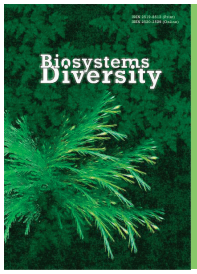

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Biochemical markers of vital biodestruction in common oak (*Quercus robur*)

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The wood of the common oak (*Quercus robur* L.) has high mechanical strength, elasticity and resistance to fracture. However, constitutional stability is not always able to provide the plants with reliable protection from wood-decay fungi, and the initial stages of biodegradation are difficult to determine. Therefore, this study concerns research on appropriate biochemical markers for early diagnostics of wood defects. The total content of phenolic compounds in leaves and wood was determined by a spectrophotometer Optizen Pop using Folin & Ciocalteu's phenol reagent; the flavonoid content in leaves – by adding solutions of aluminum chloride and sodium acetate to methanolic extracts; catechins content – by the reaction with vanillin reagent; the concentration of phenolic antioxidants – by Brand Williams; chlorophyll and carotenoids' contents in leaves – by the formula for methanol extracts; the qualitative composition of phenolic compounds – by high performance liquid chromatography and highly effective thin-layer chromatography. During the planned felling of oak trees on the territory of the Boyar Forest Research Station, trees were found with signs of brown streak and biodestruction of wood. Brown streak in wood is caused by a polycondensation of phenolic compounds, which are deposited on the internal surfaces of tracheal elements. In cases of an increase in the total amount of oxidized polyphenols, the cell walls are also stained. Active oxidation processes in wood have a systemic nature for the plants and affect the physiological state of the assimilation apparatus. We determined that in leaves of the trees with signs of brown streak the total phenol content increases in comparison with the control by 1.6 times, as well as flavonoid and catechin content. Our research has shown that the complex of plastid pigments in common oak leaves does not significantly change in the early stages of destructive processes. Increase of brown streak and appearance of rot in wood are associated with slight increase in chlorophyll a to b ratio in leaves. Chromatographic profiling of the leaves showed that the presence of brown streak changes the content of individual phenolic compounds. The trees with brown rot have more substances with UV spectrum characteristic for kaempferol glycosides compared to the control. The results have shown that the biochemical profiles of the trees with signs of brown streak and brown rot differ from the control by the composition of low and medium polar compounds. The absence or presence of some individual phenolic components and their ratio in the leaves are considered as biochemical markers of hidden wood defects.

Keywords: common oak; rot; xylem; phenolic compounds; markers.

Introduction

The wood of common oak (*Quercus robur* L.) is highly valued for its high mechanical strength, elasticity and resistance to destruction. These properties are mainly provided by the structure of xylem cells, as well as by a complex of chemical compounds that is a part of cell walls.

Masson et al. (1995) have shown that plants of the genus *Quercus* are characterized by high variability of biochemical content. In addition, the distribution and content of ellagitannins (the main phenolic compounds) has a spatial and age-related dependence. At the same time, according to their data, the tannins' composition practically does not depend on the sampling height. The variability of wood's biochemical composition is more related to wood ageing and the ratio of basic xylem tissues. Destruction of common oak wood,except for mechanical destruction, is accompanied by a biochemical transformation of tissues. First of all, it is associated with ageing processes, oxidative degradation (Zaprometov, 1993), auto-fermentation with the formation of important biopolymers for the plant (Volynets, 2013), or as a result of its hydrolysis, which leads to the appearance of oligomeric ellagitannins from the products of prepolymerization, also with enzymatic activity of wood-decay fungi (phenol heterosidase, etherase, depsidase) with the conversion of heterosidic phenolic structures such as coumarins and hydrolyzed tannic compounds. Moreover, fungal invasion is often accompanied by appropriate protective reactions in plants. As a result, the enzyme systems of phenylpropanoid synthesis are activated, and the total pool of oxycinnamic acids and p-hydroxycinnamic alcohols increases. Under the impact of oxidase, they are polymerized with the formation of lignin precursors, which are an important part of induced plant protection.

A significant amount of polar components contained in wood cells in cases of contact with water rapidly diffuses and can be transferred by the xylem and phloem transport system. Mainly this concerns tannins (Moutounet et al., 1989; Puech et al., 1990; Viriot et al., 1993). These compounds can be accumulated in large quantities in the heartwood, and sometimes they may represent up to10% of the dry material. Among the water-soluble compounds, eight ellagitannins were found: castalagin and vescalagin (Mayer et al., 1967), grandinin (Nonaka et al., 1989), and roburins A-E (Herve du Penhoat et al., 1991). In studies of Nonaka et al. (1990) the castalagin and vescalagin structures, first proposed by Mayer et al. (1971), were revised. Castalin and vescalin have also been classified as ellagitannins, despite fact that they do not release ellagic acid under hydrolysis.

Phenolic compounds in wood and leaves perform quite different functions. Under stress conditions the intensity of respiration and associated oxidation and reduction processes are increased in plants. This is

accompanied by a formation of reactive oxygen species and free radicals, which causes lipid peroxidation (LPO). In the conditions of general decrease in the antioxidants pool, which neutralize the negative effect of free radicals, the prooxidant-antioxidant balance in cells is violated.

The effects of oxidative stress are neutralized by non-enzymatic antioxidant system, which includes phenolic compounds (flavonoids, tannins, conjugates of oxycinnamic acids, catechins, etc.) (Zaprometov, 1993). In cases of plant infections, they perform protective functions, are components of chemical and tissue barriers, affect the cell regeneration processes. Polyphenols are characterized by the ideal chemical structure for neutralization of oxygen radicals. They are highly active donors and acceptors for protons and electrons that allow them to stabilize free radicals and take part in chelating of transition metal ions (Fenton reaction). In particular, flavonoids can reduce the kinetic energy of LPO reactions and regulate flow in cell membranes. Common oak plants are able to synthesize a significant amount of phenols in vegetative and generative organs, which are essential for formation of systemic plant resistance. Thus, common oak bark contains 0.04–0.08% of ellagic acid – a terminal product in ellagitannins synthesis (Gudzenko, 2013).

