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Bacterial wetwood of silver birch (*Betula pendula* roth): symptomology, etiology and pathogenesis

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Abstract

The article is focused on microbiological and silvicultural properties of bacterial wetwood of silver birch (*Betula pendula*), also known as European white birch. During the active phase of the disease, bacterial wetwood (i.e. bacterial dropsy, vascular parenchymatous bacteriosis or flux slime) is characterised by crust and periderm bloating, necrotic wet stains and abundance of exudate. The disease is more likely to occur in older ($r = 0.56$, $p < 0.01$) and less-dense ($r = -0.29$, $p < 0.01$) stands.

The statistical model showed that the chance of bacterial wetwood increases with birch age by 0.36% per year. The stands with birch proportion of over 70% demonstrated 15.3% lower infection rate compared to the stands with lower birch presence. The stands with lower stocking demonstrated a higher proportion of infected tree distribution by 7.5% compared to the stands with higher birch representation. The most vulnerable were larger, older *B. pendula* trees with longitudinally fissured bark that grow on poorer soils and experience frequent water stress.

Birch associations with *Pteridium aquilinum* and *Vaccinium myrtillus* were more susceptible to infection (31.6% and 44.3%, respectively), whereas associations with *Brachypodium sylvaticum, Sphagnum palustre* and *Calluna vulgaris* were at lower risk. Strong ecological and trophic association of bacterial wetwood was present between silver birch and *Tremex* spp., particularly *Tremex fuscicornis*. Mycobiota was represented by *Rhizopus microsporus, Mucor mucedo, Penicillium aurantiogriseum, Penicillium purpurogenum* and *Acremonium strictum*.

Enterobacter, Xanthomonas, Pantoea and *Bacillus* spp. associated with bacterial wetwood of silver birch were isolated. *Enterobacter nimipressuralis* was found to be the primary causative agent through means of artificial infection, while other bacteria were found to be either weak pathogens or concomitant. *E. nimipressuralis* formed the largest number of colony-forming units (CFU) for bark and cambium (164 and 127 CFU, respectively) and was also found in a small amount as a vital obligate in the automicrobiota in healthy birch trees.

Key words

artificial inoculation experiment, *Enterobacter nimipressuralis*, regression modelling, correlogram, integrated pest management, *Tremex fuscicornis*, vital obligates, cause, symptoms, microbiota

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Introduction

Bacterial wetwood, also known as bacterial slime, bacterial dropsy, flux slime, etc., is a common tree disease linked to a variety of near- and fully anaerobic bacteria. Bacterial fermentation of the sap produces gases (often methane), the pressure of which forces the sap out by the path of least resistance. Thus, a common symptom of the disease is the bleeding of sap from the trunk or limbs of a tree. This ooze prevents callus formation over lesions, increasing the susceptibility to infection by other pathogens (Baker and Tainter 1996; Lyon and Sinclair 2005; Schink et al. 1981).

Bacterial wetwood greatly contributes to its host's dieback and death. There is virtually no known effective control measure to avoid infection and spread of the disease, which makes it a particularly dangerous phenomenon. Some management recommendations include chemical spraying, sanitary cuttings and soil fumigation in combination with proper fertilisation (Goychuk 2020). To alleviate the buildup of gas pressure and other by-products of bacterial activity, it is recommended to drain the liquid using pruning or bark perforation. However, applying these methods in birch stands is often not practical or cost-efficient, especially at a large scale (Agrios 2005; Lyon and Sinclair 2005; Smith 2012).

There is a substantial lack of scientific data on the symptomatic, etiology and pathogenesis of bacterial wetwood of silver birch (*Betula pendula* Roth), especially for different locales and biomes. The mechanism of infection is not well understood partially because there are multiple species of bacteria directly or indirectly associated with the disease. The pathogenic bacteria are commonly found in both water and soil and even in healthy trees as vital obligates, an essential automicroflora component. They can naturally be spread by absorption through mechanical abrasions or wounds by wood-boring insects. When a causative event occurs, different bacteria could have different roles as pathogens or concomitants, depending on alternative environmental scenarios or specific tree hosts (Agrios 2005; Baker and Tainter 1996; Infectious Forest Diseases 2013).

