ISSN 1644-3985

Instytut Technologii Drewna

DREWNO
WOOD

VOL. 51 POZNAŃ 2008 NR 179

DREWNO – WOOD 2008, vol. 51, nr 179

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ON YELLOW DISCOLORATIONS WHEN DRYING OAK, *QUERCUS ROBUR*

Growing trees many times contain more water than wood. When wood is to be utilized in the form of furniture it must therefore be dried in a kiln-dryer. In such a dryer the climate is very humid and warm which is ideal for many microorganisms such as bacteria and fungi. During their metabolism they change the chemical environment which sometimes lead to undisireable effects such as discolorations. In this paper we have examined specimen from oak, Quercus robur, collected from some parquet floor factories in Sweden. During drying some of these wood battens were affected with yellow streaks and spots which made the wood impossible to use for flooring. By examining small samples of the battens in light, as well as in scanning electron microscopes, we found that fungi grew inside the wooden tissue. By cultivation on agar plates we found several species where one has been identified as Penicillium roqueforti and another one as Paecilomyces variotii. We have also found that these fungi are extremely sensitive to high pH-values, so by spraying the wood with solutions of high pH already in the sawmill will probably make this problem much smaller.

Keywords: discolouration, oak, fungi, identification, staining properties

Introduction

Trees growing in the forest contain a lot of water that facilitates the transportation of nutrients that these trees need for living. When a tree is cut and machined into logs, the wooden tissue cannot hold this large amount of water and the logs start to dry. Green wood can contain more water than wood, i.e. the moisture content, M.C., is over 100%, see e.g. [Kollmann, Côté 1984], p. 421. M.C. shows the ratio between the total water amount and the amount of absolutely dry wood. Different parts of the trunk have different M.C. Heartwood is somewhat dryer, with a M.C. of about 50%, while sapwood normally contains higher

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amounts of water. Because of the inevitable drying, starting when the tree is cut, M.C. will decrease until it reaches the so-called equilibrium point. This is set by nature according to the surrounding relative humidity, R.H. in the air. Therefore, a piece of wood has an equilibrium point of only about 6% during indoor winter use, while it is leveled at about 18% outdoors during summer. When M.C. is high, such as in newly felled trees, free water is present in the cellular structure. At a certain level, for a M.C. of about 30%, the so-called fibre saturation point is passed. Now, there is no free water in the cell lumina but water is still present in the cell walls. When also this water evaporates the cell structure starts to shrink. It is natural that the outer part of a wooden piece will dry first. M.C. will therefore show a gradient where the highest M.C. is present in the middle of the piece. The outer part of the piece will therefore start to shrink, before this happens in the core, leading to tension in some parts while others are subject to compression. This will lead to severe cupping, warping and other defects in the timber. Wood must therefore be dried before it is taken into service, and the M.C. level must be set according to the climate surrounding the wooden structure. Nowadays this drying takes place in kiln-dryers where it is possible to achieve climates with different R.H. If the wood is dried too quickly in the dryer, stress is introduced in the wood which eventually will cause warp, cracks etc. If, on the other hand, the wood is dried too slowly other undesireable things might happen.

Drying oak for use in parquet floors

In Europe there are but a handful of different species of oak, and in Sweden only one has industrial significance, viz. *Quercus robur*. This type of oak is the industrial base for several companies, such as Kährs, Tarkett, Forbo, Rappgo, just to mention a few. All these companies have their own kiln-dryer equipment, because they must have perfect control over M.C. in the wood during the whole manufacturing chain. Sometimes, however, nature will implement obstacles in the form of discolorations of oak wood pieces, or battens. If this happens it will be impossible to use the wood and, instead, it must be thrown into the wood-fired boiler. Beautiful oak wood has a price of 500 – 1000 Euro/m^3, so these undesireable effects have large economic implications in this industrial branch.

One of the most evident errors that might occur is a yellow discoloration. The difference in color is easy to observe but the question now is what has happened to the wood and if it is possible to avoid such an error. Just by looking more carefully at the oak wood battens we found that this error is best observed in both ends of a piece. Firstly it was therefore presumed that the color came from the sap transported in the vessels. When this liquid evaporates in the vessel

orifices, the yellow substance is left on the wood surface. One other explanation is that the color change starts where the vessels debouch into the surrounding air and that chemical reactions start when oxygen is present in "normal" concentrations. Even if these explanations are plausible it must have been other things that changed the sap and by studies of the wood in the microscope we found that something was growing in the wooden tissue, see "Results and discussion" below.