However, constitutional stability, one of whose components is a phenolic compounds complex, is not always able to provide plants with reliable protection from wood-decay fungi. At the initial stages, it is difficult to determine the biodegradation processes and defects in wood. The development of a methodology for timely detection of hidden tree defects for use by forestries has a practical and economic value. The reconstructing of secondary metabolism in plants under the impact of fungal invasions does not exclude the formation of specific biochemical profiles that could be considered as a potential marker signs. Active transport of elicitors (oligosaccharides, glucans, chitosans), which are formed during the cell walls fragmentation process in plants and fungi, provides the beginning for appropriate physiological reactions, particularly in secondary metabolism, which can potentially be identified even in spatially distant metamers.

The purpose of this work was to conduct the biochemical profiling of leaves and wood in plants with different defects in order to identify potential markers for its early diagnostics.

Materials and methods

The plant material of common oak leaves and wood with signs of brown streak and brown rot was investigated in the areas of the NULES of Ukraine at Boyar Forest Research Station, which is located on the territory of Kyiv-Svyatoshinskyi and Makariv administrative districts. The samples of leaves and wood were taken from 10 freshly cut trees.

The total content of phenolic compounds in leaves and wood was determined by the spectrophotometric method (spectrophotometer Optizen Pop, South Korea) using the Folin &Ciocalteu phenol reagent (Sibgatullina et al., 2011). The calibration curve was based on gallic acid.

The quantitative content of flavonoids in leaves was measured at $\lambda = 419$ nm. To 300 µL of the extract, 200 µL of 0.1 M aluminum chloride solution ($AlCl_3$) and 300 µL of 1 M sodium acetate (CH_3COONa) were gradually added. The calibration curve was based on quercetin (Sigma, Germany). The catechins' content was determined by the reaction on vanillin reagent. 900 µL of methanol, 2.5 mL of 1% vanillin solution and 2.5 mL of 9 N•H_2SO_4 in methanol were gradually added to 100 µL of the extract. The optical density (D) of reaction mixture was determined after 30 minutes at $\lambda = 500$ nm (Nonaka et al., 1990). The phytochemical studies were repeated five times.

The concentration of phenolic antioxidants in extracts was determined spectrophotometrically by Brand Williams method using 2,2-diphenyl-1-picrylhydrazyl (DPPG) free stable radical (Brand-Williams et al., 1995). Water soluble vitamin E (Trolox) was used as a standard for calibration curve construction. To obtain the initial solution, 6 mg of vitamin E ($M_{Trolox} = 250.29$) was dissolved in 2.4 mL of 80% ethanol. The reaction mixture contained 0.25 mL of plant extract, 1.75 mL of 80% ethanol and 2 mL of 0.2 mM DPPH ($M_{DPPH} = 394.33$). In control samples, 2 mL of 0.2 mM DPPH solution was added to 2 mL of 80% ethanol. The reaction began after the addition of DPPH solution. The tubes were intensively shaken and left for 30 minutes in the dark at room temperature. The optical density of reaction mixture was determined at a wavelength of 517 nm. Inhibition of DPPH (InDPPH) in percentages was calculated by the formula:

$$In_{DPPH} = 100 \times \frac{Dk - Do}{Dk}$$

where: D_k – optical density in the absence of antioxidants (control); D_o – optical density in the presence of antioxidants (for a calibration curve – Trolox in known concentrations). The antioxidant activity of plant extracts was expressed in µM Trolox equivalent (Sibgatullina et al., 2011).

The chlorophyll *a*, *b* and carotenoids' content in leaves was determined in methanol extracts, which were obtained in the ratio of 1 : 10 (sample of plant material : methanol). The chlorophyll (C_a and C_b) and carotenoids' ($C_{(x+c)}$) quantitative contents were determined by the scanning spectrophotometer OptizenPop (South Korea) using the formula (Wrolstad et al., 2005):

$$C_a\left(\frac{mg}{ml}\right) = 16.72A_{665}.2 - 9.16A_{652}.4$$

$$C_b\left(\frac{mg}{ml}\right) = 34.09A_{652}.4 - 15.28A_{665}.2$$

$$C_{(x+c)}\left(\frac{mg}{ml}\right) = [(1000A]_{700} - 1.63C_a - 104.96C_b) \div 221$$

Qualitative analysis of phenolic compounds in methanol extracts was investigated by the method of high performance thin-layer chromatography (HETLC) on Silicagel G60 (Merck, Germany) plates in a solvent system: chloroform – acetic acid – methanol – water (vol./vol./vol./vol. – 60/32/12/8) (Kovalev et al., 2003). Individual products on the chromatogram were detected in UV ($\lambda_{max} = 365$ nm) and analyzed using the Sorbfil TLC Videodensitometer program.

The separation of the substances was carried out using a reverse-phase high performance liquid chromatography (HPLC) in the Agilent 1260 system equipped with a four-channel pump, a vacuum degasser, an autosampler, a column thermostat, and a diode-matrix detector. Immediately prior to analysis, the samples were filtered through a 0.2–0.5 µm syringe filter. The 2-eluent scheme was used (eluent I – 5 g/L aqueous orthophosphoric acid solution; eluent II – acetonitrile) on the Agilent Zorbax SB-C18 column, 5 µm, 4.6 × 250 mm. Sample volume was 5 µL, the column was thermostatted at 20 °C, flow rate was 1.0 mL/min, analytical time was 35 min, elution profile – isocratically 15% eluent II in eluent I for 5 minutes, further – linear gradient from 15% to 35% II in I for 20 min, at the end of isocratic – 35% II in I for 7 min or more. The basic detection was carried out at wavelengths of 205 and 254 nm, additional wavelengths were chosen as necessary (300 and 325 nm for hydroxycinnamic acids and 350 nm for flavonoids).

The processing and visualization of chromatographic data (including absorption spectra) were performed using Agilent Chem Station and Corel Draw X3 software.

