FUNGI AND MINERALS OCCURRING IN HEARTWOOD DISCOLORATIONS IN *QUERCUS ROBUR* TREES

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ABSTRACT

The studied *Quercus robur* trees were oaks protected as monuments of nature (200-350 years old) and oaks growing in forest stands (200-300 years old). The most visible symptoms occurring in aboveground organs included defoliation (25-75%), dying of twigs and branches. Discoloured heartwood, obtained with an 80-cm Pressler borer, differed in colour intensity from normal wood and was divided into two groups: stained brown and dark brown. The brown-stained heartwood was a more active site, considering the number of fungi and their ability to produce enzymes (phenoloxidase, pectinase and cellulase) in comparison with the dark-brown-stained heartwood. This brown-stained heartwood contained also statistically more Ca, Mg and Mn.

KEY WORDS: pedunculate oak, trunk heartwood, fungi, enzymes, minerals.

INTRODUCTION

Discoloration and decay of wood are serious defects of timber trees. The development of the discoloration begins with an injury of the tree, e.g. dying or breaking off of branches, attack of insects or other factors, e.g. exposure to outdoor weathering and artificial (UV) light (e.g. Shigo 1972; Shortle and Cowling 1978; Hon and Feist 1986; Grosclaude 1993; Lilja and Heikkilä 1995; Dujesiefken et al. 1998). Discoloration originating as a result of tree response to injuries are colonized by nonhymenomycetous fungi and bacteria (e.g. Tattar et al. 1971; Hallaksela and Niemistö 1998). Decay fungi inhabited tissues earlier changed by those microorganisms, which are able to detoxify the phenolic substances (Shortle and Cowling 1978; Rayner and Boddy 1988; Grosclaude 1993). Fungi most often isolated from discoloured wood of trees e.g. of birch, maple, yellow-poplar, sweetgum and white oak belong to the genera *Phialophora*, *Epicoccum*, *Gliomastix*, *Fusarium*, *Coniothyrium*, *Nodulisporium*, *Ophiostoma*, *Alternaria*, *Trichocladium* and *Cladosporium* (Shortle and Cowling 1978; Shortle 1979; Arnold and Gramss 1992; Przybył 2001).

An increase in inorganic components in discoloured sapwood was reported by many workers. The proportion of inorganic material is higher in discoloured and decayed wood than in normal wood (Shigo and Sharon 1970; Shigo and Hillis 1973; Przybył and Mańka 2000), although its content depends on wood structure (Penninckx et al. 2001). Some studies indicate that mean nitrogen content varied among decayed wood samples but was always higher in decayed wood than in normal wood (Fukasawa et al. 2005). Many investigations on wood discoloration of relatively young trees without and with heartwood (e.g. *Q. rubra* and *Q. alba*), i.e. 20-50 years old, contrast with the rare knowledge about old trees over 200 years old. Poland is one of the few countries in Europe where large numbers old oak stands and monumental trees have survived (Kasprzak 2001). My observations of the condition of old forest stands and monumental trees made in the last three years prompted me to study wood discoloration.

The purpose of this investigation was to identify the fungi occurring in discoloured heartwood of old specimens of pedunculate oak (*Q. robur*) and the possible effects of those fungi on the heartwood by analysing: (1) phenoloxidase (laccase), which is involved in detoxification of phenols, and (2) enzymes that can participate in decomposition of pectin and cellulose. Moreover, mineral concentrations in discoloured and normal wood were also investigated and compared in relation to intensity of discoloration.

MATERIAL AND METHODS

Plant material and wood collection

The studied trees of *Quercus robur* were grown on sites belonging to 19 Forest Districts. Among these trees, 12 oaks protected as monuments of nature were 200-350 years old and 21 oaks growing in forest stands were 200-300 ye-

ars old (Ufnalski, in preparation). The most visible symptoms occurring in aboveground organs included defoliation (25-75%), and dying of twigs and branches. Moreover, the following types of wounds were observed on the trees: broken branches, broken tops, branch wounds and wounds resembling frost cracks.

Samples of stem wood were obtained with an 80-cm Pressler borer 1.3 m above ground. These samples comprised mostly heartwood; the sapwood was composed of only 5-20 youngest annual rings (Ufnalski, in preparation). Discolorations of heartwood (starting most often from pith) were observed on 80% of studied trees. Discoloured heartwood differed in colour intensity from normal wood and was divided into two groups: stained brown or dark brown. Decay of wood was not observed at the studied level of stem.