Microbiological attack

When drying conditions are to be changed in a dryer this is achieved by adding heat to outdoor air and blowing this into the dryer by a fan. When the air gets warmer, the R.H. of the air decreases. The dry air now flows through the stacked timber and water starts to evaporate from the wood. When the water from the timber comes into the air the R.H. in this air increases. At a certain level it is therefore necessary to change the air in the dryer. The warm and humid air is therefore led out, and outdoor air is led into the drying chamber, and warmed up. The warm and humid climate in such a dryer is ideal for many microorganisms, such as bacteria and fungi. These organisms are abundant everywhere in nature and if the conditions are right, from the organisms' point of view, they will start to grow. This growth is a normal process when plants are degrading in nature. It is a bad idea to try to stop this process but we only want to use the timber a short time, before this degrading process takes place. We therefore wanted to find out why the oak battens were yellow and what we could do to prohibit this color. By looking at small pieces in a microscope, *vide infra*, we soon suspected that fungi and/or bacteria were the origin of the problems and started to scan the literature on oak wood, bacteria and fungi. By searching in the Science Citation Index database on the Internet, we found some interesting clues.

Initially we found one paper, [Bauch 1984] published some 25 years ago, where it was shown that yellow discolorations in oak originated from a fungus called *Paecilomyces variotii*. This fungus is part of the *Fungi imperfecti*, i.e. fungi which reproduce only asexually. The authors of [Bauch 1984] also reported that a mixture of acetone and water had to be applied in order to extract the yellow color while pure water was inefficient. Further research in the database showed that other scientists also had studied yellow discolorations during drying, but with some contradictory results. In Wegener and Fengel [1987], it was stated that bacteria and/or fungi were not involved in the process. They also found that the yellow color was soluble in water and that it had a low pH (3.1). In France several scientists had studied the color of oak wood and how it was related to the extractives in the tree. According to [Klumpers et al. 1994] it was found that oak wood contains about 10% extractives in the form of elagitanni-

nes. They showed that part of these were soluble in water while other parts had to be dissolved in an acetone/water mixture. The reason for the yellow color, therefore, they said, was probably hidden in chemical reactions of those elagitannines. They also showed that the differences in solubility were dependent on the age of the heartwood. Older heartwood had more elagitannines which were harder to solve in water. In [Charrier et al. 1995] such chemical compounds have been studied in more detail but mostly for brown discolorations. In Canada some researchers had examined the extractives in *Q. robur and petrea* by using mass spectrometry [Mosedale et al. 2002]. They found over 50 different compounds, e.g. elagitannines which could be involved in the process of discolorisation. There were also papers showing that bacteria might be responsible for the change in color. In [Stankiewich et al. 1971] *Clostridium quercicolum* bacteria are reported to have invaded living oak trees. Just for information on the large interest in this field [Shiego et al. 1973] gives an impressive list of about 150 papers on microorganisms in living trees.

According to literature *P. variotii* fungus seems to be of special interest. We therefore tried to find more information on this, and closely related, species. In [Brown, Smith 1957] it was found that the problem with yellow discoloration was known already in 1923 but many authors called the fungus *Penicillium divaricatum*. This has also happened later see e.g. [Hubert 1929] p. 23, where the fungus is reported to grow on birch, oak, hickory and maple. Some fungi can have different stages and *P. variotii* also has a stage when it reproduces sexually. In such a case it is called *Byssochlamus* and it is then a part of *Ascomycetes* or sac fungi. Other such fungi are the more well known truffles and morels, see [Campbell 1999] p. 580. In [Brown, Smith 1957] it is further reported that the two fungi *Paecillomyces* and *Byssochlamus* have conidial states, i.e. where asexual spores are produced whose forms are indistinguishable from each other, and the conclusion is that they are two forms of the same fungus.

In [Cartwright, Findlay 1936] it is reported that many fungi can attack oak wood. One of these were able to produce golden oak and the authors reported that it was identified as *Eidamia catenulata* by H.S. Williamson already in 1923. She also did connect the yellow color with the Death Watch beetle. By this survey it was obvious that microbiological life was involved and we started to experiment on our own.

Material and methods

Because of the lack of experience in the field of mould fungi we started with some traditional microbiological methods.

Cultivation of fungi from discoloured wood
Pieces of yellow stained wood were added to SAB-agar plates and fungal colonies were allowed to grow on the agar plate. (SAB, i.e. "Sabouraud", is a special recipe for agar nutrition.) After initial fungal growth, colonies were selected based on morphological appearance and incubated on individual agar plates to achieve pure cultures.