Obtained results were presented as mean ± standard error (x ± SE). The data was analyzed in Statistica 7 (StatSoft Inc., USA, 2004). The significance of the differences between the values ($P < 0.05$) was determined by the analysis of variance (ANOVA) method in the XLSTAT (Addinsoft Inc., USA, 2010). The data were compared using Tukey's test (with Bonferroni correction).

Results

The trees with defects of wood and signs of partial destruction are detected during the planned felling of common oak in the Boyar Forest Research Station (Fig. 1). One of the typical defects is so-called "pidpar" – a continuous or sporadic brown streak of wood without a clear spatial orientation. Brownstreak in wood is caused by a polycondensation of phenolic compounds, usually catechins into phlobaphenes, which are deposited on the inner surfaces of xylem cells (Fig. 2). In the case of an increase in the total amount of oxidized polyphenols, cell walls are also stained. In these conditions, brown streak is determined visually. First of all, it negatively affects its decorative qualities, and the commercial value of the products, respectively. The reason for occurrence of such a defect is not completely understood. However, during a detailed study of xylem tissues, signs of partial damage to cell walls' integrity was revealed (Fig. 2b, d). Defects of this kind are usually typical for the vital activity of xylotrophs, which hydrolyze the components of cell walls by exogenous enzymes. Deposition on the inner surface and impregnation of cell walls with polyphenolic compounds creates some obstacles for the pathogen and decelerates the destruction.

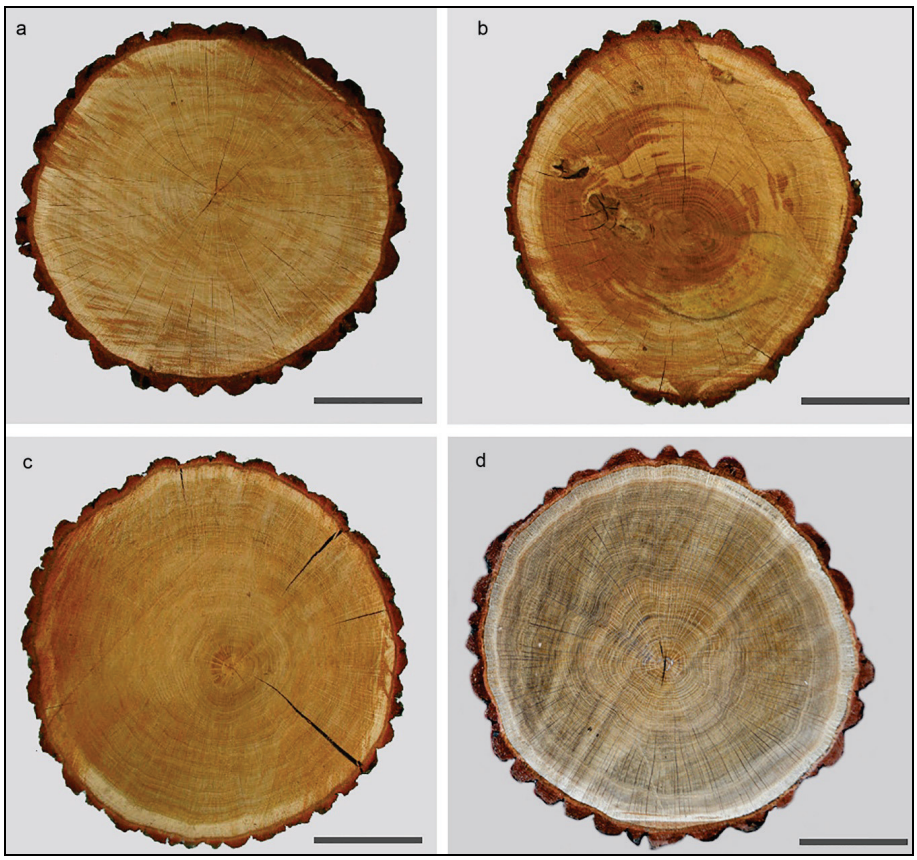

Fig. 1. Transverse section of *Quercus robur* trunk and its colour in the norm (*a*) and in pathogenesis: brown rot (*b*), brown streak (*c*, *d*); bar – 100 mm