Silver birch is among the ecologically and commercially important tree species in Europe that is affected by bacterial wetwood. Being an essential component of temperate and boreal forests, it creates habitat and food source for many important insects and fungi. As one of the most enduring pioneer species with wide ecological amplitude, birch is extremely valuable for soil protection and reforestation projects. Its wood is widely used in pulp, veneer, plywood and fuelwood production. The species is known to tolerate a broad range of growing conditions; even so, it is sensitive to long periods of drought (Atkinson 1992; Beck et al. 2016)

The relevancy of research on bacterial wetwood of *B. pendula* lies in both the importance of the birch species on a landscape scale and the accelerating rate of climatic change in recent years. The weakening of birch forests is largely associated with synoptic anomalies such as increased water stress levels and elevated average temperatures. Hundreds of thousands of hectares of silver birch stands are affected by this disease, and it continues to spread at an alarming pace (Beck et al. 2016; Cherpakov 2012; Sagitov et al. 2005; Shvets 2016b).

The objective of this publication is trifold. The first element is to assess the specific symptomatology of bacterial wetwood occurring on silver birch in order to allow for its better assessment for field practitioners. The second element is to address the etiology of the disease and determine its primary causative agents and concomitant bacteria in the laboratory settings and via artificial infection. The third element is to analyse multiple biotic and abiotic factors that contribute to bacterial wetwood emergence and pathogenesis and develop a set of recommendations on future microbiological research and on how to better counter the spread of the disease.

Material and methods

The research is based on a systems approach using generally accepted methods in the field of forestry, forest phytopathology and forest statistics: methods of forest-pathological examinations (reconnaissance and detailed) and plant pathological studies, as well as special methods of experimental bacteriology, mycology, parasitology and laboratory analyses (Beltyukova 1968; Chumaevskaya and Matveeva 1986; Jance 2005; Patyka and Pasichnyk 2017; Tkachik 2014).

A set of 45 sample plots is established for a variety of common edaphic and hydrological conditions for silver birch. The sample plots average 1.92 ha and include pure silver birch, mixed birch–pine and birch–aspen forests. The total study area is 86.6 ha and it spreads across a large region of Zhytomyr Polissya located in northern Ukraine, featuring a considerable variability in forest land use and forest characteristics.

Our sample plots are located at seven ranger stations of Yemilchinsky, Korostensky, Bilokorovytsky, Slovechansky, Olevsky and Luhynsky State Forest Enterprises. The field surveys cover stands of different ages, stand density and volume, average heights and diameters, site index classes (i.e. bonitet) and birch participation in the stand composition. The main characteristics of the study plots summarised by ranger stations are shown in Table 1.

For assessment of types of forest site conditions, we used a widely accepted classification system, where all forest sites are classified into four main groups by nutrient status (Pogrebnyak 1955) (A – poor, B – relatively poor, C – relatively rich and D – rich) with six soil moisture classes in each (0 – very dry, 1 – dry, 2 – fresh, 3 – moist, 4 – damp, 5 – wet or swamps). For this research, all sample sites fall within six classes: A_2, A_3, B_2, B_3, C_2 and C_3, which is typical habitat for silver birch trees in the Right-Bank Ukraine (Chuprov 1986).

For each sample plot, the trees were counted and categorised based on bark appearance, thickness and the colour of the leaves. The presence and distribution rate of bacterial wetwood was determined for each tree based on visual appearance (Shvets 2016a). Distribution rate (or infection rate) of disease is the process characteristic of a certain type of plant population that determines the number of new infections per unit time (in our case, for 1 year). It was measured as a proportion of infected *B. pendula* trees to the total count of *B. pendula* in the study area.

As *B. pendula* is characterised by wide intraspecific variability, the type and colour of the bark is considered as one of the most reliable signs of the wood quality and overall tree health (Kleshcheva 2007). As part of the research, we identified five classes of birch bark (coarse fissured, longitudinally fissured, rhomboid fissured, unclearly fissured and plain) and assessed their different exposure to bacterial wetwood and their role in the distribution rate of the disease (Shvets 2016a).

The characteristic of the territory's moisture supply level was calculated using Selyaninov's hydrothermal coefficient of hydration (HTC), where the following zones were identified: excess moisture, or drainage zone (HTC > 1.3), sufficient hydration (1.0–1.3), arid (0.7–1.0), dry (0.5–0.7) and irrigation (HTC < 0.5) (Selyaninov 1928; Goychuk et al. 2018).

The count of insects was carried out according to the adopted methodology for the analysis of pest population of model trees (Meshkova 2006). For each 10 trees within different phytosanitary classes, the total number of flight holes was counted at a height of 1.3–1.5 m on an area of 50 cm^2, and their average number was estimated.