Fungal staining properties
To evaluate the ability of different fungal strains to produce the yellow staining, pieces of autoclaved wood were inoculated with fungal strains from the pure cultures. The inoculated wood pieces were incubated until fungal growth was macroscopically visible. To evaluate the staining inoculated pieces of wood were compared to controls.

Morphological studies
Morphological studies of the fungi were carried out to differentiate between fungal strains. Studies were also performed to elucidate the location of the fungi in the wood.

Macroscopic studies
The initial discrimination between the fungal strains was performed based on the macroscopic appearance of the colonies (colour and shape). The different strains were documented with a digital camera (Canon PowerShot G3).

Microscopic studies
After macroscopic evaluation the different fungal strains were further studied for microscopic differences of hyphae and conidia in order to differentiate between the fungal strains.

Light microscope
Parts of the fungal colonies were picked up from the agar plates with a pipette tip and suspended in a droplet of Türks reagent on a slide. The dye was allowed to penetrate the cells for some minutes after which time the sample was covered with a slip and examined in a Carl Zeiss light microscope with 40–100 × magnification. The examination was documented with microphotographs.

Scanning electron microscope (S.E.M.)
The S.E.M. was used for two purposes:
1. To find out if and where the fungi was located in the stained wood.
2. To see the ultrastructural appearance of conidia and hyphae.

In the fungal location studies the wood was dried and prepared with spraying before examination in the S.E.M. In the evaluation of fungal ultrastructual appearance parts of the fungal colonies used for S.E.M examination where suspended and fixed in glutar aldehyde and cytospun onto round coverslips. In preparation for S.E.M examination the samples were initially dehydrated with a series of ethanol baths, 50%, 66.6%, 75%, 90%, 95% and 99.5%. To avoid rehydration the samples where kept in ethanol all the time. After the samples were dehydrated they were inserted into a critical point drying (CPD) pressure chamber. In the chamber the liquid carbon dioxide (CO_2) was substituted for ethanol. When all the ethanol had been substituted for, the temperature was increased which promotes the transition from liquid to gas phase of the CO_2. After the transition the chamber can be opened and the dry samples can be further prepared for examination. After dehydration and drying the samples were sprayed with platinum which finished the preparation. The samples were examined in a JEOL scan electron microscope.

pH tests

Fungal colonies were incubated on SAB-agar plates with varying pH to evaluate if and how the growth rate was affected by changes in pH. The tests were conducted on SAB-agar plates on which pH had been set by addition of 1M sodium hydroxide (NaOH) or 1M hydrochloric acid (HCl). The plates were produced with the following pH values: ≈ 4, ≈ 5, 5.6 (control), ≈ 7 and ≈ 8. pH values on the plates were assessed with universal indicator strips. The plates were incubated for ≈ 48 hours. After incubation the plates were evaluated for fungal growth. The growth was documented with a digital camera (Canon PowerShot G3).

Results and discussion

When we had collected our first piece of yellow oak we achieved our first result just by putting a small sample of the discolored oak wood into our S.E.M., see fig. 1.

The S.E.M. has an ability to magnify the specimen up to 200,000 times, and hence it is possible to examine the cells in the wood, or even details on the cell wall itself. In fig. 1 a part of a vessel is shown and further it is obvious that something was present in the vessel that normally should not be there. We suspected that it was a hypha from a fungus and we started to read about fungi and how to cultivate them on a more practical substrate than wood battens. After some days fungi were growing at our agar plates. We were, however, a little confused because we found that at least ten different fungi were present on our plates. On another type of agar plate we even found one colony of a bacterium.

Two of our fungi showed large differences in habiti. One of them grew with white colonies while the other one showed a greenish appearance. Because of limited funding we had to concentrate our efforts and we chose two fungi for further studies. Now our goal was to identify these two species. This, however, showed up to be a very difficult task.