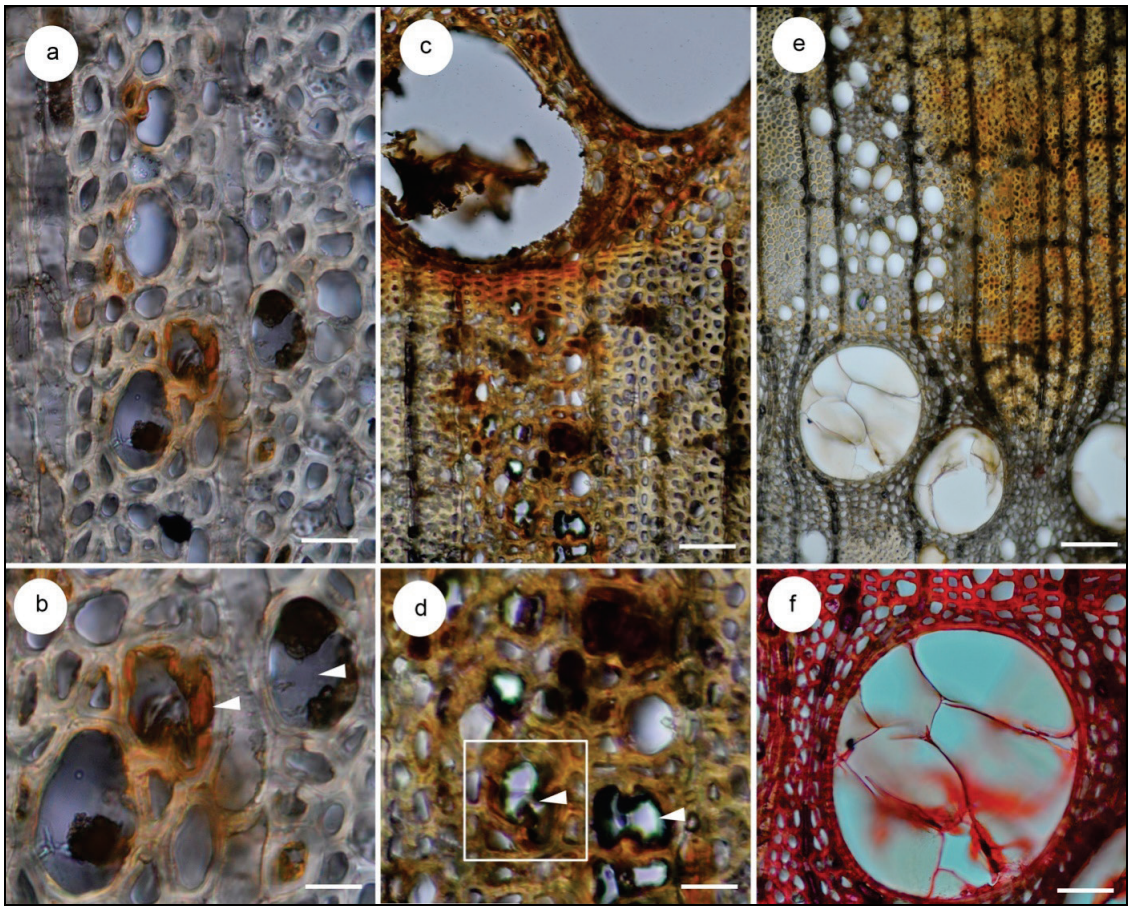

Fig. 2. Cross section of *Quercus robur* xylem: *a*, *c* – deposition of polyphenolic compounds on the inner surface of cell walls; *b*, *d* – cell walls destruction, typical for fungi lesions; *e*, *f* – process of body formation, thickening and colouring of cell walls; bar: *a*, *b*, *d* – 20 μm; *c*, *e* – 100 μm; *f* – 50 μm

It is known that oligosaccharides, which are formed during the process of enzymatic hydrolysis, can perform elicitors function. During its translocation by the conducting system, they reach the leaves and cause appropriate physiological reactions, including activation of the secondary metabolism. Study of the total phenolic compounds' content in leaves of the trees with brown streak has shown that the total phenols' content increased by 1.6 times in comparison with the control. The flavonoids' and catechins' contents also increased. Meanwhile, the total antioxidant activity of phenolic compounds did not significantly change (Table 1).

Table 1

The content of the main classes of phenolic compounds in *Quercus robur* leaves in the presence of hidden wood defects (x ± SE, n = 5)

Samples	Concentration, mg/g			Antioxidant activity, μMeq
	phenols	flavonoids	catechins	
Control	155.1 ± 5.57	6.36 ± 0.20	6.76 ± 0.27	65.8 ± 3.3
Brown streak	247.4 ± 8.07**	7.54 ± 0.36*	9.26 ± 0.32*	66.3 ± 3.3
Brown rot	108.8 ± 3.05*	6.76 ± 0.16	12.78 ± 0.42**	63.8 ± 3.2

Note: significance of differences compared to the control was assessed by one-way ANOVA; * – significant differences at $P < 0.05$, ** – significant differences at $P < 0.01$(with Bonferroni correction).

Other results were obtained when studying leaves of trees with signs of brown rot. The total phenols' content in organs decreased by 1.3 times, and catechins' content increased almost by two times. A similar reaction of plants suggests that the synthesis of phenolic compounds depends on the degree of wood destruction. In the initial stages of fungal invasion the phenylpropanoid synthesis in plant leaves is activated with the formation of gallo- and ellagitannins. This class of compounds has a high antioxidant potential and expressed bacteriostatic and fungistatic properties. It remains unclear why the antioxidant potential does not change by the apparent increase of phenolic compounds in the leaves. If the effectiveness of plant response to the impact of xylotroph was insufficient and the total destruction of xylem cells began, the mechanisms of secondary synthesis switched to the formation of catechins, which have the ability to non-enzymatic polycondensation with the formation of water-insoluble phlobaphene. These compounds are deposited in the protoplast, as well as on the inner surface of cell walls in vessels, fibers and parenchyma and perform the function of histochemical barriers.

Since most phenylpropanoid synthesis enzymes are concentrated in plastids and its synthesis depends on the photosynthesis intensity, it was also important to find out the qualitative composition of plastid pigments, in particular chlorophyll (Chl) *a*, *b* and carotenoids. As a result, we found that the plastid complex in common oak leaves does not significantly change and does not depend on invasive or destructive processes, at least at its early stages (Table 2). According to our data, the chlorophyll *a* and *b* content in plants with brown streak directly depended on the intensity of wood browning in the trunk. If, in the case of brown streak formation an increase in the total amount of photosynthetic pigments is obvious, an increase in the amount of chlorophyll *a* on the background of decrease in chlorophyll *b* content raises questions, because under stress conditions the fraction of supporting green pigment is usually increased.