An ordinary least squares (OLS) statistical model was developed to establish significant causal relationships between the distribution rate of bacterial wet-

Table 1. Main silvicultural characteristics of study plots, by ranger station

Forest enterprise	Ranger station	Sample plots, count	Size, ha	Age, years	Height, m	DBH, cm	Volume, m^3/ha	% birch component	Bonitet	Regeneration type	Infection rate, %
Yemilchynsky	Korolivske	7	2.1	47.6	19.1	20.6	152.9	64.29	I–III	Natural/artificial	24.46
Yemilchynsky	Gartivske	9	1.5	48.3	17.4	18.7	167.8	77.78	I–IV	Natural/artificial	29.01
Korostensky	Behivske	7	1.6	52.9	19.9	22.9	147.1	65.71	I–III	Natural/artificial	42.67
Bilokorovytsky	Zubkovytske	7	1.6	41.9	19.0	19.1	155.7	71.43	I–II	Artificial	29.89
Slovechansky	Kovanske	5	2.4	50.6	21.0	23.2	190.0	72.00	I–II	Artificial	40.94
Olevske	Snovydovycke	6	2.5	56.3	20.8	22.7	193.3	61.67	I–II	Natural/artificial	37.12
Luhynsky	Lypnycke	4	2.4	47.3	21.8	23.0	203.8	85.00	I–Ia	Artificial	15.73

wood in silver birch stand and associated silvicultural parameters. All data were checked for normality using Anderson–Darling in combination with box plots and tested using the Box–Cox procedure. The Pearson and Spearman bivariate correlations were applied for normally distributed and non-parametric data, respectively. Based on the results of correlation analysis, multicollinearity and heteroscedasticity analysis, the final model was selected. The final choice of a functional form, variable selection and their transformations were made using a backward selection process on standardised analysis of variance (ANOVA) tests and residual analysis. The statistical analysis and modelling were done using the R programming language (R Core Team 2013).

For mycological and microbiological analysis, 187 samples of wood were collected from affected trees of different age groups and from different parts of the trunk. Forty-two cultures of fungi and bacteria were isolated in pure culture. Potato agar (PA) was used as a nutrient medium for isolating bacteria, while meat-peptone bouillon (MPB) was used for accumulation cultures. To isolate phytopathogenic bacteria, we introduced samples of exudate and infected tissues into the nutrient medium in Petri dishes (Gvozdyak and Goychuk 1991).

The infected tissues were introduced in the form of saw dust, which was extracted with a sterile lancet from a wood sample treated with ethyl alcohol and burned on all sides. In most cases, when sowing sawdust on a nutrient medium, intensive overgrowth of bacterial mass was observed. *In vitro* and *in vivo*, 141 artificial inoculations of trunks and leaves of *B. pendula* were conducted. The anatomic–morphological and physiological–biochemical properties of phytopathogenic isolates of bacteria were studied (Patyka 2017). During the artificial inoculation, the trunks of five model birch trees aged 35–40 years were mechanically damaged. The suspension of microbial culture (8.6–9.9 million colony-forming units [CFU]/ml) was injected in the amount of 5 ml per test plant directly under the bark. The experimental part of the work was performed at the Institute of Microbiology and Virology, National Academy of Science of Ukraine.

Results

Symptomatology

Symptoms of bacterial wetwood are directly related to the moisture content in the birch trunk and include the formation of a wet pathological core, cracks and ulcers, necrotic wet spots (especially in places of external infection) and copious exudation. On the trunks of affected trees, numerous epicormic shoots are formed, which testify to the deep pathology of birch. The bark of affected trees exfoliates, exposing the sapwood. Chronic pathogenesis is accompanied by dieback of the upper part of the plant and eventual death of the weakened tree (Alizadeh 2017; Hanson 2018; Jacobi 2009) (Fig. 1).

The early diagnostic sign of bacterial wetwood is the appearance of dry treetops for some of the birch