Fungi isolation

Sections of heartwood (5 mm long) showing brown and/or dark brown discolorations were surface sterilized with the use of 0.5% cupric chloride (1 min) and 70% alcohol (1 min). After rinsing in sterile water and dried in sterile filter paper, the sections were placed in Petri dishes containing 2% (w/v) malt extract agar (MEA; Merck, Darmstadt, Germany). The dishes were incubated at room temperature (22-25˚C). Fragments of the medium with mycelium were transplanted onto fresh MEA immediately after the mycelium appeared, and the fungi were identified as soon as sporulation occurred.

Isolation frequency was defined as the percentage of isolates of individual species in relation to the total number of isolates obtained.

Cellulase and pectinase assay

Cellulolytic and pectolytic enzymes were studied by using the solid media, whose composition in g per litre of distilled water was as follows:

1) for cellulase: medium with carboxymethylcellulose (CMC; Sigma-Aldrich Chemie, Germany, C – 5678, sodium salt, low viscosity) 10, ammonium sulfate 0.5, L – asparagine 0.5, potassium dihydrogen phosphate 1.0, potassium chloride 0.5, magnesium sulfate 0.2, calcium chloride 0.1, yeast extract (Difco) 0.5 (pH 6.2; Kjøller and Struwe 1980);

2) for pectinase: polygalacturonic acid (PA; Sigma-Aldrich Chemie, Germany P-3850, sodium salt) 5 or citrus pectin (CP; pectin from citrus fruit, Sigma-Aldrich Chemie, Germany, P 9135) 5, potassium sulfate 0.5, potassium chloride 0.2, calcium chloride 0.2, magnesium sulfate 0.1, ferrous chloride 0.01 (pH 5; Kjøller and Struwe 1980).

Cellulose degradation was estimated by measuring the light zone around colonies after 1 month of incubation at 10˚C. Pectin degradation was estimated after 7 days of incubation at room temperature by covering the cultures with 1% ethylhexadecyldimethyl ammonium bromide (cetavlon; Sigma-Aldrich Chemie, Germany, 5335) for 30 min.

Ten-day-old fungal cultures were transferred to Petri dishes containing the above-mentioned substrates. Three replicates were made for each isolate. The following scale was used to classify the reactions of the various fungal species to cellulase and pectinase: light zone 1-2 mm (slight reaction, +), light zone 3-4 mm (moderate reaction, ++), light zone 5-6 mm (strong reaction, +++).

Bavendamm's test

Bavendamm's test was used for estimation of phenoloxidase (laccase) production. Fungi were grown on a medium of the following composition: gallic acid (Ubichem, Hampshire, UK) or tannic acid (Sigma St Louis, MO, USA) 5 g, agar 20 g, malt extract (Merck, Darmstadt, Germany) 15 g per liter of distilled water (Kjøller and Struwe 1980; Rayner and Boddy 1988). Three isolates were tested for each fungus. Observations of the intensity of reaction and radial growth of mycelium were made after 7 days of incubation at room temperature (about 22˚C). The following scale was used to estimate the reactions of the various fungal species to gallic and tannic acid: – without brown discoloration (negative), + light brown to brown discoloration formed at the center of mycelium, ++ light to dark brown discoloration formed under mycelium but not extending to the mycelium margin, +++ light to dark brown discoloration extending a short distance (up to 1 cm) beyond the mycelium margin, ++++ dark brown discoloration extending considerably beyond the mycelium margin (1-2 cm), +++++ dark brown discoloration forming a wide zone (over 2 cm) around inoculum, without mycelium growth.