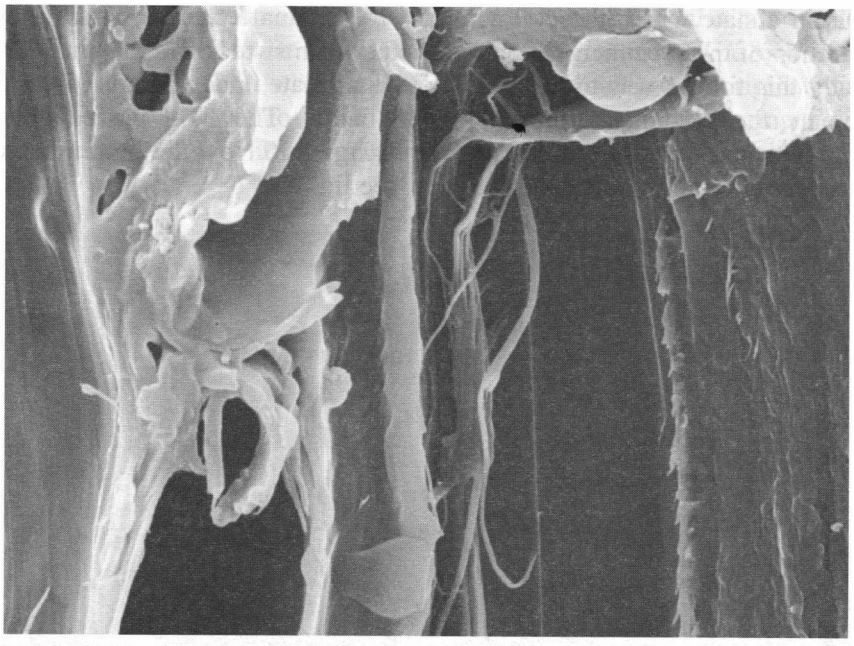

Fig. 1. S.E.M. picture of yellow colored oak wood. Original magnification 4,000×
Rys. 1. Zdjęcie zabarwionego na żółto drewna dębu wykonane przez skaningowy mikroskop elektronowy. Oryginalne powiększenie 4,000×

We started to examine our fungi in a light microscope, see "Material and methods" above. Some of our first microphotographs are shown in fig. 2.

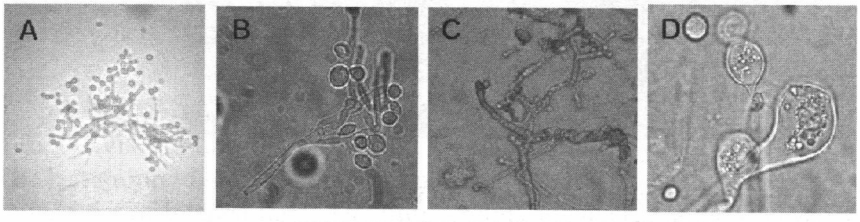

Fig. 2. Microphotographs of an unknown fungus cultivated from yellow oak
Rys. 2. Zdjęcia mikroskopowe nieznanego grzyba wyhodowanego z żółtego dębu

Now a normal procedure implemented by botanists is to use a "key" or a special scheme in which different features are described. Sometimes, pictures accompany this text in the form of drawings or photos. However, the pictures in fig. 2 could not be used for such an identification purpose. This is because some of the structures needed for identification were missing. It was obvious that we had to look at larger parts of the fungi and at the same time try to keep the fungus in a state where these structures were as unaffected as possible by the preparation of the specimen. After some experiments we found that the best way to study this fungus was to put the whole agar plate under the microscope and illuminate the specimen with light from below. The light passed not only through the agar but also the specimen growing on it. Now the light microscope showed better correspondence to the keys, see fig. 3.