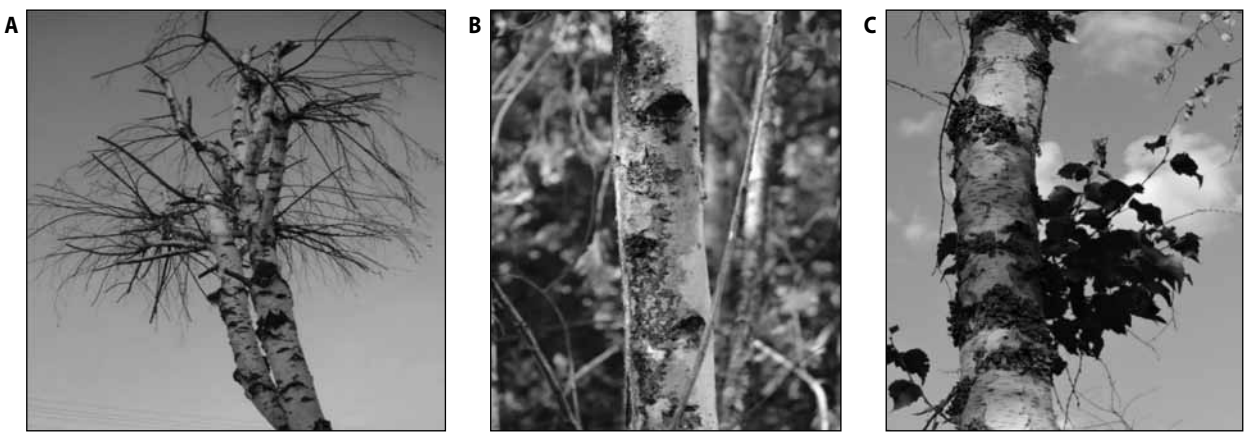

Figure 1. Symptoms of bacterial wetwood: dry top of *Betula pendula* affected by *Enterobacter nimipressuralis* (A); exudate of *E. nimipressuralis* on the trunk of *B. pendula* (B); water shoots on the trunk of birch affected by *E. nimipressuralis* (C)

plants in stands with low crown density. Signs of plant damage by the causative agent of bacterial wetwood usually appear in spring or early summer, when swellings of various sizes can be noticed, mainly on the trunks under the bark. Birch trees with roughly fissured and longitudinally fissured bark feature peculiar fermentation odours and brown exudate coming out of fissures. The birch with smooth bark usually ruptures under the pressure of gases released by bacteria, particularly hydrogen sulphide. Exudate darkens, becomes dark brown or sometimes even black. The leakage is brief, observed mainly in May–June, and then it dries out. Re-discharge of the exudate sometimes occurs in the fall. On the transverse sections of the affected trunks, wet areas of wood of various brown shades can be noted.

The leaves of infected trees are substantially smaller than those of healthy trees, often exhibiting chlorotic condition. Usually, below the dying crown, numerous epicormic shoots appear, which eventually also rot off usually in a year or two (in some cases, as soon as a few months). About one-third of birch trees die from bacterial wetwood in mature and over-mature stands in the studied region (Goychuk and Shvets 2017).

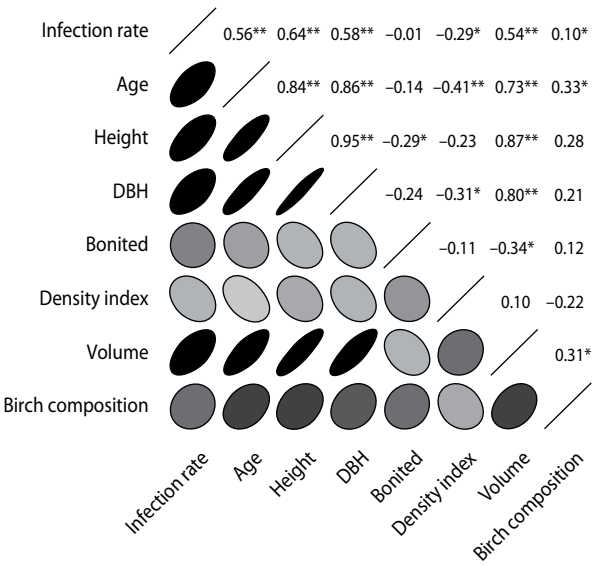

Figure 2. Correlogram for bacterial wetwood spread and associated forest taxation parameters. (Note: bonitet is also known as site index, and density index is also known as relative density of stocking)

**Significant at 0.01 level, *significant on 0.05 level.

Effect of stand characteristics on bacterial wetwood

The research revealed some relations between surveyed stand characteristics and the spread of bacterial wetwood. Bacterial wetwood distribution correlated with average age, height, diameter at breast height (DBH) and volume of growing stock per hectare at 0.01 level, and with a relative stocking density[1] and proportion of birch in the stand composition at 0.05 level (Fig. 2). As anticipated, strong correlations were also found among age, height, DBH and tree volume. Relative density of stocking negatively correlated with age ($p < 0.01$), and positively correlated with DBH ($p < 0.05$).

Due to the high degree of multicollinearity among age, height, DBH and volume, only four variables were included into the model: the spread of bacterial wetwood in % (i.e. change of infection, or infection rate), age of the silver birch stand in years, relative stocking density in % and birch participation in the stand composition in %. The relative stocking density and birch participation in the stand composition were transformed into dichotomous variables (0 as 0%–69.9% and 1 as 70%–100%) due to their non-parametric nature. The variables were coded as DAMAGE, AGE, BIRCH70to100 and DENSITY70to100, respectively. Their descriptive statistics are shown in Table 2.

