, Monsieur Roger Cailleux cultive artificiellement tous les Pleurotus du groupe ostreatus, ainsi que ceux du groupe eryngii. Je lui ai donc confié une sporée d'un pulmonarius à hyphes épaissies qu'il a cultivée sur compost. Et, de fait, les hyphes à parois épaissies qui se trouvaient dans le carpophore trouvé dans la nature ne se trouvaient plus, ou tout au moins ce caractère était extrêmement atténué. Il est donc clair, comme vous le pensez, que ce caractère est en partie lié à l'environnement. Cependant, j'ai fait personnellement dans une forêt des environs de Paris la récolte simultanée, sur un même tronc de Fagus abattu, la récolte d'une grande série de carpophores ayant des hyphes à paroi mince et, au bout, un petit groupe de trois ou quatre carpophores qui avaient des hyphes à paroi épaissie. Dans ce cas il n'est pas possible d'évoquer l'envi-

ronnement ou des conditions extérieures puisqu'elles étaient les mêmes. Deuxièmement je voudrais vous demander quelques précisions sur la façon dont vous concevez le Pleurotus columbinus. Est-ce que vous avez récolté cette espèce en hiver?

BRESINSKY: Pl. columbinus should be a species in accordance with Bresadola, Iconographia, tab. 291. Our Pl. columbinus has been collected in November (1n; Karlsruhe, leg.Fuhrmann) and in October (4t; Poland; leg.M.Moser). I don't think that sclerified hyphae are an absolutely reliable character for Pl. pulmonarius.

ROMAGNESI: Je veux vous donner la raison de la question. Je ne sais si vous connaissez une thèse sur le Pleurotus ostreatus qui a été soutenue, il y a un ou deux ans, au Laboratoire de Cryptogamie de Monsieur Heim à Paris. Il s'agissait d'une étudiante Sud-américaine, dont j'ai malheureusement oublié le nom compliqué, mais qui avait étudié surtout le comportement du groupe ostreatus et eryngii en culture portant principalement sur l'incidence des caractères de température et d'autres facteurs physiologiques qui accompagnaient ces cultures. Il a été ainsi trouvé d'une façon extrêmement claire que dans les récoltes de Pleurotus ostreatus il y avait deux groupes d'espèces qui se différenciaient très nettement par la température optimale du développement. Et ce que les mycologues français ont toujours considéré comme Pleurotus ostreatus est une espèce d'hiver dont l'optimum de développement est relativement bas, tandis que pulmonarius, cornucopiae et les autres espèces qui poussent au printemps ou en automne ont un optimum de température beaucoup plus élevé. Or, des formes d'hiver que nous avons toujours considérées comme étant le type Pleurotus ostreatus sont des formes qui présentent des colorations bleues puisque les carpophores virent au brun dans la vieillesse. Je me demande si vos Pleurotus columbinus ne seraient pas nos Pleurotus ostreatus au sens des mycologues français.

BRESINSKY: I am sorry that I cannot give a final answer, since this question should be solved on the base of confrontation experiments. What we certainly know is that there are at least two different dark-colored species which are fruiting in the late season and which we are calling P. ostreatus and P. columbinus.

ROMAGNESI: Sur l'interprétation de Pleurotus columbinus il y a des difficultés. En effet, je crois vous rappelez que la planche d'Icones

de Bresadola, dans les fungi tridentini vous avez Pleurotus ostreatus, représante un champignon qui n'est pas bleu, ni bleu d'acier, mais qui est vert fuligineux.

BRESINSKY: Ce n'est pas ostreatus, c'est columbinus.

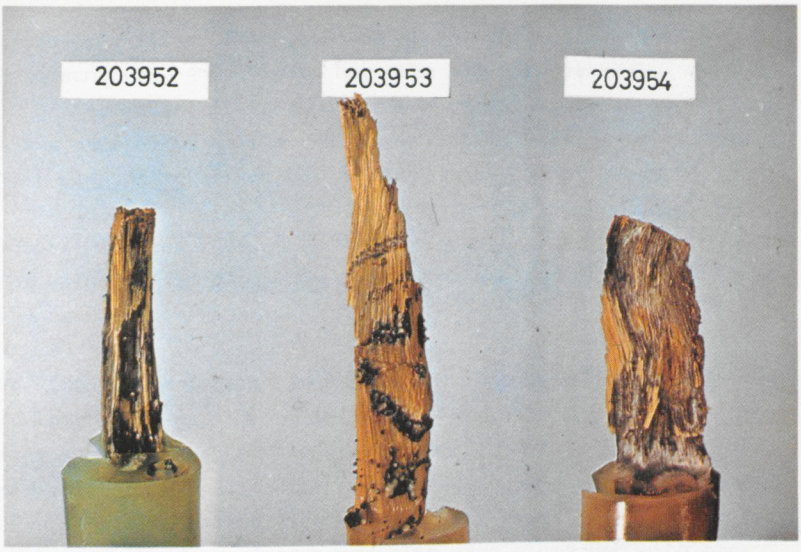
ROMAGNESI: Or, il y a peut-être quarante années qu'au jardin des plantes de Paris j'ai récolté sur un Cercis siliquastrum ce que je pense être le columbinus de Bresadola. Eh bien, ce champignon, lorsqu'il est jeune a un chapeau fauve ou ocre, et quand il se développe il prend exactement la couleur gris-olivâtre verdâtre de la Planche de Bresadola. Et sur la planche même de Bresadola la couleur rougâtre à laquelle vous faites allusion est très proche, en effet, de celle que j'observe. D'autre part, vous savez qu'il y a dans la littérature des données sur la toxicité de Pleurotus columbinus. Or, je puis vous affirmer, que le Pleurotus bleu qui pousse en hiver est parfaitement comestible. Je me demande donc, si en fait, votre columbinus n'est pas le véritable ostreatus et si votre ostreatus n'est pas le champignon qu'à tort ou à raison j'ai donné dans mon travail sur ce groupe sous le nom de salignus, lequel est en effet un champignon brun, et qui pousse aussi bien sur feuillu que sur conifères. Il aurait la peut-être un problème de systématique à résoudre.

