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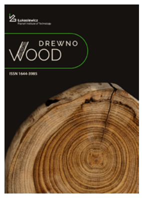

The Resistance of Subfossil Heartwood of Oak (Quercus Robur L.) from Two Sites in Poland to Destruction by Subterranean Termites

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The resistance of subfossil heartwood of European oak (Quercus robur L.) was examined in experiments conducted in accordance with the ASTM D 3345-08: 2009standard. The subfossil heartwood came from Dołhobrody (5100 +/-50 BC) and Pułtusk (13th–14th century). Subfossil heartwood of Q. robur is more susceptible to deterioration by subterranean termites than the recent heartwood of this species. The average weight losses of subfossil heartwood of oak were 0.306 g (Dołhobrody) and 0.475 g (Pułtusk). The average degree of damage to the subfossil heartwood was at the level of moderate attack, penetration. The differences in the degree of damage and weight losses of wood blocks for subfossil heartwood from Dołhobrody and Pułtusk were statistically insignificant. Termite mortality was slight grade (Pułtusk) or slight/moderate grade (Dołhobrody).

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Introduction

The heartwood of European oak (*Quercus robur* L.) is considered to be durable wood and has high resistance to biotic and abiotic degradation factors (Wagenführ and Scheibler 2007). The heartwood of this species can survive for many years in relatively good condition in water or moist soil. Such wood is also called waterlogged oak wood or "black oak" and is often found in archeological excavations in Europe [Dzbeński 1971, Kokociński 1999, Reinprecht 1999, Babiński et al. 2011, Kránitz et al. 2012, Mańkowski et al. 2016]. Black oak was also used in past centuries in the production of furniture, table cutlery, flooring, and luxurious construction joinery. The heartwood of oak is rated as medium durable (M) [PN-EN 350: 2016-10] against degradation by termites. Much work has been done on the chemical and mechanical properties of archeological oak wood. Some of these studies have concerned archeological oak from Poland [Dzbeński 1971, Babiński et al. 2011, Kránitz et al. 2012, Mańkowski et al. 2016]. The influence of the structure [Dzbeński 1971, Kokociński 1999, Kránitz et al. 2012, Mańkowski et al. 2016], humidity, and lignin, holocellulose and ash compound contents [Kúdela and Reinprecht 1990, Reinprecht 1999] on the mechanical properties of fossil oak wood has been investigated [Kúdela and Reinprecht 1990, Reinprecht 1999]. There are fewer publications on the biodegradation of archeological oak wood. These publications deal with abiotic conditions as well as bacteria and fungi [Singh 2012]. The most numerous are publications on the conservation of waterlogged oak wood [Hoffman 1981, Jenssen and Murdock 1981, Keene 1981, McCawley et al. 1981, Murray 1981, Schweingruber 1981,

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Watson 1981 and 1984, Drocourt and Morel-Deledalle 1984, Hug 1984, Jagielska 2004, Babiński et al. 2011]. Among them are publications concerning the freeze-drying method. They show the results obtained in the archeological conservation of oak wood in terms of physical and mechanical properties.

The problem of termites has received much attention from researchers in Europe, tracking its spread and persistence in some cities, and the effectiveness of eradication methods. European termite sites include Atlantic regions of France and Portugal, Italy and the Mediterranean regions of Spain and France, and regions on the Adriatic and Black Sea coasts. Among the northernmost European cities where termites have been found are Hamburg, Munich, Mannheim, Hallein, Vienna–Schönbrunn, and Berlin [Weidner H. 1954, Becker 1970, Becker and Kny 1977, Seelensschlo 1988]. The spread of subterranean termites in Great Britain is of particular significance [Laine 2002].

Archeological waterlogged wood is sometimes buried in moist sand or soil prior to proper conservation to prevent degradation [Babiński 1999]. There have been periods in the history of Europe when tangible cultural goods had to be moved from museum exhibitions and museum warehouses to alternative locations (for example, during the Second World War). These were most often shelters in basements. Under these conditions, termites may be a problem in some European cities. Subterranean termites can pose a threat to waterlogged wood, and in particular to subfossil heartwood of European oak from archeological finds, and other artifacts that contain this material. The freeze-drying method, despite certain reservations, has been used for the conservation of many archeological objects in the past half-century [Keene 1981, McCawley et al. 1981, Murray 1981, Schweingruber 1981, Watson 1981, Amoignon and Larrat 1984, Drocourt and Morel-Deledalle 1984, Hug 1984, Jagielska 2004]. Some facilities did not use PEG or other chemicals in addition. The slow-drying method has also been successfully used [Jenssen and Murdock 1981]. The authors found no published study evaluating the resistance of subfossil European oak, freeze-dried or slow-dried, to deterioration by subterranean termites. The purpose of this paper is to provide information on this topic.