No transformation for DAMAGE and AGE is needed. Anderson–Darling A-value for AGE is 0.528 ($p = 0.169$) and for DAMAGE is 0.519 ($p = 0.177$). After introducing factor interactions and backward stepwise model selection, the final model looks like:

$$\begin{aligned} \text{DAMAGE} = {} & 26.632 + 0.362*\text{AGE} + \\ & - 15.231*\text{BIRCH70to100} + \\ & - 7.495*\text{DENSITY70to100} \end{aligned} \quad (1)$$

The OLS model summary and ANOVA are provided in Table 3. The estimated goodness of fit R^2 for the model is 0.5 and its standard error is 13.529. The model

[1] Relative stocking density is estimated as the sum of the cross-sectional areas of individual trees at breast height, referred to the sum of the cross-sectional areas of the tabular etalon stand of the same species under identical growing conditions. It is expressed in fractions of a unit (0.10, ..., 1.00), where 100 is an etalon value (Anuchin 1982).

Table 2. Descriptive statistics for model variables

	DAMAGE	AGE	BIRCH70to100	DENSITY70to100
Mean	31.7889	49.1333	0.5333	0.6000
Standard error	2.7425	2.9673	0.0752	0.0739
Median	33.3000	51.0000	1.0000	1.0000
Mode	6.1000	75.0000	1.0000	1.0000
Standard deviation	18.3971	19.9050	0.5045	0.4954
Sample variance	338.4524	396.2091	0.2545	0.2455
Kurtosis	−1.1123	−0.6105	−2.0752	−1.9085
Skewness	0.0632	−0.4663	−0.1383	−0.4225
Range	59.9000	75.0000	1.0000	1.0000
Minimum	0.9000	5.0000	0.0000	0.0000
Maximum	60.8000	80.0000	1.0000	1.0000
Confidence level (95.0%)	5.5271	5.9801	0.1516	0.1488

Table 3. OLS model summary and ANOVA

	df	SS	MS	F	*p*-Value
Regression	3	7387.035074	2462.345025	13.45208	0.000
Residual	41	7504.869370	183.0455944		
Total	44	14891.90444			
	Coefficients	Std. error	t Stat	***p*-Value**	VIF
Intercept	26.63152004	7.871756676	3.383173685	0.002	
AGE	0.361819972	0.111072883	3.257500487	0.002	1.2017
BIRCH70to100	−15.23073127	4.255736666	−3.578870701	0.001	1.1334
DENSITY70to100	−7.494992915	4.270371858	−1.755114815	0.087	1.1004

and each variable are significant at 0.01 level, except DENSITY70to100 which is marginally significant at 0.05 level. The variance inflation factor (VIF) for independent variables is within the 1.1–1.2 range, demonstrating very little collinearity in the model. Analysis of the fit plots and residual distributions allows for the analysis of trendlines and demonstrates that heteroscedasticity is not a problem (Fig. 3).

The model demonstrates that the chance of bacterial wetwood infection increases with birch age, roughly by 0.36% per year. The distribution of bacterial wetwood is smaller for the stands with higher concentration of birch. The stands with a birch proportion of 70% or more demonstrate 15.3% lower infection rate, on average. This result may be partially explained by the fact that silver birch often creates pure stands in the most favourable growing conditions, where trees demonstrate high vigour and survivability. The bacterial wetwood is also less likely to occur in stands with higher relative density of stocking. For stands with tree density ranging from 0.70 to 1.00, the spread of disease is on average 7.5% lower when compared to the stands with tree density under 0.70.

Effect of forest type conditions, bark classes and plant associations

Our research demonstrated that birch trees that grow on wetter soils and on depressions are subject to more intensive dieback. This phenomenon is associated with the shallow surface root system of birch and elevated water stress during a sharp decrease in the level of ground water during dry periods. For drier sites, especially those with richer soils, the percentage of damaged trees is lower (Fig. 4, left). This is particularly true

