WOOD RESEARCH
58 (4): 2013
591-598

WOOD DECAY CHARACTERIZATION OF A NATURALLY-INFECTED OAK WOOD BRIDGE USING PY-GC/MS

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(Received May 2013)

ABSTRACT

Decayed-wood samples were collected from a naturally-infected bridge made of *Quercus robur*. Fruit-bodies of the white-rot basidiomycetes *Hymenochaete rubiginosa* and *Stereum hirsutum* were identified. Presence of *Fuscoporia ferrea* and *Mycena galericulata* was analysed by rDNA-ITS sequencing. The degradation patterns was studied by analytical pyrolysis combined with gas chromatography/mass spectrometry (Py-GC/MS). Relative peak areas were calculated for pyrolysis products arising from carbohydrates and lignin. The pyrograms of control and decayed wood showed the same degradation products but with different quantities. The lignin/carbohydrate ratio increased, whereas the lignin syringyl/guaiacyl ratio decreased. This was due to the preferential degradation of the carbohydrates and syringylpropanoid structures.

KEYWORDS: *Quercus robur*, white-rot fungi, rDNA, Py-GC/MS, lignin/carbohydrate ratio, syringyl/guaiacyl ratio.

INTRODUCTION

Wood degradation occurs through brown-, white- and soft-rot fungi (Eaton and Hale 1993, Schmidt 2006). White-rot predominates in hardwoods (Gilbertson 1980). It is divided into simultaneous white rot and selective delignification (Liese 1970, Nilsson 1988). Soft rot is mainly caused by ascomycetes and deuteromycetes. Characteristic is their preferential growth within the cell wall and the formation of cavities (Findlay and Savory 1954, Liese 1955, Levy 1965). White-, brown- and soft-rot fungi as well as staining fungi have been found in wooden bridges

(Schmidt and Huckfeldt 2011), as also in the currently investigated bridge (Karami et al. 2013a, b). In Germany, the main wood species for decks and railings of bridges is Bongossi followed by oak (Dinger 1997).

Lignin is a large, complex, three-dimensional polymer, composed of three main types of phenyl-propane units, i.e., *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) linked by various types of bonds (Galletti and Bocchini 1995). Analytical pyrolysis combined with gas chromatography/mass spectrometry (Py-GC/MS) is a reliable technique for characterization of lignin and carbohydrate degradation by different fungi (del Rio et al. 2001). Decay of *Eucryphia cordifolia* wood by white- and brown-rot fungi was reported by Mulder et al. (1991). Camarero et al. (1994) investigated degradation of phenolic lignin units by two white rot fungi. Martinez et al. (2001) studied wheat lignin degradation by *Pleurotus* species. Vane (2003) examined the molecular composition of lignin in spruce wood after white-rot decay. Alves et al. (2006) measured the quantification of pyrolysis-lignin of *Pinus pinaster* and *Picea abies* wood samples. Vinciguerra et al. (2007) analysed wood decay of a naturally-infected *Platanus acerifolia*. The present study uses gas chromatography/mass spectrometry to provide information on the structural components of lignin and carbohydrate in naturally-infected *Quercus robur* wood which can help to better understand the fungal degradation of lignin and carbohydrate, and to characterize the degradation pattern.

MATERIAL AND METHODS

Sampling

Samples of about 15 cm length were collected in 2011 from the deck of a bridge made of oak wood (*Quercus robur* L.). The bridge of 18 m length and 2.2 m width crosses the stream Bille between Hamburg and Schleswig-Holstein and was built in 1998 for pedestrians and cyclists (Fig. 1). Some parts of the bridge showed decay and fruit bodies on the deck and railings (Lerche 2010). Fruit-bodies were identified macro- and microscopically. From the inner part of rotten samples, fungi were determined by rDNA-ITS sequencing according to Schmidt et al. (2012).

Pyrolysis–gas chromatography/mass spectrometry

Wood specimens were milled in a cryogenic mill (SPEX CertiPrep Freezer/Mill 6750, 3 cycles, 2 min. each) cooled by liquid nitrogen and portions of accuracy 70–90 μg were weighed. Analytical Pyrolysis was conducted at 50°C with a Frontier Lab Micro furnace Double-shot Pyrolyzer (Py-2020iD) connected with an autosampler (AS-1020 E). The interface of the pyrolyzer was kept at a temperature of 360°C. The pyrolysis products were transferred via a constant helium flow (split 1:15) onto the capillary column (Varian FactorFour VF1701, 60 m × 0.25 mm, 0.25 μm) which was attached to an Agilent 5975C Mass Selective Detector (MSD) and an Agilent Flame Ionization Detector (FID) with the aid of a column splitter. The injection port of the Agilent 6890 GC System was kept at 250°C. Gas chromatography was performed at a flow rate of 2 ml.min^{-1}. The oven programme started off with 4 min at 45°C, rose then with a rate of 3°C.min^{-1} to 280°C and was kept at 280°C for 15 min. For mass selective detection, electron impact ionisation energy was 70 eV, scan rate was 1.41 scans/sec and the scan range adjusted to 15–550 Da. All measurements were performed in TIC (total ion chromatogram) mode. Duplicate analyses were performed in arbitrary order. Identification of the products was carried out using an in-house library, NIST 02 MS library, and literature comparison.