Materials and methods

The experiments were conducted in accordance with the procedure defined in ASTM D 3345-08 [2017] for testing the durability of wood and wood-based materials against subterranean termites.

The heartwood of subfossil European oak (*Quercus robur* L.) coming from two stands (archeological sites) was used for the tests. The first site was Dołhobrody (5100 +/-50 BC) on the Bug river, and the second was the castle hill in Pułtusk (13th–14th century). Additionally, recent sapwood of Scots pine (*Pinus sylvestris* L.) was used in the experiment as a reference material, as required by ASTM D 3345-08: 2009.

Subfossil oak and Scots pine wood was used to make seasoned samples of 7% ± 1% moisture content. Blocks with dimensions of 25.4 x 25.4 x 6.4 mm were made from subfossil oak from Dołhobrody and sapwood of Scots pine. Blocks with dimensions of 18.8 (+/-0.4) x 20.9 (+/-0.4) x 8.6 (+/-0.4) mm were made from subfossil oak from Pułtusk. The choice of these dimensions results from the shape of the available wood from that location.

The dry state of the wood was achieved by the freeze-drying method. The dry wood density of subfossil European oak was 0.54 g/cm^3 (Dołhobrody) and 0.64 g/cm^3 (Pułtusk). The dry wood density of Scots pine was 0.51 g/cm^3.

Each block was placed individually on the bottom of a glass vessel and sprinkled with 200 g of river sand, which was sieved, washed and thermally sterilized. The volume of the glass was 450 ml. The amount of water used to moisten the sand in the testing container was then reduced by 7% of the saturation point of the sand. Each glass was filled with 1 ± 0.05 g of termites. The termite species used was *Reticulitermes lucifugus var. santonensis* de Feyteaud. This name is considered by many entomologists to be a synonym for *R. flavipes* Kollar. The termites were collected from a laboratory culture at Warsaw University of Life Science, Institute of Wood Science and Furniture, Department of Wood Science and Wood Preservation. Pseudergates accounted for over 90% of the individuals in each glass. The containers with wood blocks and termites were placed in an incubator at 27 °C for four weeks. The water content of the testing containers was replenished weekly.

Approximate termite mortality was estimated after four weeks according to the procedure of ASTM D 3345-08 [2017] on a scale of slight (0–33%), moderate (34–66%), heavy (67–99%), and complete (100%).

The wooden blocks were weighed after being removed from the containers and cleaned to remove termites and sand. The blocks were photographed and reweighed after freeze-drying. The degree of damage to the wood blocks was classified visually based on the rating recommended in ASTM D 3345-08 [2017]: 10 – sound, surface nibbles permitted; 9 – light attack; 7 – moderate attack, penetration; 4 – heavy; 0 – failure. In ambiguous cases, the intermediate value was recorded: 10/9 = 9.5, 9/7 = 8, 4/0 = 2.

The average degree of block destruction was calculated for each category of wood (species and stands). The block weight losses and the average weight losses for the individual wood categories were also calculated.

The significance of the difference of obtained average results was verified statistically. Chebyshev's inequality was used to evaluate the significance of the average degree of destruction and average weight losses for subfossil oak and Scots pine. If the absolute value of the difference of arithmetic mean values for both wood species was greater than or equal to three times the standard error of the difference, then the difference of mean values was recognized as statistically significant.

Chemical analyses were also performed for quantitative determination of cellulose, holocellulose, lignin, and ash content. The wood material was fragmented on a mill (Retsch SM 200) and sorted on shives. The chemical analysis used material from 0.43 to 1.02 diameter. Before analysis the material was extracted in a Soxhlet apparatus with a mixture of chloroform and ethanol, 93:7 by volume [Antczak et al. 2006] for 10 h. Cellulose was obtained by the Kürschner–Hoffer method (4 cycles) [Kürschner and Hoffer 1929], and holocellulose was determined according to Wise et al. [1946] (5 cycles). Mineral substances were obtained in accordance with PN-92/P-50092 on the dust fraction (under 0.43 mm), and lignin was determined according to the ASTM D 1106-96 [2013] standard. All measurements were performed in three replicates per sample.