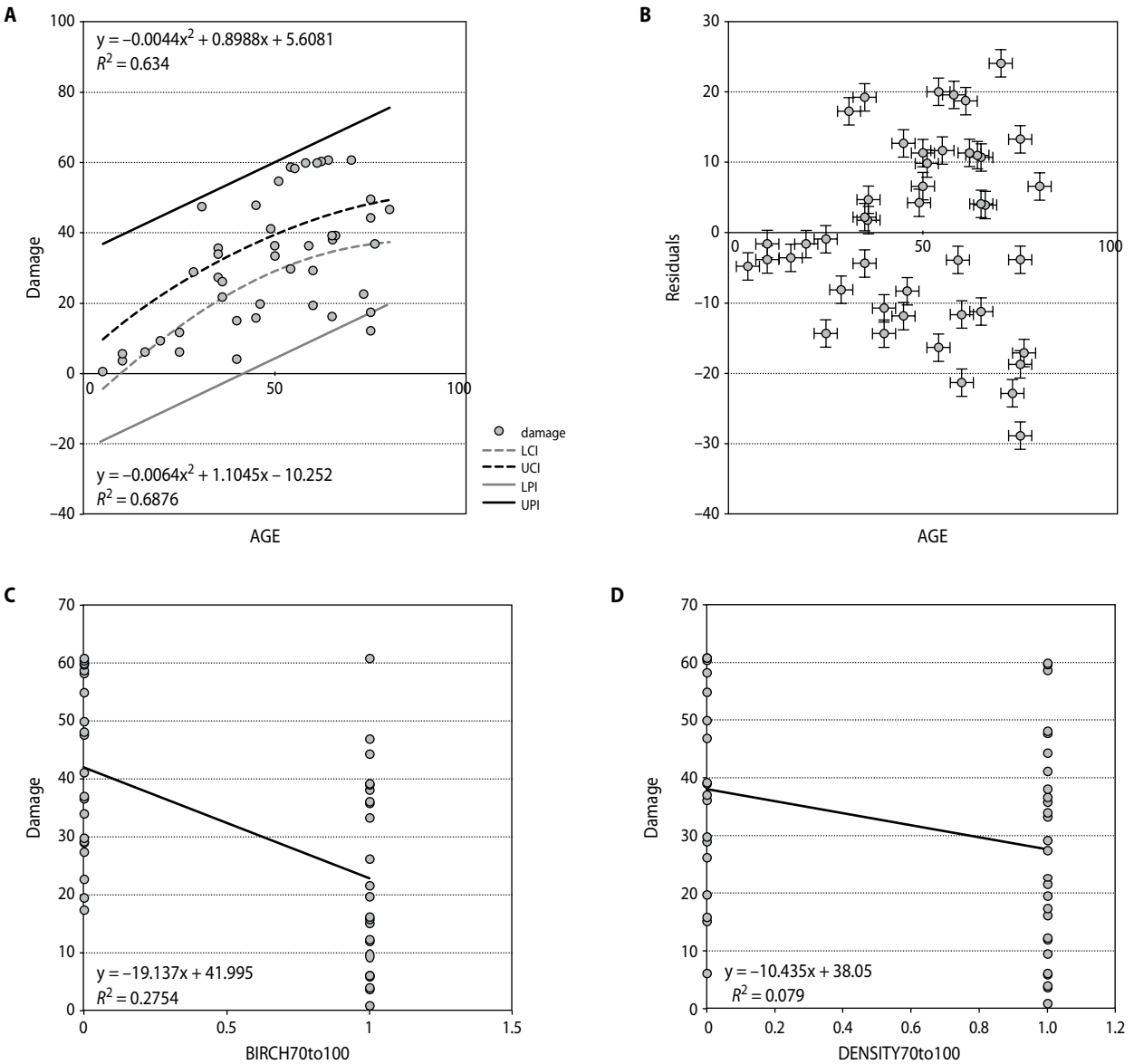

Figure 3. (A) AGE linear fit plot; (B) AGE residual values; (C) BIRCH70to100 linear fit plot; (D) DENSITY70to100 linear fit plot

LCI – lower confidence interval, UCI – upper confidence interval, LPI – lower prediction interval, UPI – upper prediction interval.

for trees featuring longitudinally fissured (20.2%) and coarse fissured (17.4%) bark, and noticeably less for trees with plain and rhomboid fissured bark (12.1% and 5.1%, respectively).

Birch associations with *Pteridium aquilinum* (L.) Kuhn (PTER) in conditions B_2, B_3 and C_3 and with *Vaccinium myrtillus* Linnaeus (VACC) in conditions B_3 and C_3 were more affected by bacterial wetwood (31.6% and 44.3%, respectively). At the same time, birch associations with *Brachypodium sylvaticum* (Hudson) Beauv. (BRACH) in conditions B_2 and C_2 and with *Sphagnum palustre* Linnaeus (SPHAG) and *Calluna vulgaris* Linnaeus (Hull) (CALL) in conditions A_2, A_3 and B_2 experienced lower damage (Goychuk et al. 2018). To a certain degree, these data correlate with forest site conditions, where the greatest