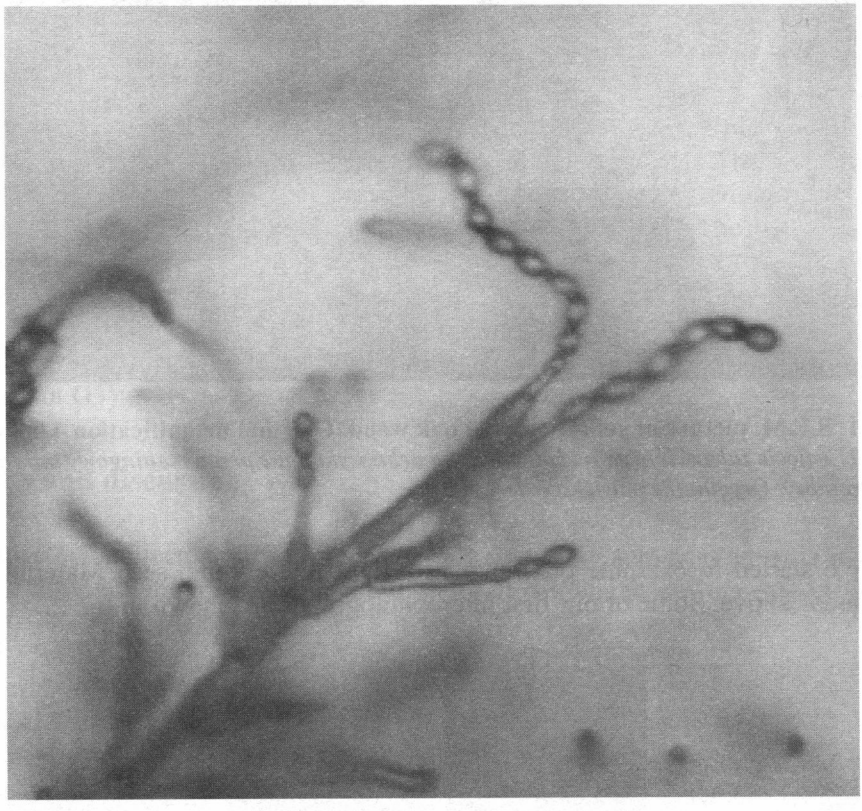

Fig. 3. An unknown fungus as it shows up in the microscope
Rys. 3. Nieznany grzyb tak, jak go widać pod mikroskopem

Now we were able to start the identification. In [Campbell et al. 1999] the kingdom of fungi is divided in four divisions, *Chytridimycota*, *Zygomycota*, *Ascomycota* and *Basidiomycota*. The species in the first division normally live in water and have flagellated cells. Such cells are not present in our fungi, see both fig. 2 and 3. The second division includes, e.g. mold found on bread, and they have hyphae that are only septate, i.e. divided in small compartments, where sexually reproductive cells are formed. Such a fungus was found in an Erasmus student project in the department of Mechanical Engineering. The two students from Belgium studied soft wood with black dots and these dots originated from a fungus. This was found to be of *Mucor* genus and in our Scanning Electron Microscope, S.E.M., it showed up as visible in fig. 4.

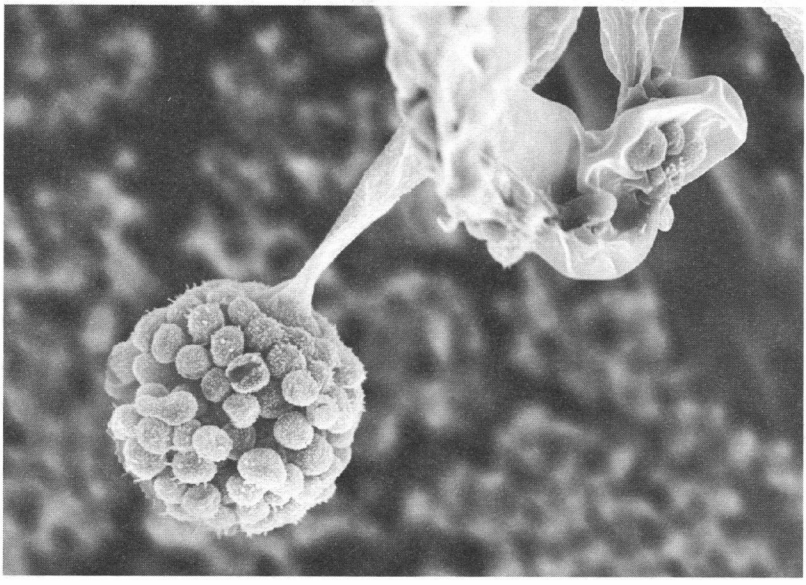

Fig. 4. A sporangium on *Mucor plumbeus* of the *Zygomycota* division (photo: Simon van Remoortel and Yves van Poucke)
Rys. 4. Zarodnia na Mucor plumbeus z podziału Zygomycota (fot.: Simon van Remoortel i Yves van Poucke)

Our fungi, causing yellow discolorations on oak, do not seem to come from that division, and they also had clearly septate hyphae, see fig. 5.

The last group in our list contains "normal" fungi such as mushrooms, which is likewise not of interest here. The third group, however, has asexual reproduction in the form of spores growing in long chains or clusters, *vide infra*. It is therefore plausible that we might deal with fungi from the division *Ascomycota*.

There are also fungi that cannot be classified in these divisions because of lacking sexual reproduction. These are called *Deuteromycetes* or *Fungi imperfecti*. If, and when sexual reproduction is observed, the fungi from this group is moved accordingly to the appropriate division, and most often this has been the *Ascomycetes*.