Wood decomposition test

An in vitro decomposition test was carried out to assess the effect of fungi dominating in one or both groups and differing in cellulase and pectinase activity: *C. herbarum* (positive cellulase and pectinase), *P. melinii* (positive cellulase and pectinase), *P. jensenii* (positive cellulase and pectinase), *A. alternata* (positive pectinase), *Cryptosporiopsis* sp. (positive pectinase), *P. rubrum* (positive pectinase), *C. virescens* (negative cellulase and pectinase), *P. funiculosum* (negative cellulase and pectinase) (Tables 1 and 2). The common original weight was determined for five wood blocks (ca. 0.5×1 cm) being one replicate for each species. Four replicates were used for each fungal species. The blocks were inserted into moist sand on Petri dishes (five blocks per one Petri dish) and autoclaved at 120˚C for 30 min. Each block was inoculated with 0.2 ml water suspension of mycelium of the fungi that were grown for 7 days on malt extract agar (MEA; Merck, Darmstadt, Germany). The suspensions exhibited the same density (0.04 at a wavelength of 520 nm, Specord UV VIS Carl Zeiss, Jena, DDR). Control wood blocks were inoculated with 0.2 ml of sterile water. After incubation for 2 months at ca. 23˚C, the wood blocks were removed and cleaned of adhering sand and mycelium. They were then dried at 105˚C to constant weight. Weight losses were expressed as percentage of the original weight (Nilsson et al. 1989). The data were subjected to an analysis of variance and Tukey test (JMP version 4 – Academic AD00007160, USA 2001).

Chemical analyses

The milled wood samples of 11 trees showing discoloration and contiguous normal wood were analysed for total concentrations of the elements N, Ca, Mg, Mn and C.

Nitrogen was determined by a semi-microkjeldahl procedure after "wet" mineralization with sulfosalicylic acid. For analysis of Ca, Mg, Mn samples were digested in a 3:1 (v/v) mixture of nitric and perchloric acid. For Ca a flame photometric procedure was performed, whereas Mg and Mn were determined by atomic absorption spectrophotometry (Zeiss 3, Germany). Organic C was determined by

TABLE 1. Fungi identified in the heartwood showing brown discoloration.

Fungi	Fungi frequency (%)	Enzymes			Bavendamm's test			
		Pectinase		Cellulase	Gallic acid		Tannic acid	
		CP	PA	CMC	Reaction	Growth (mm)	Reaction	Growth (mm)
Acremonium sp.	2.8	–	–	–	–	7.2	–	7.0
Alternaria alternata (Fr.) Keissler	4.4	+	+	–	+	8.4	+	8.0
Aureobasidium sp.	1.6	–	–	–	–	–	–	–
Chloridium virescens (Pers.: Fr.) W. Gams	7.2	–	–	–	–	16.7	–	**2.0***
Cladosporium herbarum (Pers.) Ling ex Gray	17.6	++	++	+	++++	1.9*	++++	4.8*
C. macrocarpum Preuss	2.8	+	–	–	++	2.0*	++	3.3*
Cladosporium sp.	1.2	–	–	–	++++	2.7*	++++	5.7*
Coleophoma empetri (Rostr.) Petrak	1.6	–	–	–	–	3.4	–	4.2
Diplodia sp.	0.4	++	++	–	–	4.2	–	5.2
Endomyces sp.	1.2	–	–	++	+++	2.0*	+++	2.0*
Epicoccum purpurascens Ehrnb. ex Schlecht.	4.4	+	+	–	+	5.8	+	6.2
Gonytrichum caesium C.G. Nees et T.F.L. Nees	7.2	–	+	+	++++	3.5*	++++	3.7*
Hormonema dematioides Lagerb. et Melin	2.4	–	++	–	–	9.5	–	9.8
Hyphomycetes 1	1.6	–	–	–	+++++	0.0*	+++++	0.0*
Hyphomycetes 2	0.8	–	–	–	+++++	0.0*	+++++	0.0*
Mucor mucedo L. ex Fries	1.6	–	–	–	–	9.2	–	9.9
Nodulisporium sp.	0.8	–	–	–	–	3.7	+++++	0.0*
Ophiostoma quercus (Münch) H et P. Sydow	1.6	+	+	–	–	6.5	++++	2.8*
Penicillium daleae Zaleski	0.4	+	+	–	–	7.8	–	6.4
Penicillium granulatum Bain	1.2	–	–	+	–	**7.5***	–	**7.5***
Penicillium jensenii Zaleski	6.8	–	+	+	–	9.0	–	8.5
Penicillium purpurogenum Stoll	0.4	+	+	+	–	7.1	++++	2.0*
Penicillium rubrum Stoll	5.2	+	+	–	–	14.0	–	14.1
Penicillium sp.	0.4	–	–	–	–	7.3	–	7.0
Phialocephala sp.	1.6	+	+	–	–	11.6	+++++	0.0*
Phialophora melinii (Nannf.) Cohant	4.0	+++	+	+++	+	5.3	++	**6.7**
Phialophora sp.	0.8	++	++	–	–	5.5	–	5.9
Sporothrix sp.	0.8	–	–	–	–	6.4	++++	2.7*
Staphylotrichum sp.	1.6	–	+	+	–	17.7	–	26.2
Thielaviopsis sp.	1.6	–	–	+	–	32.3	–	24.8
Trichoderma sp.	2.4	–	+	–	–	6.9	–	7.2
Non-sporulating 1	5.6	–	–	–	+++	4.7*	–	9.0
2	2.4	–	–	–	+++++	0.0*	+++++	0.0*
3	1.6	–	–	–	+++++	0.0*	+++++	0.0*
4	0.8	–	+	–	++++	3.7*	–	6.2
5	1.6	–	+	–	+	13.3	+++	6.8*
Total number of inocula	274							
Number of isolates examined (100%)	250							