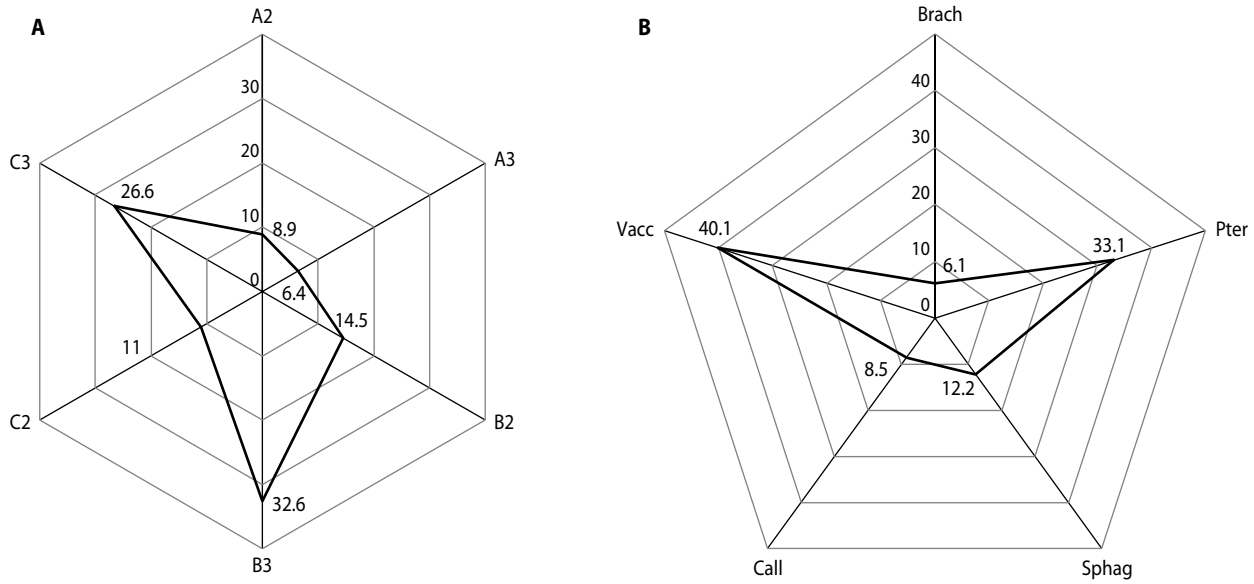

Figure 4. Bacterial wetwood distribution (%) depending on forest site conditions (A) and plant associations (B)

Table 4. Dynamics of bacterial wetwood spread in birch stands depending on meteorological factors

Year	Average annual air temperature/during the growing season, ºC	Annual precipitation/during the growing season, mm	Moisture supply index V/during the growing season	Infected area, ha
2007	7.8/20.8	552/65	3.1/2.1	876
2008	7.1/19.3	568/77	3.4/2.6	732
2009	7.4/20.1	550/79	3.2/2.6	708
2010	6.8/18.9	625/92	3.7/3.2	689
2011	7.1/19.0	587/73	3.4/2.5	797
2012	7.0/21.1	560/59	3.3/1.9	911
2013	7.6/19.4	562/51	3.2/1.7	1023
2014	8.3/20.5	510/46	2.8/1.5	1180
2015	8.9/21.2	491/44	2.6/1.4	1327
2016	8.7/20.9	524/38	2.8/1.2	1419
2017	8.8/20.4	519/43	2.8/1.4	1372
2018	8.7/20.1	534/59	2.9/2.0	1259

Note: Meteorological data obtained from https://www.worldweatheronline.com. The moisture supply index V (aka hydrothermal coefficient of moisture Selyaninova) is calculated as V = R/(t + 10), where R is the average precipitation in cm during growing season and t is the average temperature in °C for the same period (Selyaninov 1928).

distribution of bacteriosis is noted in wet edatopes (Fig. 4, right).

Effect of meteorological factors and entomofauna

Our research confirmed that catalysing factors of bacterial wetwood of birch in the study region included meteorological and related abiotic factors such as temperature, humidity, solar radiation, water, texture and soil aeration. The calculated moisture supply index is on a continuous downward trend in recent years, while the spread of bacterial wet wood is demonstrating explosive growth. The decrease by half of the moisture supply index correlated with a near doubling of the increase in the forest area affected by the disease (Tab. 4).

Linked to meteorological condition, the role of entomofauna as a potential vector for the spread of

bacterial wetwood was also investigated. *Tremex fuscicornis* (Fabricius) was found to be the dominant xylophagous species in affected birch stands with the level of dominance being 82.4%. *Tremex magus* (Fabricius), *Xiphydria camelus* (Linnaeus), *Xiphydria prolongata* (Geoffroy) and *Xiphydria longicollis* (Geoffroy) were also present. The present entomofauna is abundant, which is attributed to favourable hydrothermal conditions and the absence of specialised entomophages.