Results and discussion

The average weight losses of subfossil heartwood of oak were 0.306 g (Dołhobrody) and 0.475 g (Pułtusk). The average degrees of damage to the subfossil oak heartwood were at the level of moderate attack, penetration. Termite mortality was graded as slight (Pułtusk) or slight/moderate (Dołhobrody). The results of the test of the possibility of termites feeding in the heartwood of subfossil European oak and sapwood of Scots pine are presented in Table 1 and Fig. 1.

Table 1. Block weight losses, degree of damage to wood blocks, and termite mortality

No of block	Block weight losses [g]	Degree of damage of the wood block	Termite mortality [%]
heartwood of subfossil Quercus robur from Dołhobrody			
1	0.51	7	slight
2	0.37	7	slight
3	0.14	7	moderate
4	0.18	7	moderate
5	0.33	7	slight
average	**0.31**	**7**	
heartwood of subfossil Quercus robur from Pułtusk			
1	0.52	4	slight
2	0.47	7	slight
3	0.39	7	slight
4	0.47	7	slight
5	0.52	7	slight
average	**0.47**	**6.4**	
sapwood of contemporary Pinus sylvestris			
1	0,87	0	slight
2	0,54	4	slight
3	0,75	4	slight
4	0,56	0/4=2	slight
5	0,83	0	slight
average	**0,71**	**2**	

Fig. 1. Condition of wood samples after 4 weeks of feeding by termites: top – heartwood of subfossil *Quercus robur* from Dołhobrody; center – heartwood of subfossil *Quercus robur* from Pułtusk; bottom – sapwood of contemporary *Pinus sylvestris*

Table 2. Statistical verification of the results

Variants of the experiment	Absolute value of the difference of arithmetic average values to both category of wood	Triple value of standard error of the difference	Evaluation
on the basis of degree of damage to the wood block			
subfossil oak Dołhobrody and-subfossil oak Pułtusk	0.6 <	1.8	statistically insignificant difference
subfossil oak Dołhobrody and contemporary sapwood of Scots pine	5.0 >	2.7	statistically significant difference
subfossil oak Pułtusk and contemporary sapwood of Scots pine	4.4 >	3.2	statistically significant difference
on the basis of weight losses of wood block			
subfossil oak Dołhobrody and-subfossil oak Pułtusk	0.169 <	0.214	statistically insignificant difference
subfossil oak Dołhobrody andcontemporary sapwood of Scots	0.404	0.287	statistically significant difference
subfossil oak Pułtuskand recent sapwood of Scots pine	0.235	0.217	statistically significant difference

Table 3. Contents of holocellulose, cellulose, lignin and ash

The origin of subfossil oak	Content of extractives [%]	Content of holocelulose [%]	Content of cellulose [%]	Content of hemicellulose [%]*	Content of lignin [%]	Ash content [%]
Dołhobrody	14.3	52.4	42.4	10.0	31.2	2.1
Pułtusk	11.9	54.1	44.8	9.3	31.0	3.0

The statistical verification of the results obtained is presented in Table 2.

The difference in the degree of damage to the wood blocks and weight loss of wood blocks for subfossil heartwood from Dołhobrody and Pułtusk proved statistically insignificant. The differences relative to recent sapwood of Scots pine are statistically significant.

The results of chemical analyses of the wood used in the tests are presented in Table 3.

Both examined oaks have a similar chemical composition. Particular attention is drawn to the low content of hemicelluloses, which is around 10%. Such a low content of this polysaccharide is unusual for native wood, where the content of hemicelluloses is reported to exceed 30% [Geffertová et al. 2006]. In the case of waterlogged wood, this is easily explained. The reduction in the content of polysaccharides with a low degree of polymerization is explained by the action of bacteria causing degradation mainly through erosion and tunneling, and by the interference of cavitation bacteria. This process of deterioration occurs very slowly and results in the gradual decline of cellulose and hemicelluloses content [Helms et al. 2004, Kim and Singh 2000]. In addition, the humid environment of the wood may lead to leaching of water-soluble compounds and the transfer of compounds from the environment to the wood (increased ash content).