Radial growth (mm) on MEA (control): *Acremonium* sp. – 7.4, *A. alternata* – 9.6, *Aureobasidium* sp. – 3.1, *Ch. virescens* – 16.5, *C. herbarum* – 16.0, *C. macrocarpum* – 11.9, *Cladosporium* sp. – 12.2, *C. empetri* – 4.0, *Diplodia* sp. – 4.1, *Endomycetes* sp. – 5.5, *E. purpurascens* – 6.2, *G. caesium* 9.8, *H. dematioides* 10.0, *Hyphomycetes* 1 – 9.0, *Hyphomycetes* 2 – 8.0, *M. mucedo* – 9.6, *Nodulisporium* – 4.0, *O. quercus* – 6.9, *P. daleae* – 9.5, *P. granulatum* – 16.0, *P. jensenii* – 9.8, *P. purpurogenum* – 7.9, *P. rubrum* – 14.2, *Penicillium* sp. – 8.6, *Phialocephala* sp. – 12.0, *Ph. melinii* – 6.0, *Penicillium* sp. – 7.4, *Phialophora* sp. – 6.0, *Sporothrix* sp. – 10.1, *Staphylotrichum* sp. – 27.1, *Thielaviopsis* sp. – 13.0, *Trichoderma* sp. – 10.0, non-sporulating: 1 – 8.2, 2 – 9.1, 3 – 3.9, 4 – 5.9, 5 – 14.8

* white field; the statistical differences (Student's test) in radial growth of mycelium; medium brownig occurred,
* in bold; the statistical differences (Student's test) in radial growth of mycelium without medium brownig,
in bold without asterix; no statistical differences (Student's test) in radial growth of mycelium; medium browning occurred.

the method of Lichterfeld in a modification of Alten. These analyses were carried out at the Department of Horticultural Plant Fertilization, University of Agriculture, Poznań. The data were subjected to an analysis of variance and Tukey test (JMP version 4 – Academic AD00007160, USA 2001).

RESULTS

Fungi isolated from brown discoloration

The 274 samples of brown-stained wood yielded 250 fungal isolates. This group was represented by 36 fungi, although 16 of them could only be identified to species level. Within this group, five of the fungi differing in their culture morphology belonged to non-sporulating fungi. The most common fungus isolated from brown discoloration was *Cladosporium herbarum* (over 40 isolates; 17.6%). Relatively frequent, occurring in 10 – 18 isolates, were: *Gonytrichum caesium* (7.2%), *Penicillium jensenii* (6.8%), non-sporulating no. 1 (5,6%), *P. rubrum* (5.2%), *Alternaria alternata* (4.4%), *Epicoccum purpurascens* (4.4%), and *Phialophora melinii* (4.0%). Twenty-eight isolates occurred in fewer than 7 isolates (3%). Within this subgroup of fungi isolated from brown discoloration, the total frequency of 6 species belonging to genus *Penicillium* were 14.4% (Table 1).

TABLE 2. Fungi identified in the heartwood showing dark brown discoloration.