RESULTS AND DISCUSSION

Investigated bridge and fungi

The bridge made of *Quercus robur* wood showed severe degradation after only 13 years in service, particularly in the deck and railings (Fig. 1). Although laboratory tests with basidiomycetes classified European oak according to EN 350-2 (CEN 1994) as a durable timber species (Durability Class 2), this was neither attested by laboratory soil box tests (DC 5) nor in field studies in ground (DC 5) and above ground (DC 4) (Brischke et al. 2009).

The surface of the attacked bridge wood areas showed a thin layer of soft rot. The following four basidiomycetes were identified: *Hymenochaete rubiginosa* (Dicks.: Fr.) Lév., and *Stereum hirsutum* (Willd. Fr.) S.F. Gray were collected as fruit bodies growing on the wood surface. *Hymenochaete rubiginosa* is a common fungus growing on used wood of *Quercus* species. *Stereum hirsutum* occurs on dead hardwoods including oak (Breitenbach and Kränzlin 1986). In addition to the macroscopic analysis, DNA was obtained from the inner portions of decayed wood pieces, the rDNA-ITS amplified by PCR and then sequenced. Sequence comparison with sequences in the DNA databases (BLAST) identified them as *Fuscoporia ferrea* (Pers.) G. Cunn., which produces white-rot in broad-leaved and coniferous trees, and as the secondary saprobiont *Mycena galericulata*. An earlier investigation found *H. rubiginosa* and *S. hirsutum* on the same bridge (Lerche 2010). Dinger (1997) identified two brown-rot fungi and four white-rot species, including *H. rubiginosa* in a study on 84 wooden bridges in Hamburg, *M. galericulata* and *S. hirsutum* were found by Schmidt and Huckfeldt (2011) in several bridges. Fig. 1 shows the investigated bridge.

Fig. 1: Investigated bridge (photo: Lerche 2010).

Py-GC/MS

The degradation patterns of oak wood were studied by analytical pyrolysis coupled with gas chromatography/mass spectrometry. In Fig. 2 the pyrograms of control and degraded oak wood are illustrated.

The fungi causing rot in the decayed sample are unknown. The pyrograms show several major peaks from lignin and carbohydrate breakdown products. The identities of the lignin- and carbohydrate-derived peaks are given by numbers (Tab. 1). The pyrolysis compounds were rather similar in the control and decayed wood, but differences occurred in their relative abundances.

Tab. 1: Semiquantitative analysis of the lignin and carbohydrate components detected by Py–GC/MS of control and degraded wood. Gl = guaiacyl lignin, Sl = syringyl lignin, Ps = polysaccharide.