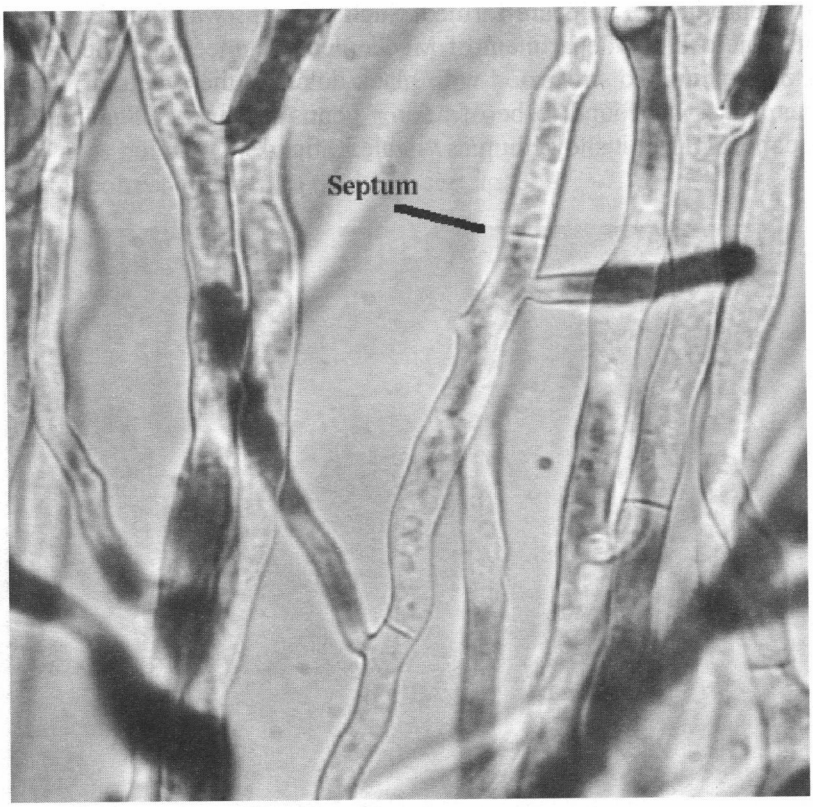

Fig. 5. Septate hyphae in an unknown fungus
Rys. 5. Strzępki z przegrodami w nieznanym grzybie

By use of [Campbell et al. 1999] we were able to identify two plausible divisions for our fungi. Hence, we consulted a book on *Ascomycetes*, [Ainsworth et al. 1973], and by looking at the pictures we found that *Penicillium* and *Paecilomyces* were two genera of interest. The mycologists, however, are not always unanimous in identification of species, and in fact not even the genus. Just as an example, *Paecilomyces* genus was set up by Banier in 1907 on the foundation of only one species. This according to [9] and these authors added some 15 new species to the genus themselves. Further amendments were made by the author

of [Sanson 1974] who doubled the number and an investigation on the Internet showed up to 114 different species in the genus. A number of these species have been sorted in other genera over time. Further, *Ascomycetes* and *Fungi imperfecti* are very large classes of fungi which contain several thousands of species, 45,000 and 30,000 respectively. In e.g. [Ainsworth et al. 1973] there are 67 pages with pictures but they show only a few of them. Many of these species are also closely related and for non-experts they look practically the same.

In our struggle with finding more pictures on the interesting genera we found that a special form *Penicillium digitatum* was a very common green mould fungus on oranges. Such a fungus was easy to obtain and in fig. 6 the mould growing on this orange can be compared to our unknown fungus.

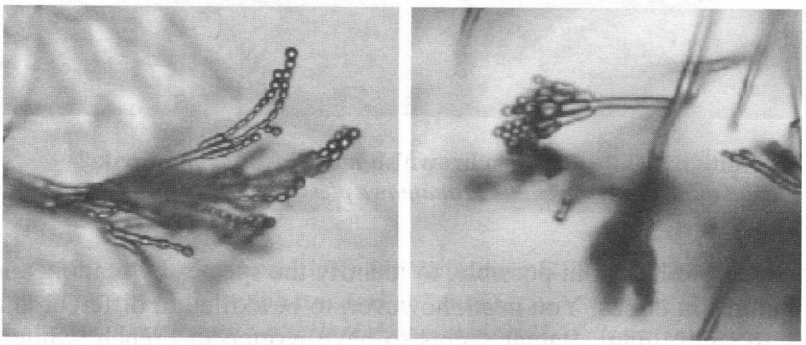

Fig. 6. Green mould, probably *Penicillium digitatum* on an orange to the left, and unknown fungus on yellow oak to the right
Rys. 6. Zielona pleśń, prawodpodobnie Penicillium digitatum na pomarańczy po lewej oraz nieznany grzyb na żółtym dębie po prawej

It is obvious that the two types show many similarities, e.g. the spores or with a more strict language the conidia, i.e. asexually formed "spores", grow in long "chains" at the end of a hypha. The fungus found in yellow oak, however, has such chains much shorter. The color of the conidia on the agar plates was also of a deeper shade of green than this of the type on the yellow oak. They can therefore be of the same genus but it is probable that they are of different species.