Fungi	Fungi frequency (%)	Enzymes			Bavendamm's test			
		Pectinase		Cellulase	Gallic acid		Tannic acid	
		CP	PA	CMC	Reaction	Growth (mm)	Reaction	Growth (mm)
Alternaria alternata (Fr.) Keissler	6.7	+	+	–	+	8.5	+	8.3
Aspergillus niger van Tieghem	1.2	+	++	–	–	14.3	–	14.4
Chalara sp.	0.8	–	–	–	–	7.5	–	**5.3***
Chloridium virescens (Pers.: Fr.) W. Gams	1.6	–	–	–	–	18.6	–	**3.3***
Cladosporium herbarum (Pers.) Ling ex Gray	2.4	++	++	+	++++	1.9*	++++	4.8*
Cryptosporiopsis sp.	3.2	+	+	–	–	23.9	–	29.0
Gonytrichum caesium C.G. Nees et T.F.L. Nees	3.2	–	–	+	–	9.9	–	8.5
Humicola sp.	8.5	–	+	–	++	2.0*	+	6.9
Mortierella humilis Linn. et Gams	8.5	–	–	–	–	**13.4***	–	**7.1***
Mortierella isabellina Oudem.	2.8	–	–	–	++	28.0	++	25.0
Mortierella vinacea Dixon-Stewart	0.8	–	–	–	–	**15.3***	–	25.0
Ophiostoma quercus (Münch) H. et P. Sydow	0.4	–	–	–	–	6.5	++++	2.8*
Paecilomyces variotii Bain	1.2	–	–	–	–	**7.0***	–	14.6
Penicillium frequentans Westling	0.8	+	+	+	–	14.3	–	10.3
Penicillium funiculosum Thom	9.4	–	–	–	–	10.3	–	10.0
Penicillium jensenii Zaleski	7.2	–	+	+	–	9.0	–	8.5
Penicillium rubrum Stoll	8.0	+	+	–	–	10.6	–	10.6
Penicillium steckii Zaleski	0.8	–	+	–	–	7.5	–	6.8
Penicillium sp.	1.6	–	–	–	–	7.8	–	6.8
Sordaria fimicola (Rob.) Ces. et de Not.	3.2	–	–	–	–	11.9	–	**6.5***
Trichothecium roseum (Pers.) Link ex Gray	1.2	–	–	–	–	**1.6***	–	**1.0***
Trichoderma pseudokoningii Rifai	1.6	–	–	–	–	15.6	–	10.3
Non-sporulating 1								
Non-sporulating 1	0.8	–	–	–	+++	4.7*	+++	8.2*
5	2.8	–	–	+	+++	**13.3**	+++	**14.8**
6	0.8	–	–	+	–	10.2	+	**6.7**
7	4.4	–	–	–	–	4.5	–	5.8
8	2.4	+	–	+	–	4.7	–	3.4
9	0.8	+	+	–	–	5.7	–	6.2
10 (basidiomycetes)	0.4	–	–	–	+++	0.0*	+++	0.0*
11	12.6	–	–	–	–			
Total number of inocula	204	–	–	–	+	18.9	+	14.8
Number of isolates examined (100%)	223							

Radial growth (mm) on MEA (control): *A. alternata* – Table 1, *A. niger* – 15.0, *Chalara* sp. – 10.1, *Ch. virescens* – Table 1, *C. herbarum* – Table 1, *Cryptosporiopsis* sp. – 26.2, *G. caesium* – 9.8, *Humicola* sp. – 8.3, *M. humilis* – 27.0, *M. isabellina* – 28.4, *M. vinaceae* – 25.0, *O. quercus* – Table 1, *P. variotti* – 15.0, *P. frequentans* – 15.2, *P. funiculosum* – 13.9, *P. jensenii* – Table 1, *P. rubrum* – Table 1, *P. steckii* – 9.8, *S. fimicola* – 13.7, *T. roseum* – 20.0, *T. pseudokoningii* – 16.5, non-sporulating: 1 – Table 1, 5 – Table 1, 7 – 8.8, 8 – 5.1, 9 – 5.5, 10 – 5.8, 11 – 4.9, 12 – 17.4.

* white field; the statistical differences (Student's test) in radial growth of mycelium; medium brownig occurred,
* in bold; the statistical differences (Student's test) in radial growth of mycelium without medium brownig,
in bold without asterix; no statistical differences (Student's test) in radial growth of mycelium; medium browning occurred.

Pectinase activity was shown by 17 sporulating and identified fungal species (47.2%) and 2 non-sporulating fungi (5.5%). Twelve fungi were active in medium containing CP and 18 in medium containing PA. The reaction classified as strong (+++) was shown by *P. melinii* in medium with CP. The majority of studied species showed a low activity in CP as well as in PA (e.g. *A. alternata*, *E. purpurascens*) or in one of those substrates (e.g. *G. caesium* and *P. jensenii*) (Table 1).