No	RT	Compound	Control	Degraded wood	*Hymenochaete rubiginosa*	*Stereum hirsutum*	Origin
1	5.10	Propanal-2-one	0.474	0.166	0.0723	1.04	Ps
2	7.68	Hydroxyacetaldehyde	0.583	0.403	0.101	0.567	Ps
3	9.07	Acetic acid	1.15			0.00242	Ps
4	10.39	1-Hydroxy-2-Propanone	0.295	0.0763	0.024	0.521	Ps
5	13.96	? 2-Propenoic acic methyl ester	0.0858	0.0182		0.0202	Ps
6	15.10	3-Hydroxypropanal	0.402	0.182	0.00542	0.079	Ps
7	15.40	Butenal-(3)-2-one	0.102	0.0107		0.0124	Ps
8	15.72	Furan-(3H)-2-one	0.0696	0.019	0.00304	0.015	Ps
9	16.53	Furan-(2H)-3-one	0.0524	0.108	0.139	0.0552	Ps
10	17.23	Butanedial	0.251	0.0455	0.016	0.0873	Ps
11	17.40	2-Hydroxy-3-oxo-butanal	0.292	0.0234	0.112	0.187	Ps
12	18.09	2-Furaldehyde	0.188	0.272	0.0217	0.0873	Ps
13	23.92	2-Hydroxycyclopent-2-ene-1-one	0.0624	0.0587	0.0054	0.245	Ps
14	24.89	Dihydro-methyl-furanone	0.0569	0.0477	0.00276	0.0255	Ps
15	25.38	Isomer of 4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	0.08	0.161	0.0153	0.0436	Ps
16	26.75	2(5H)-Furanone	0.172	0.053	0.0501	0.024	Ps
17	27.84	4-Hydroxy-5,6-Dihydro-Pyran-(2H)-2-one	0.155	0.501	0.00321	0.0124	Ps
18	31.26	Guaiacol, 2-Methoxy-phenol	0.122	0.151		0.00479	GL
19	35.05	? gamma-Lactone derivative	0.21	0.0368	0.000454	0.00193	Ps
20	36.09	4-Methylguajakol	0.0544	0.154		0.0153	GL
21	36.37	unknown	0.077		0.00041	0.00229	Ps
22	36.73	2,4-Dimethylphenol	0.000797	0.00463		0.0129	P
23	40.07	?? 1,4:3,6-Dihydro-alpha-d-glucopyranose				0.0596	Ps
24	40.18	? Furoic acid methyl ester		0.0485	0.00219	0.0403	Ps
25	40.36	unknown Furan derivative	0.103	0.0111	0.164	0.0696	Ps
26	41.54	1,4:3,6-Dihydro-alpha-d-glucopyranose	0.0211	0.0398			Ps
27	41.98	1,5-Anhydro-beta-d-xylofuranose	0.0196	0.195	0.00434	0.0135	Ps
28	42.41	Vinyl guaiacol	0.179	0.315			GL
29	43.42	Eugenol	0.02	0.0289	0.116	0.0423	GL
30	44.23	? 5-Hydroxymethyl-furaldehyde-2	0.0696	0.149		0.0189	Ps
31	44.69	Syringol	0.366	0.278			SL
32	45.25	unknown				0.00481	Ps
33	45.76	Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-	0.0447	0.02			GL
34	46.42	?? 3-Methyl guaiacol			0.349	0.0191	GL
35	47.10	2-Hydroxymethyl-5-hydroxy-2,3-dihydro-(4H)-pyran-4-one	0.138	0.381		0.00468	Ps
36	47.87	Isoeugenol (trans)	0.0973	0.188			GL
37	48.19	1,5-Anhydro-beta-d-xylofuranose	0.0748	0.339		0.0031	Ps
38	48.43	4-Methylsyringol	0.16	0.262			SL
39	48.88	Vanillin	0.0929	0.0753		0.057	GL
40	49.29	Benzofuran derivative					GL
41	49.71	Benzofuran derivative	0.0568				GL
42	51.18	Homovanillin	0.0632	0.0218		0.00223	GL
43	52.05	Acetoguaiacone	0.422	0.105	0.00648		GL
44	52.25	1,6-Anhydro-beta-d-mannopyranose		0.118	0.00433	0.0203	Ps
45	53.57	4-Vinyl syringol	0.0235	0.478			SL
46	53.99	Guaiacyl aceton	0.0694	0.0426			GL
47	54.23	4-(1-Propenyl)-2,6-Dimethoxyphenol (trans)	0.003	0.0746	0.339	0.00994	SL
48	55.12	1,6-Anhydro-beta-d-mannopyranose	0.669	0.0633			Ps
49	57.42	unknown		0.0856	4.29	0.809	Ps
50	57.93	Levoglucosan	0.273	2.22		0.00957	Ps
51	58.29	4-(1-Propenyl)-2,6-Dimethoxyphenol (trans)	0.307	0.402		0.0015	SL
52	59.47	Syringaaldehyde	0.0787	0.163			SL
53	60.98	Homosyringaaldehyde	0.153		0.00126	0.00698	SL
54	61.81	Acetosyringone	0.273	0.157		0.0154	SL
55	62.38	Coniferyl alcohol (trans)	0.0682	0.116			GL
56	63.09	Coniferyl aldehyde	0.0768	0.0231	0.508	0.00315	GL
57	63.25	Syringylacetone	0.0567	0.0838			SL
58	68.16	Sinapylalcohol (cis)	0.273	0.0072		0.00137	SL
59	70.97	Sinapyl alcohol (trans)	0.25	0.0141			SL
60	71.34	Sinapaldehyde		0.0692			SL

Fig. 3 shows the pyrograms of fruit bodies. Both fungi produce white-rot in oak. There were however differences in their pyrograms. Remarkable are the higher peaks of compounds 50 (levoglucosan) and 57 (syringylacetone) in the *H. rubiginosa* pyrogram compared to *S. hirsutum*. When compared with the decayed sample (Fig. 2 right), the heights of the peaks were generally smaller.