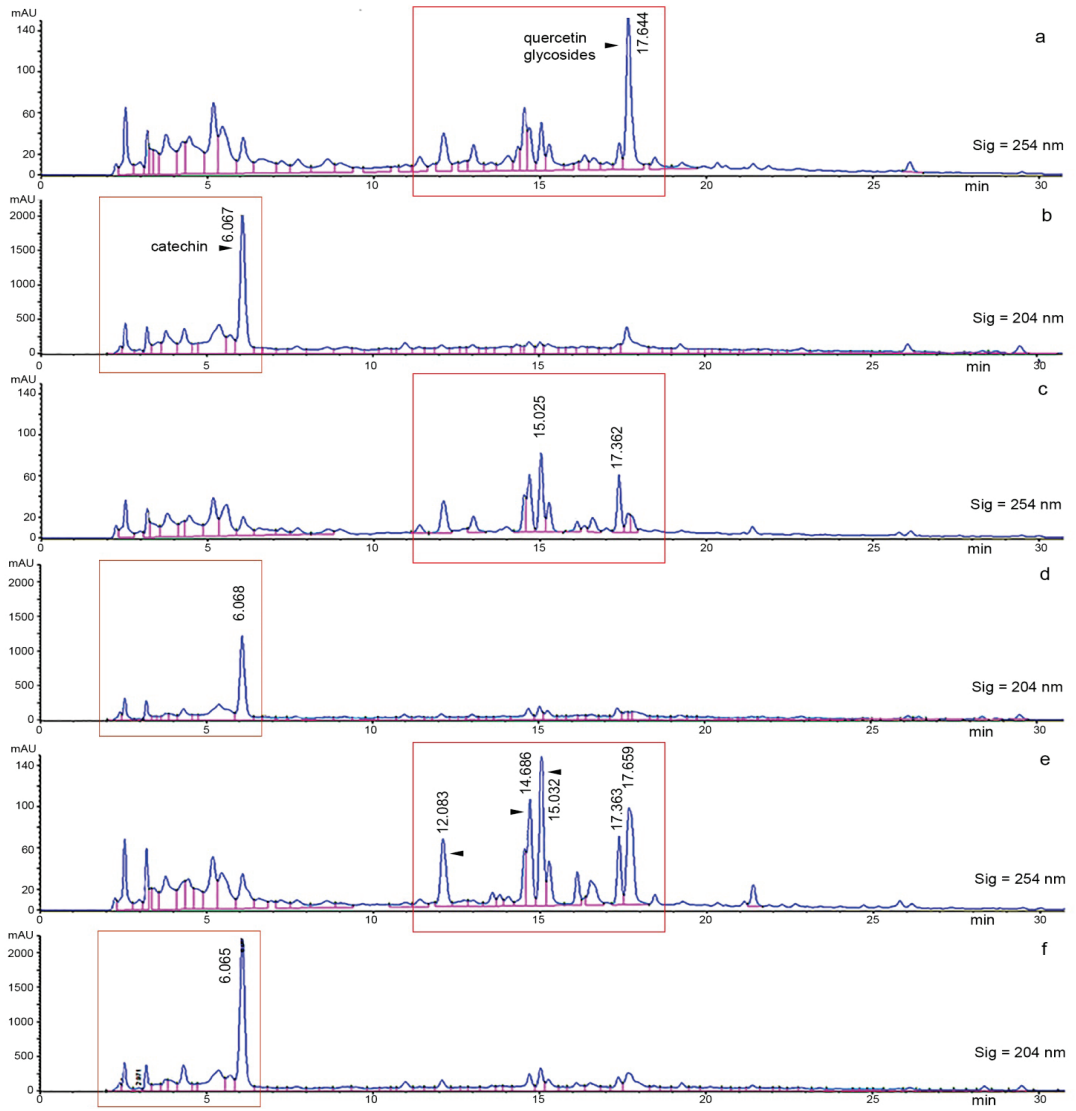

Fig. 3. Chromatogram of the leaves of *Quercus robur* tree with different conditions of trunk wood: *a*, *b* – trees without signs of wood destruction and browning; *c*, *d* – wood with brown streak; *e* – trees with brown rot in wood; arrows show the peaks that are significantly different in the control and in the presence of pathologies

Table 2
The plastid pigments content in *Quercus robur* leaves under the presence of hidden wood defects (x ± SE, n = 5)

Pigments and their ratio	Control	Brown streak I	Brown streak II	Brown rot
Chl*a*, mg/g	4.3 ± 0.22	4.8 ± 0.34	5.2 ± 0.26*	4.9 ± 0.27
Chl*b*, mg/g	1.8 ± 0.10	2.1 ± 0.16	2.0 ± 0.10	1.7 ± 0.11
Chl*a* + Chl*b*	6.1 ± 0.36	6.9 ± 0.44	7.2 ± 0,36*	6.6 ± 0.33
Carotenoids, mg/g	1.8 ± 0.09	1.7 ± 0.09	1.6 ± 0.08	1.7 ± 0.08
Chl*a* /Chl*b*	2.4 ± 0.11	2.3 ± 0.12	2.6 ± 0.13	2.8 ± 0.14*

Note: see Table 1.

According to chlorophyll *a* to *b* ratio in the leaves, there was a tendency to its increase in the case of increased browning and appearance of rot in wood of the trunk. Also noteworthy is the tendency to decrease in the total amount of carotenoids in the leaves under the conditions of destructive processes. Thus, the photosynthetic apparatus of the leaves in the trees with brown streak by the composition of the pigment complex was not significantly changed. Since the synthesis of phenolic compounds in the leaves of the trees was significantly different, biochemical profiling was carried out. Chromatographic analysis of the leaves showed that the presence of trunk browning signs significantly reduces the content of quercetin glycoside (peak 17.647 min) and catechin (peak 6.037 min) (Fig. 3). In the trees with brown rot, these indices were at the same level as the control, however, the intensity of the peak signal with retention time of 17.367 minutes was significantly increased. According to the characteristic UV spectrum, this substance is defined as kaempferol glycoside (Fig. 4). In addition, a new peak (21.385 min) appeared on the chromatographic profile, which can also be attributed to kaempferol glycoside by the UV spectrum. The ratio of the signal intensity between two flavonols also changed (Fig. 3). In the norm and in the trees with brown rot quercetin glycoside content considerably dominated. In the leaves of the trees with brown streak the ratio was shifted to kaempferol glycoside. The difference between the biochemical profiles in the control and in pathogenesis is presented on the chromatograms, which are imposed on one another (Fig. 5).