Nine species (25.0%) were able to produce cellulase in medium containing CMC. The most active (strong reaction) was *P. melinii* (Table 1).

Besides, 11 species (30.5%) and 15 species (41.6%) showed significant inhibition of radial growth of mycelium together with browning of medium containing gallic or tannic acid, respectively. The maximum inhibition related to the largest discoloured zone (+++++) of the medium was observed in *Nodulisporium* sp. (tannic acid), *Phialocephala* sp. (tannic acid), *Hyphomycetes* nos. 1 and 2 (both acids) and non-sporulating nos. 2 and 3 (both acids). A high phenoloxidase activity (++++) was exhibited also by *C. herbarum*, *Cladosporium* sp. and *G. caesium* in both used substrates; by non-sporulating no. 4 in gallic acid; and by *Ophiostoma quercus*, *Penicillium purpurogenum*, *Sporothrix* sp. in tannic acid. The radial growth of *C. virescens* and *P. granulatum* in the medium containing one of the two used acids was inhibited but no browning was noticed. The growth of *Phialophora melinii* was not inhibited in any of the two phenolic substrates, but in the case of tannic acid the medium browning zone (++) was observed (Table 1).

Fungi isolated from dark brown discoloration

The 204 samples of dark-brown-stained wood yielded 223 fungal isolates. This group consisted of 30 fungi, however 18 of them could only be identified to species level. From dark-brown-stained wood, 26.6% of fungi were non-sporulating. The following fungi occurred most frequently (in over 15 isolates): non-sporulating no. 11 (12.6%), *P. fu-*

niculosum (9.4%), *Humicola* sp. (8.5%), *Mortierella humilis* (8.5%), *P. rubrum* (8.0%), *A. alternata* (6.7%) and *P. jensenii* (7.2%). Twenty two fungi (73.3%) occurred in fewer than 10 isolates (4.4%). In this fungal group, *Penicillium* spp. were found in 27.8%, and *Mortierella* spp. in 12.1% of total number of isolates (Table 2).

Pectinase activity was shown by 11 fungal species (36.6%), of which 8 were active in medium containing CP and 10 in medium containing PA. The activity designated as moderate (++) exhibited *C. herbarum* (in both substrates) and *Aspergillus niger* (in PA).

Cellulase activity was shown by 7 fungal species (23.3%). A slight reaction (+) was observed in *C. herbarum*, *G. caesium*, *P. frequentans*, *P. jensenii* and non-sporulating nos. 5, 6 and 8.

Besides, six fungal species (20.0%) showed browning of medium with significant inhibition of radial growth of mycelium on medium containing gallic and/or tannic acid. In this group of fungi, the greatest inhibition related to the zone of extensive browning of medium (++++) was observed in *C. herbarum* (in both acids) and *O. quercus* (in tannic acid). The growth of some species in gallic or tannic acid was inhibited but no browning was noticed, e.g. *C. virescens* (tannic acid), *M. humilis* (both acids), *M. vinacea* (gallic acid), *S. fimicola* (tannic acid).

Decomposition test

In comparison with the control, only the species *C. herbarum* and *P. melinii* caused significant weight loss of inoculated wood blocks: 7.9% and 8.1%, respectively (Table 3).

TABLE 3. Weight loss of wood caused by tested fungi.

Fungal species	Weight loss (%)
Alternaria alternata	4,4 b
Cladosporium herbarum	7.9 a
Chloridium virescens	5.7 b
Cryptosporiopsis radicicola	5,4 b
Penicillium funiculosum	4,7 b
P. jensenii	6.0 ab
P. rubrum	4.7 b
Phialophora melinii	8,1 a
Control	4,5 b

Means values marked with the same letter do not differ significantly (P<0.001; LS Means Differences Tukey HSD)

Mineral concentrations

The brown-discoloured wood contained significantly more Ca (0.51%), Mg (0.022%), and Mn (57.4 mg/kg^{-1}) in comparison with dark-brown-stained wood and the control (Table 4).

TABLE 4. Concentrations of minerals in trunk heartwood of *Q. robur*.