We also had a white type. Noteworthy is that when this fungus grew older we observed a brownish color of the surface of the fungal colony. In fig. 7 this type is shown in the form of two pictures from the light microscope.

Also for this fungal type we see that the conidia grow in long chains, typical for the asexual reproduction of *Ascomycetes*, see [Campbell et al. 1999] p. 580, but they have more oval forms and the chains do not grow so closely together as

in *Penicillium* species shown in fig. 6. In fact this type looks very similar to some of *Paecilomyces* species found in [Brown, Smith 1957]. It should be noted here that *Penicillium divaricatum* as shown in [Hubert 1929] also is quite similar to the types in fig. 7, and the authors of [Brown, Smith 1957] suspect that this in fact is a *Paecilomyses*.

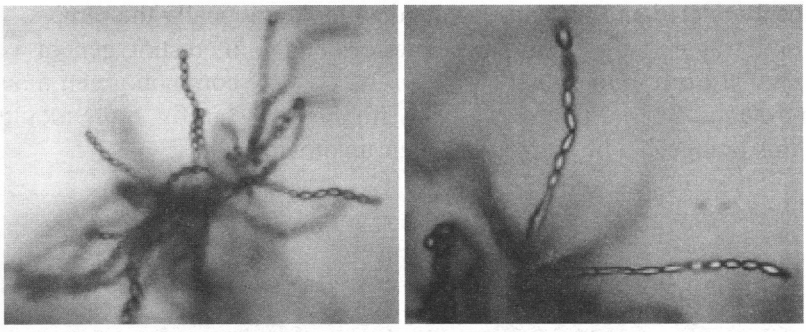

Fig. 7. The initially white and then brownish mould from yellow oak
Rys. 7. Początkowo biała a następnie brunatnawa pleśń z żółtego dębu

It is therefore hard, but possible, to identify the species by reading and looking at pictures in books. You need, however, to be skilled in different languages in order to study fungi. Banier seems to have written in French, while the authors of [Brown, Smith 1957] wrote their paper in English and Latin. German and Italian are also common and even Swedish is present in literature. A much safer method is to compare an already identified fungus with the unknown species, as we compared the orange mould with our unknown species above. Such identified fungal "strains" can be bought from different laboratories, such as the Centraalbureau voor Schimmelcultures in Holland. It is also possible to send your fungus to the bureau and in our case, they have identified the fungus to the right in fig. 1 as a *Penicillium roqueforti* which normally is found in a famous type of cheese. The other one was identified as a *Paecilomyces variotii* (This bureau also identified the *Mucor* above.)

pH tests

Fortunately, we were able to identify the fungi using "normal" references, and also the species with some help from specialists. Our second aim was to find ways to make it harder for the fungi to grow and prosper. Some ways were found in literature. In [Cartwright et al. 1936] it is proposed that the timber should be dipped into a solution of sodium flouride (2%) or in borax (6%) but they also suggested that initial steaming for few hours with live steam at 100°C would be harmful to the fungi.

We, however, followed another thread, viz. to experiment with different pH on the agar itself. The tests were conducted on SAB-agar plates with different pH, see "Materials and methods" above. Our results are shown in fig. 8 and 9.

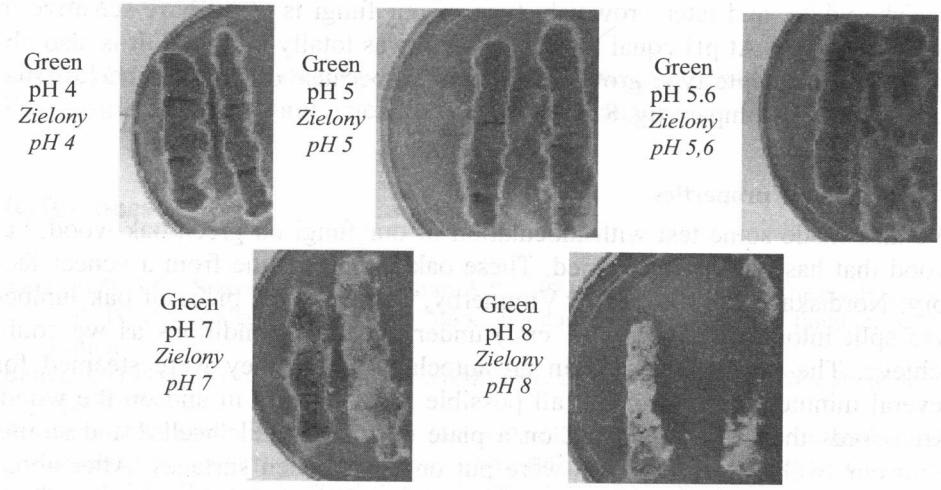

Fig. 8. Green type fungus. Sensitivity for different pH. Images from a representative experiment out of three (photo: Jerker Linné)
Rys. 8. Grzyb typu zielonego. Wrażliwość na różne pH. Zdjęcia z reprezentatywnego eksperymentu jednego z trzech. (fot.: Jerker Linné)