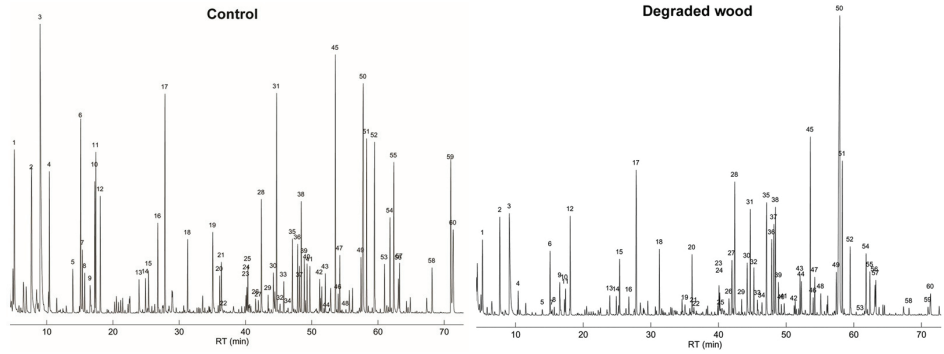

Fig. 2: Pyrograms of control and degraded oak wood.

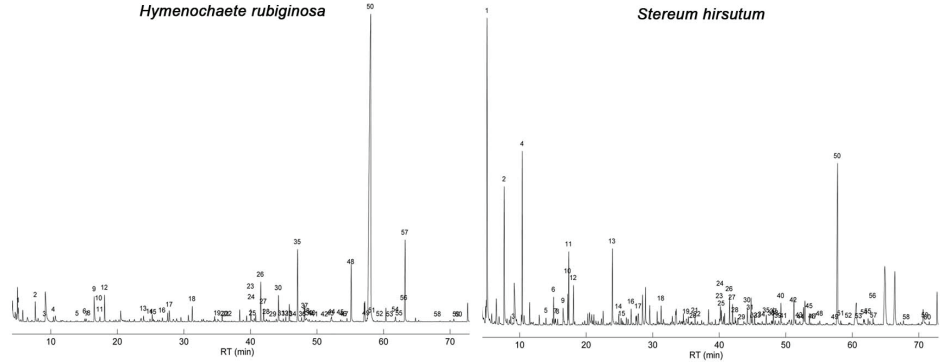

Fig. 3: Pyrograms of fruit bodies of Hymenochaete rubiginosa and Stereum hirsutum.

Tab. 1 shows the components deriving from lignin and carbohydrate detected by Py–GC/MS.

Various white- and soft rot fungi showed a decrease of S/G ratios during degradation (Faix et al. 1991, del Río et al. 2001, 2002, Martınez et al. 2001). Because of its higher condensation degree, G-lignin is more resistant to fungi than S-lignin (Lewis and Yamamoto 1990; Faix et al. (1985)). Preferential removal of lignin was found by del Río et al. (2001), indicating the selective type of white-rot. Tab. 2 shows the S/G- and lignin/carbohydrate ratios of control and degraded oak wood.

Tab. 2: Syringyl/guaiacyl and lignin/carbohydrate ratios of control and degraded wood.

Sample	Syringyl/guaiacyl ratio	Lignin/carbohydrate ratio
Control	1.9	0.4
Degraded wood	1.4	0.5

Our Py–GC/MS results revealed an increased lignin/carbohydrate ratio, indicating the preferential degradation of carbohydrate and a decrease of S/G ratio. This corresponds to the GC/MS results with soft-rot fungi by del Río et al. (2001). However, further fungi may have been present in our samples. Schwarze (1995a) discussed for *Inonotus hispidus* the influence of aeration in the wood on the rot type, causing either soft or white rot. Also the basidiomycete *Meripilus giganteus* (Schwarze and Fink 1998) showed a soft-rot decay pattern. Worral et al. (1997) presumed that white rot and brown rot fungi have arisen from the soft-rot fungi and so they still possess some genes, which enable them to cause a soft rot under certain conditions.

CONCLUSIONS

The Py-GC/MS study as well as our electron microscopic and UMSP investigations (Karami et al. 2013a, b) on the naturally rotten oak-wood bridge revealed the presence of white- and soft rot. White-rot fungi were detected by fruit-bodies and by their DNA in the rotten wood. Soft-rot was only microscopically proven. A decreased syringyl/guaiacyl ratio can occur both in white and soft rot decay. It remains therefore open, whether the increased ratio of lignin to carbohydrate was caused by one of the identified basidiomycetes or by not detected soft-rot fungi. Pyrolysis-GC/MS has proven to be a valuable analytical tool to analyse fungal degradation of lignocellulose.

ACKNOWLEDGMENT

The support by Dr. Dietrich Meier and Dr. Jürgen Odermatt is gratefully acknowledged.

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