Minerals	Heartwood discoloration		Normal heartwood (control)
	Brown	Dark brown	
%N	0.45 a	0.36 a	0.39 a
%C	43.9 a	45.7 a	50.8 a
%Ca	0.51 a	0.25 b	0.22 b
%Mg	0.022 a	0.009 b	0.005 b
mg Mn kg^{-1}	57.4 a	35.6 b	40.4 b

Means values marked with the same letter within lines do not differ significantly (P=0.2 for N; P=0.06 for C; P=0.03 for Ca, P=0.04 for Mg; %P=0.03 for Mn; PLS Means Differences Tukey HSD)

DISCUSSION

The discoloration of heartwood in studied *Q. robur* trees can be a consequence of dieback of some aboveground organs and of wounds occurring on trunk. Heartwood contains enzyme systems that can function after injury (Shigo and Hillis 1973). Brown or dark brown heartwood discolorations, differing from the natural colour, were observed on samples obtained from trees by use of a Pressler borer. Hence, in this work it was impossible to observe the columns of discoloration extending from dead branches and wounds, or the discoloration patterns on stem in cross section. The trees were not felled, considering their age and special protection.

The accumulation of minerals in trunk wood depends usually on stages of wood decomposition (e.g. Shigo and Hillis 1973; Krankina et al. 1999). In studied *Q. robur* trees the levels of Ca, Mg and Mn in brown-stained heartwood were statistically higher than in normal or dark-brown-stained wood. In the case of *Acer saccharum*, Shortle and Shigo (1973) found the highest concentration of Mn, responsible for detoxification of phenolic compounds (Shortle et al. 1971), in the interface between decayed wood and discoloured wood. The concentrations of the minerals studied in that paper were statistically higher in heartwood of old *Q. robur* trees showing decay caused by *Phellinus igniarius* (P) and *Fomes fomentarius* (F), as compared with normal and both types of discoloured wood in this study (%N: 1.30 P, 1.65 F;%Ca: 2.12 P, 1.72 F; %Mg: 0.069 – P, 0.13 – F, Mn kg^{-1}: 518.4 P, 299.5 F) (Przybył, unpublished data).

The brown-stained heartwood was a more active site, considering the number of fungal species isolated and their ability to produce enzymes, in comparison with the dark-brown-stained wood. In the brown-stained heartwood, the total number of fungi that are able to detoxify the phenolic substances and degrade wood by production of enzymes (cellulase and pectinase) were higher than in dark-brown-stained wood.

Nine species (e.g. *A. alternata*, *C. herbarum*, *O. quercus*, *P. jensenii*, *P. rubrum*) were common to both types of discolorations, but they differed in frequency (%). *C. herbarum* was the most common fungus isolated from the brown discoloured heartwood. The most known soft rot fungus *P. melinii* (Rayner and Boddy 1988) was isolated only from brown discoloration. This fungus was commonly found in discoloured wood of other tree species, e.g. sweetgum and yellow-poplar, and belonged to persistent colonizers, which grow at gallic acid levels inhibitory to decay fungi and persist in wood by utilizing some cell wall substrates (Shortle et Cowling 1978). Among the fungi that dominated in both types of discoloration, used in the wood decomposition test, only *P. melinii* and *C. herbarum* caused significant weight loss of inoculated heartwood blocks in comparison with the control. Some nonhymenomycetous fungi, e.g. *P. melinii*, *P. bubaki*, and *Cladosporium* sp., were found to cause significant weight loss due to wood decomposition in other tress, e.g. sweetgum wood (Shortle and Cowling 1978). The less enzymatically active *Penicillium* spp. and *Mortierella* spp. were isolated more frequently

from dark-brown-stained wood than from brown-stained wood. From dark-brown-stained wood, the basidiomycete was also isolated (at a frequency of 0.4%). Although any clamp-connexions were not observed in non-sporulating fungi occurring in brown heartwood, it is plausible that certain non-sporulating fungi could be involved in Basidiomycetes.

Generally, these investigations showed that the brown-stained heartwood contained statistically more Ca, Mg and Mn as well as a higher number of enzymatically active fungi than dark-brown-stained wood. This brown-stained trunk heartwood of old *Q. robur* trees could be regarded as a more advanced stage in discoloration and maybe in decomposition process because: (1) the accumulation of minerals in stem wood depends usually on stages of wood decomposition and (2) in agreement with the theory of succession (e.g. Shigo 1972), the pioneer fungi that are not able to produce enzymes followed by fungi active enzymatically.

Further research should be concerned with the identification of non-sporulating fungi in comparison to the commonly known fungi causing heartwood decay of oaks and their ability to decompose wood.

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