BRESINSKY: Some of the species, like Pleurotus columbinus, are very ambiguous. This shows that breeding experiments are necessary here in order to find the border lines between the species and in order to decide what is conspecific or not. We should have the opportunity to investigate your Pl. salignus.

BLAICH: Are there breeding experiments columbinus-ostreatus?

BRESINSKY: Columbinus x ostreatus ..... are in preparation.

SMITH: I am wondering if your situation here in Europe is not confused somewhat by invading American variants. Now we have columbinus in the western United States and it is blue and it is on conifers. I have never seen this blue thing on anything but conifer. I take it you get it on hardwoods here. In the spring we have what we call ochraceous to white Pleurotus and I tried to identify it with a number of European species. It has a spore print that is white yellowish to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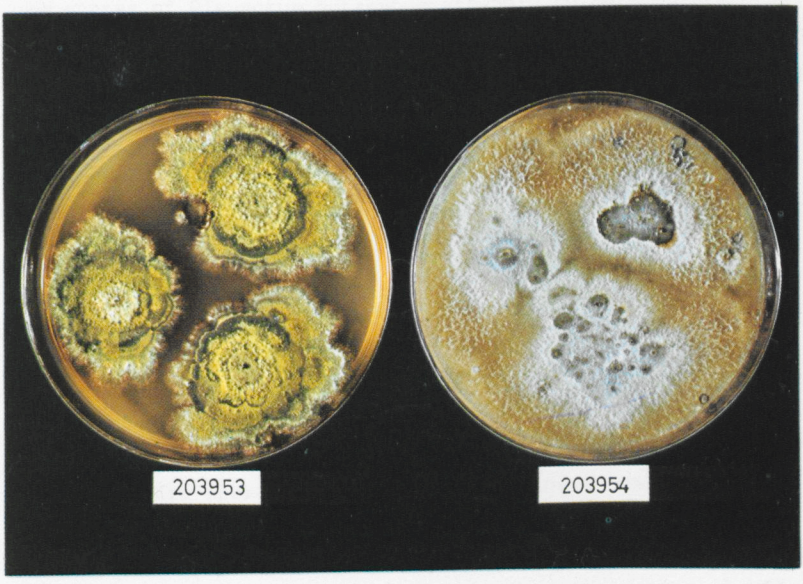
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sometimes pale lilac. This grows on aspen. This is the one we are going to send you. And then we have the big dark ostreatus with a rather bitter taste, it is almost black and it grows in the spring after the snow melts on the ski slopes. It is very distinct from the ordinary Pleurotus that we get on the elm and other hardwood.

BRESINSKY: I think that it is very interesting that we can observe so much variation in Pleurotus within one species as well as between the species.

ESSER: I think in future general regulations have to become efficient. Some years ago commercial firms have sold cultures of Pleurotus ostreatus to everybody who wanted to breed this fungus in his basement. After harvesting the fruit bodies the people has thrown the cultures substrate on the trash pile. These races of Pleurotus ostreatus became a chance to interbreed with each other and with many other fungi.
There is another question: I have heard that Pleurotus ostreatus may cause allergies. Is this a rumor? Or is this possible due to the fact that the spores of Pleurotus ostreatus are able to germinate at temperatures up to 40°C and therefore may cause after inhaling inflammations of the lung? Are these allergic reactions caused by all ostreatus strains or only by certain races? Or even by other species like columbinus?

BRESINSKY: We have put it on our program.

ESSER: What is known?

BRESINSKY: It is known that Pleurotus causes allergies. We don't know wether this is true for all species.

BAS: This is possibly a matter of the amount of spores. Pleurotus ostreatus starts to sporulate very early. They have been growing Pleurotus ostreatus in mushroom houses in the Netherlands. The workers refused to go in because there was a mist of spores. They had to wear a kind of mask because they could not stand the spores. I think if you inhale clouds of spores, there must be some effect.

SINGER: The only information I have is that in Ohio there was an

allergic reaction.