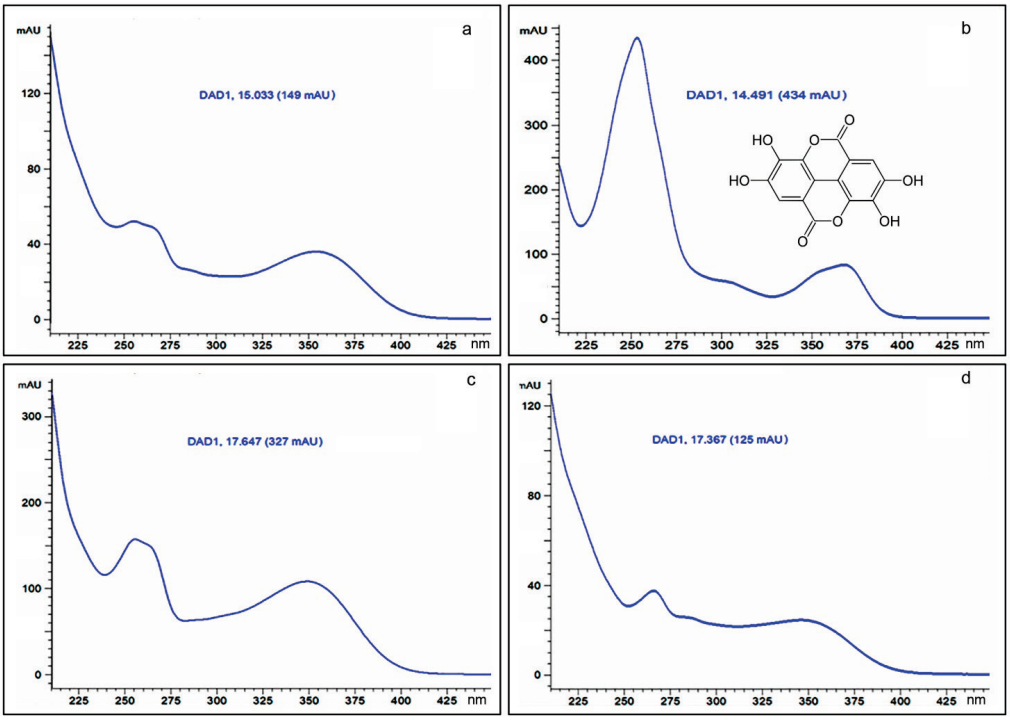

Fig. 4. The UV spectrum of peaks on chromatogram that differ in samples of wood with signs of brown streak and brown rot: *a*, *c* – quercetin glycosides; *b* – ellagic acid (maximum – 368 and 253 nm); *d* – kaempferol glycoside

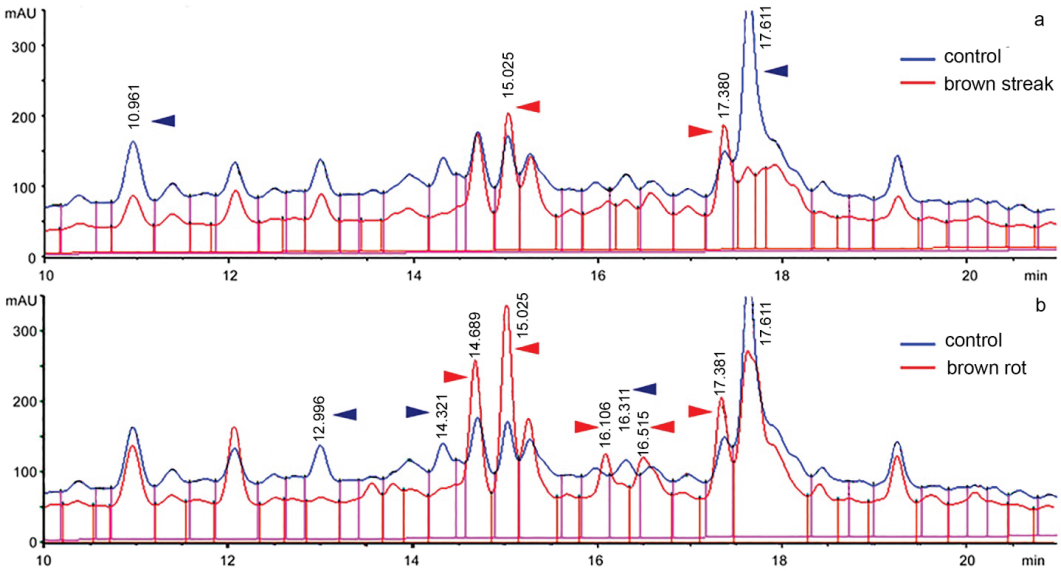

Fig. 5. The fragment of chromatogram of the leaves of *Quercus robur* tree with different condition of trunk wood (detection signal 254 nm)

The peaks profiles of common oak wood for 2–4 minutes in the chromatogram are almost identical for brown streak and the control (Fig. 6). The profiles of wood with signs of destruction (peak signals at 2.783 and 3.27 min) are much more intense. The biochemical profiles of the trees with signs of brown streak and brown rot differed from the control by the composition of medium polar compounds. Thus, on the chromatograms peaks 3 and 4 were not detected at 13.40 and 13.71 min (Fig. 7, shown by arrows). The absence of some individual phenolic compounds is a marker that should be taken into consideration.

Discussion

The effectiveness of protective functions of ellagitannins is higher than gallotannins. This is due to the ability of ellagitannins to form o-quinones with pronounced electrophilic properties (Salminen et al., 2011). Antifeedant activity of tannins is also based on protein precipitation, and is enhanced by its prooxidant activity, especially in slightly alkaline conditions (Salminen et al., 2011). In tannins synthesis, the poly- galloyl glucose with a relatively low prooxidant potential is first formed in the cells. Further transformation of gallotannins into ellagitannins leads to an increase in total prooxidant activity of cells. Ellagic acid is formed as a result of lactonization of hexaoxydiphenic acid, which is released in the case of ellagitannins' hydrolysis. Increase in the total pool of ellagic acid compensates the deposition of toxic agents that are accumulated in cells during intensive oxidative processes. Insofar as accumulation of ellagic acid occurs under plant infection by phytopathogenic fungi (Zaprometov, 1993), an increase in its concentration in wood can indicate an increase in elicitorial activity of glucosamine with the activation of the appropriate hydrolases. Quantitative changes in the concentration of ellagitannins in common oak wood in response to the impact of fungi are also associated with the activation of the appropriate enzyme systems, which leads to its hydrolysis in plant tissues (Klumpers et al., 1994).