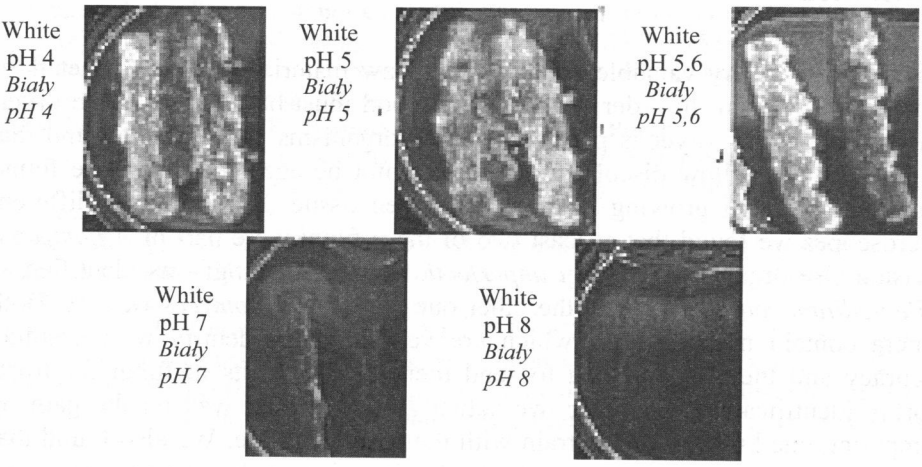

Fig. 9. White type fungus. Sensitivity for different pH (photo: Jerker Linné)
Rys. 9. Grzyb typu białego. Wrażliwość na różne pH (fot.: Jerker Linné)

It is obvious from fig. 8 that the green type fungus, i.e. the *Penicillium roqueforti* is very sensitive to high pH values. At a pH equalling 8 the fungus grows much slower and this is evident also for a neutral agar plate with a pH equal to 7.

The white, and later brownish, type of our fungi is even more sensitive to high pH values. At pH equal to 8 the growth was totally inhibited. It is also obvious that the white type grows much slower, because of smaller and less matured colonies, compare fig. 8 and 9.

Fungal staining properties

We also made some test with inoculation of our fungi on green oak wood, i.e. wood that has not yet been dried. These oak samples came from a veneer factory, Nordiska Fanérfabriken in Vimmerby, Sweden. One piece of oak lumber was split into smaller pieces (1 cm^3) under as sterile conditions as we could achieve. The pieces were put in an autoclave where they were steamed for several minutes in order to kill all possible fungi already in and on the wood. Afterwords the pieces were put on a plate with six small "wells" and strains from our twelve fungi samples were put on the wooden surfaces. After about three weeks we could see that these strains had started to infect the wood and changed the color of the wood. The fungi therefore grew in a very slow pace on wood compared to the agar, but the important thing was that we found that the wood had a significant yellow color change because of the fungal growth.

Conclusions

Oak wood is a very valuable and important raw material for the parquet floor industry in Sweden. In order to use it, the wood must be dried in a kiln dryer. The climate in the dryer is perfect for microorganisms such as fungi and that might result in yellow discolorations that cannot be accepted. We have found a number of fungi growing inside the wooden tissue. By studies in different microscopes we found that at least two of these fungi were part of *Ascomycota* division else organised in *Fungi imperfecti*. One of the fungi was identified as a *Penicillium roqueforti* while the other one was a *Paecilomyces variotii*. Both genera contain many species which are very hard to identify with absolute accuracy and therefore we had to send them to specialists in fungi for trustworthy identification. However, we actually came a long way on the path by comparing one known fungal strain with the unknown type. We also found that these fungi were very sensitive to high pH. Spraying or dipping the oak wood battens already in the sawmill might make life for the fungi so hard that they cannot inhabit the wooden structure. Furthermore, we confirmed that inoculated

fungal strains from our cultivated samples were able to change the color of not dried wood.

Acknowledgements

This work was supported by FORMAS, the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning.

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