Because the chemical composition affects the properties of waterlogged wood [Hoffman 1981], chemical analyses of the subfossil heartwood of European oak were very important in this study. Termites have endosymbionts in the digestive system [König and Fröhlich 2013], which may lead to reduced polysaccharides, increased lignin and ash, and foreign microorganisms [Schultze-Dewitz and Unger 1972]. It seems that termite mortality may be influenced by the content of chemical compounds derived from lignin [Kartal et al. 2004, Rana et al. 2010] and ash. These can negatively affect termites' endosymbionts, which they need in order to live [Rana et al. 2010, König et al. 2013]. It is important to compare the resistance of recent heartwood of European oak to termite attack.

In earlier studies [Krajewski et al. 2019] the authors determined the degree of damage to modern oak wood by termites, obtaining values for the degree of damage as 8, 9.5, 9.5, 10, 9 (average 9.2), which is above the "light attack" level and approaches the level of 10, "sound, surface nibbles permitted." Consequently, analysis of modern oak was not included in the present study. Termite mortality was at the heavy (67–99%) and complete (100%) levels. This indicates that prolonged presence in a very wet environment reduced the durability of subfossil heartwood of European oak against attack by subterranean termites.

The lignin content in heartwood of recent oak (*Q. robur*) is between 14.78–18.18% [Kolář et al. 2014] and 21.54% [Reinprecht 1999]. In subfossil heartwood of European oak in different locations it may have different values, for example between 29.0% (Zelená Voda) and 33.32% (Gabčikovo) [Reinprecht 1999], between 19.96% (Osek 945–405 BC) and 33.72% (Tovačov 2490–2190 BC) [Kolář et al. 2014]. A 31% content of lignin in subfossil heartwood of oak is therefore a large value; the content of lignin in subfossil heartwood of oak from Dołhobrody and Pułtusk was almost twice as large as in recent heartwood of oak (*Q. robur*). Termite mortality was moderate or slight in this case. The lignin content in Neolithic beech wood, where termite mortality was complete, was 2.4 times higher than in the wood of modern beech [Krajewski et al. 2015]. In the case of subfossil heartwood of European oak from Dołhobrody and Pułtusk, the content of lignin was about 1.5–2 times higher than in modern wood, and did not differ significantly from the values reported for objects from Central and Eastern Europe.

The percentage ash content in recent heartwood of European oak is reported at 0.15% [Kokociński 1999], 0.19–0.55% [Kolář et al. 2014], and 0.59% [Reinprecht 1999]. The ash content in subfossil heartwood may differ substantially depending on the age of the wood, its zone (inner/outer), and environmental conditions: 1.21% (Wisłoka valley) and 1.49% (Bóbr valley) [Kokociński 1999], 1.24% (Zelená Voda) and 2.51 (Gabčikovo) [Reinprecht 1999], 1.57–2.61% (Tovačov 2490–2190 BC), 1.37–2.15% (Osek 945–405 BC), 1.14–2.62% (Tovačov 265–50 BC), 1.90–2.47% (Osek 208 BC – 137 AD), 1.81–2.45% (Tovačov 168 BC – 214 AD) [Kolář et al.

2014], for Dołhobrody (7100 +/- 50 years) 1.11% (outer) to 3.50% (inner) [Kránitz et al. 2012], and 1.42–1.86% (Płońsk, 12th century) [Mańkowski et al. 2016]. In the subfossil heartwood of European oak studied here, the ash contents were 2.1% (Dołhobrody 5100 +/- 50 BC) and 3.0% (Pułtusk 13th–14th century), and therefore did not differ much from the values reported for subfossil oak from the aforementioned locations. This factor did not reduce the destruction of wood by termites and did not result in any significant mortality.