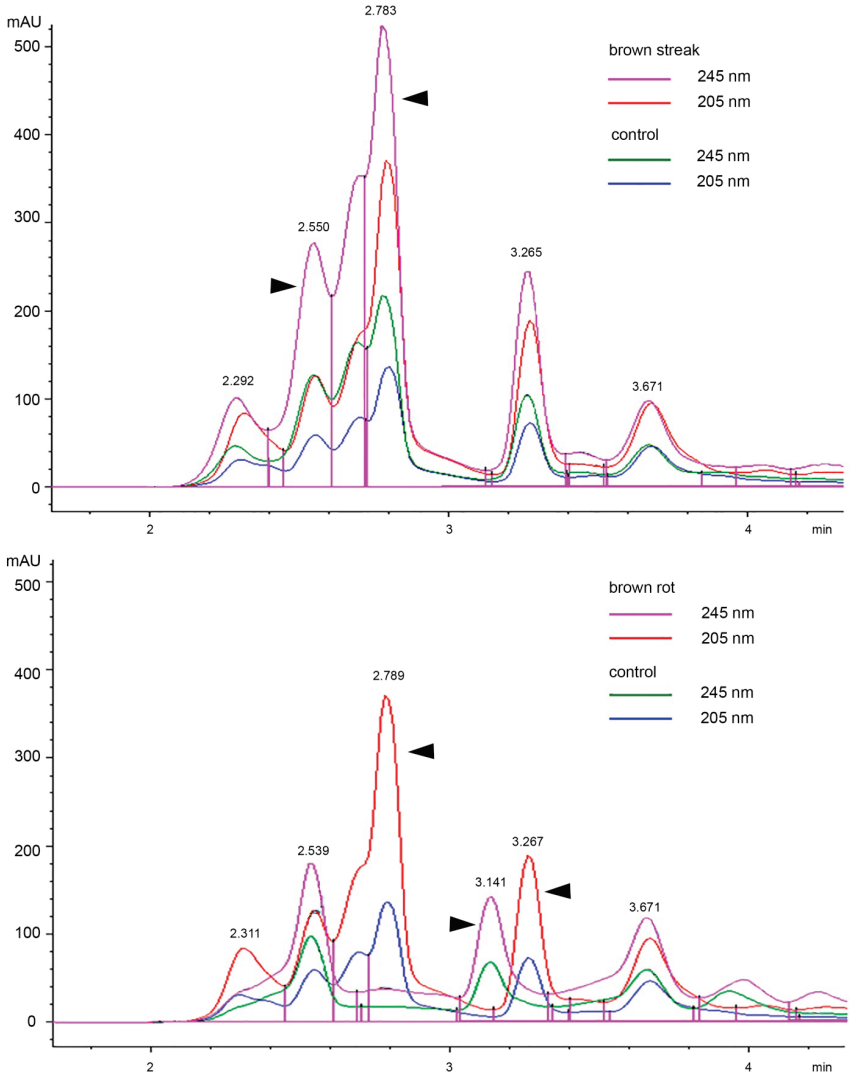

Fig. 6. Peaks profiles of polar compounds in the extracts of *Quercus robur* wood

The accumulation of phenolic compounds of a certain class in plant tissues is a stress indicator (Winkel-Shirley, 2002; Cheynier et al., 2013; Naikoo et al., 2019). Phenol synthesis is associated with an increased activity of phenylalanine ammonia lyase (PAL), chalcone synthase (CHS) and many other enzymes (Cheynier, 2013). Plant phenolics perform a wide variety of functions and help the plant organism to adapt to adverse environmental factors (Landolt et al., 1997; Lattanzio et al., 2009). They are able to regulate transcriptional processes, affect membrane permeability, vesicle trafficking and transduction of chemical signals (Naikoo et al., 2019). At the same time, in turn, sugars can act as inducers of flavonoid synthesis. Thus, the activity of CHS, which is a key enzyme in flavonoid synthesis, is affected by sucrose concentration (Solfanelli et al., 2006). That explains a slight increase in flavonoid content in the leaves at night time. The increase in the kaempferol glycosides' fraction in the leaves of common oak with wood browning may be due to the changes in regulation of the relevant enzyme systems. Such processes might be associated with the entry into the leaves via an ascending flow of specific products from wood biochemical transformation (Pakhomov et al., 2008; Brygadyrenko, 2015).

It is known that dihydrokaempferol, which is synthesized from naringenin using F3H, flavanone 3-hydroxylase, is a kaempferol precursor. The synthesis of dihydroquercetin is possible from dihydrokaempferol or eriodictyol. Both of these products are formed from naringenin. The synthesis of eriodictyol involves the F3'H enzyme, flavanone 3-hydroxylase, which also synthesizes dihydroquercetin from dihydrokaempferol. Thus, both ways of quercetin synthesis are possible with the involvement of F3'H (Deng & Lu, 2017). A decrease in the activity of this enzyme will

lead to an inevitable increase in the share of kaempferol and its glycosides in leaf tissues. Quercetin and kaempferol are known as the most important regulators of auxin transport (Murphy et al., 2000). Flavonols have an effect on the enzyme indole-3-acetic acid (IAA) oxidase, which inactivates auxins. In this case, kaempferol containing one hydroxyl group in the B-ring activates this enzyme, and quercetin with two hydroxyl groups is its inhibitor. In the synthesis of flavonols, a balance shift towards kaempferol can lead to some decrease in the active forms of auxin and, respectively, to weakened cell growth via stretching. This process, with an increase in the activity of anionic peroxidases, may have an adaptive value. Because the cells in the areas of wood browning have a smaller diameter and thickened lignified cell walls, they are more resistant to destructive processes caused by pathogenic microorganisms and fungi. In addition, it is also known that naringenin and kaempferol have antifungal activity, for example, against *Pyriculari aoryzae* (Padmavati et al., 1997). Chlorogenic acid and rutin inhibit the development of *Fusarium oxysporum*, while catechol and protocatechuic acid – *Colletotrichum circinans* (Lattanzio et al., 2006). The increase in the total phenols' content in the leaves of oaks with brown streak occurred mainly due to the accumulation of hydrolyzed tannins. The antifungal properties of tannins and proanthocyanidins are well known. They inhibit *Aspergillus niger*, *Colletotrichum graminicola*, *Gloeophyllum trabeum*, *Trichoderma*, etc. The tannins protect the heartwood against the wood-decay fungi and suppress the activity of extracellular hydrolases in pathogenic microorganisms (Lattanzio et al., 2006).

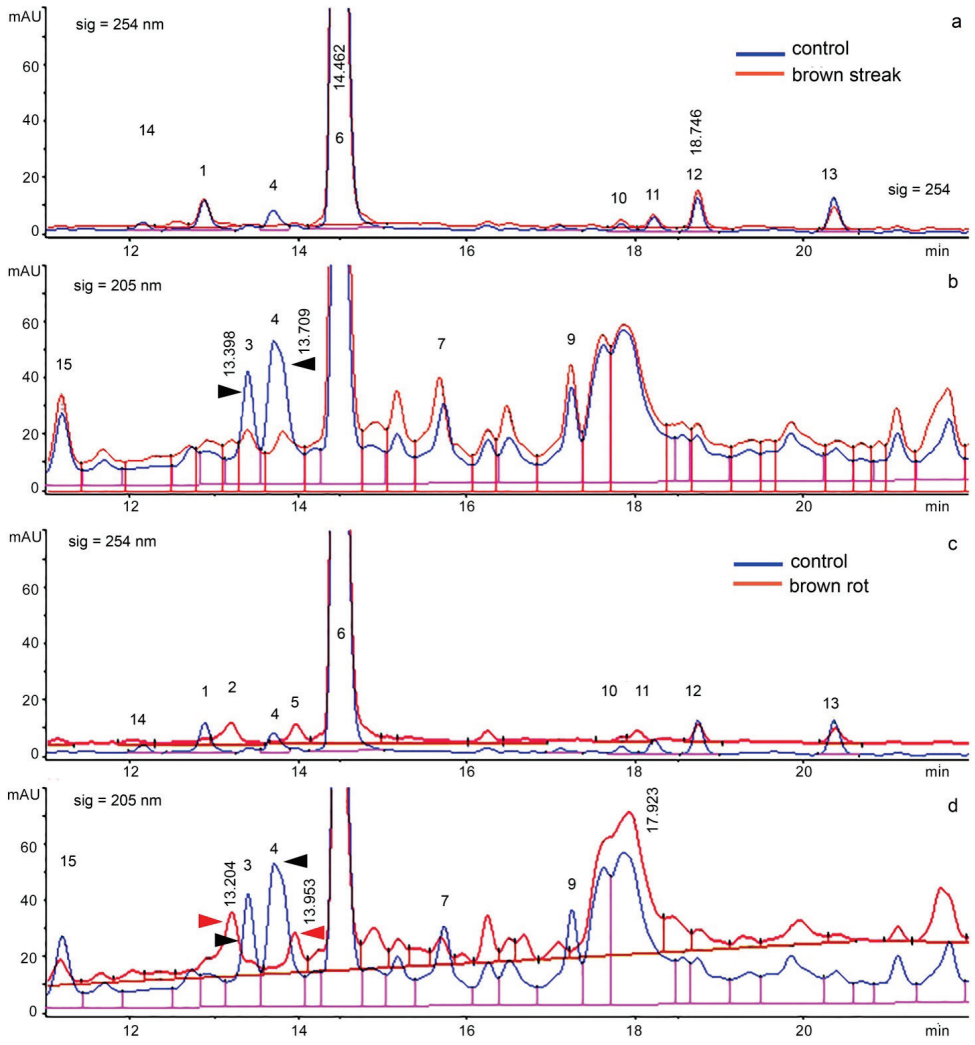

Fig. 7. Peaks profiles of low polar compounds in the extracts of *Quercus robur* wood with various defects (detection signal 205 nm, 254 nm)

Thus, an increase in the total pool of ellagic acid suggests an increase in the intensity of oxidative processes in plant tissues and can be considered as a marker of brown rot and hidden wood defects.

Conclusions

Brown streak in common oak wood is caused by intravital accumulation of oxidized polyphenols in the cell walls of tracheal elements, as well as deposition of phlobaphenes on the internal surfaces of the heartwood parenchyma. Brown streak in wood is determined visually and reduces its decorative qualities.

Microscopy of the oak wood samples with a brown streak allowed us to detect the signs of cell walls' biodegradation. At the same time, a visually distinguishable developed mycelium of xylotrophic fungi was not found. This might be due to the fact that the development of primary destructors in cell walls is inhibited by the oxidation products of phenolic compounds. Slight biodegradation of cell walls and the lack of developed mycelium in xylem suggest that the products of oxidized phenolic compounds decelerate the development of xylotrophic fungi and reduce the activity of its enzyme systems.

Protective reactions against biodestructors in common oak with signs of brown streak have a systemic nature. This is confirmed by a significant increase in the leaves of the total content of phenols, which are further transported to xylem tissues. The need for activation of the secondary metabolism explains the increase in the leaves of chlorophyll content. With the development of brown rot in the trunks, the phenolic compounds content in the leaves gradually decreases. The HPLC method shows that brown streak in wood is accompanied by qualitative changes in the flavonoids' composition in the leaves. In the leaves the content of catechin (with the retention time 17.637 min) and some quercetin glucosides (6.037 min) decreases, while the content of kaempferol glycosides (17.367 min) increases. Changes in the qualitative composition and in the ratio of

amount of individual flavonols and hydrolyzed tannins in common oak leaves are informative markers of induced protective reactions that can be used for vital detection of brown streak in wood.

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