Hart and Hillis (1972) demonstrated that tannins were responsible for oak heartwood durability, and therefore their content, as well as their qualitative variability, is significant and influences the ultimate natural durability of a wood specimen [Baar et al. 2019]. Wood durability is positively correlated with the presence of specific extractives, especially phenolic compounds, and some studies have confirmed this in the case of oak heartwood [Hart and Hillis 1972, Aloui et al. 2004, Guilley et al. 2004, Karami et al. 2014]. The natural durability of oak wood deposited underground for a long duration may be expected to be significantly lower due to the loss of toxic tannins caused by leaching or by deactivation by soil components [Baar et al. 2019]. Many authors have reported high variability in the composition of oak wood's main components [e.g. Bednar and Fengel 1974] and note that it is mainly deposition conditions, rather than time alone, that determine the mechanism and rate of wood degradation [Krutul and Kocoń 1982, Baar et al. 2019]. For example, subfossil oak samples exposed to degradation by *Serpula lacrymans* (Wulfen) J. Schröt exhibited a mass loss of 4.9%, which is twice as high as in recent oak, but is still low [Horský and Reinprecht 1986, Baar et al. 2019]. Subfossil oak has also been found to have reduced resistance to two brown rot fungi (Poria placenta (Fr.) Cooke and Laetiporus sulphureus (Bull.) Murrill) and to the white rot fungus Trametes versicolor (L.) Lloyd [Baar et al. 2019].

Unfortunately, publications referring to foraging by termites in waterlogged wood are sporadic and do not concern subfossil oak heartwood. The average degree of damage to Neolithic waterlogged beech wood (*Fagus sylvatica* L.) by subterranean termites was 0–2 (failure–very heavy). However, termite mortality was complete in that case [Krajewski et al. 2015]. The contents of wood components were holocellulose 28.1%, lignin 52.6%, extractives 1.76%, ash 3.4%. Thus, the content of polysaccharides was low, with a relatively high lignin content. In the case of the examined subfossil heartwood of oak, the holocellulose content was much higher, at 52.4% and 54.1%.

The ash content in the wood of Neolithic beech, where termite mortality was complete, was over four times that of modern beech wood and over eight times that of whitewashed wood of modern pine. The examined subfossil heartwood of oak contained 4–10 times (Dołhobrody) or 5–15 times (Pułtusk) more ash. Subfossil oak trunks go through a process of fossilization, where the types of inorganic substances that replace organic substances depend on the subsurface environment; usually calcification or silicification takes place [Fengel and Wegener 1989, Florian 1990, Baar et al. 2019]. According to Horský and Reinprecht [1986], the presence of mineral salts (based on Cu, Zn, As, Sn, or B) that are partly toxic to organisms can also positively influence the natural durability of subfossil oak. An enormous increase in iron content is linked to a high content of tannins, which are excellent chelators of metal ions [Baar et al. 2019]. With iron, they form blue-black complexes, which are largely insoluble in water [Mila et al. 1996]. Thus, the termite mortality rate may be influenced by the type of chemicals contained in the ash. Unfortunately, the authors did not investigate the ash composition. In the absence of a qualitative analysis of the ash, it is difficult to discuss further. The presence of certain microorganisms in the wood may also have an influence on termite mortality, which was also not investigated here. It is doubtful, however, that the microorganisms would survive freeze-drying.

Low resistance of subfossil oak heartwood to termite feeding may therefore be caused by a relatively higher content of lignin, high content of some mineral compounds, and degradation and leaching of tannins. The decrease in tannin content caused partly by leaching, but mainly by slow hydrolysis and the transformation of tannins into relatively ineffective ellagic acid made the greatest contribution to reduced resistance not only to fungi [Horský and Reinprecht 1986, Baar et al. 2019] but probably also termites. It has been clearly shown that subterranean termites can enter and feed on subfossil heartwood of European oak, at least in some cases. The termite plague is very difficult to control in European cities [Weidner 1954, Seelensschlo 1988, Ferrari et al. 2011]. Due to climate change, the role of phytophagous thermophilic species has increased, mainly as a result of their range shifting to the north and to higher altitudes [Jaworski and Hilszczański 2013]. Subterranean termites therefore also have an increased chance of colonizing buildings in major European cities beyond their current natural range. Therefore, under favorable conditions, they can potentially also cause degradation of subfossil heartwood of European oak.

Conclusions

Subfossil heartwood of *Q. robur* is more susceptible to deterioration by subterranean termites than the recent heartwood of this species. Subterranean termites may be a threat to objects made of subfossil heartwood of oak stored in conditions that are conducive to the development of Isoptera.

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