Based on the eight test characteristics, the life strategy of *T. fuscicornis* is subordinated mainly to K-selection (Tab. 5). The table illustrates the results of expert evaluation of common evolutionary–ecological tactics for life strategies of bacterial wetwood of birch in its relationship to the dominant xylophagous species. Based on the original test characteristics, the continuum of the life strategy of the causative agent is suggested. The determining criterion is the grading of trees by their physiological characteristics, including physiologically healthy, weakened and severely weakened trees. The life strategy of causative agents on the r-axis and K-continuum and the three tactics of disease manifestation are provided, which illustrate the potential ability of adaptive efforts to implement them.

Associated microbiota

When isolating bacteria from the initial stages of bacterial pathologies, the growth of the same type of colonies was observed in Petri dishes. This not only facilitated further work on the identification of the pathogen, but also, to a certain extent, indicated the primary role of *Enterobacter nimipressuralis* in the pathology of bacterial wetwood (Fig. 5).

To determine the number of microorganisms per unit volume, we measured the inoculum of the research material on a dense nutrient medium using Koch's method, followed by counting colonies under the assumption that single cells usually form one colony. The average number of CFU isolated from the affected tissues of *B. pendula* ranged from 2 to 164 CFU. The largest numbers (164 and 127 CFU for bast and cambium, respectively) were attributed to *E. nimipressuralis*. *Bacillus subtilis* was well represented as well. For *Xanthomonas campestris,* only two samples generated positive results (23 and 16 CFU for bark and phloem, respectively) (Tab. 6).

The obtained culture of *E. nimipressuralis* cells was characterised by small polymorphic sticks with peritrichous flagella placement, arranged singly or in pairs, rounded at the ends, ranging in size from 0.45 to 1.75 μm; it was gram-negative, motile and did not form

Table 5. Assessment of evolutionary–ecological tactics, manifestations and implementation of life strategies of bacterial wetwood

Life strategy	Energy spending of adaptive efforts on the implementation of tactics[a]			Adaptation assessment to mastering		Population density *Tremex fuscicornis*	Exit holes on birch plants, (number/dm^2)	Size of ecological niches of host plants
	Reproduction	Survival	Trophic connection	Time	Space			
Physiologically healthy trees								
Typical K	++	+++	+	+++	++	Subthreshold, with a tendency to increase	0.09	Narrow
Physiologically weakened trees								
K-r-domination of K-selection	+++	++	+	++	+++	Three and more threshold levels, with increasing numbers	10.3	Moderate
Physiologically severely weakened trees								
r-k-d domination of r-selection	+++	+	+	+	++	Uncontrolled, cascading growth	10.9	Wide

Note: [a]Energy spent on adaptive implementation efforts tactics (+++ significant (high); ++ insignificant; + moderate).

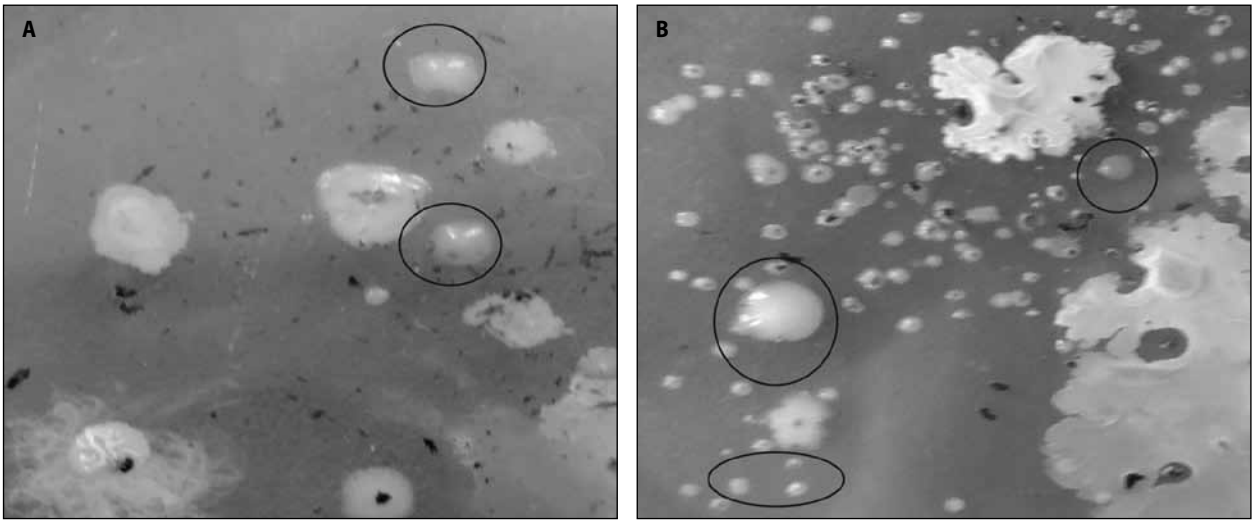

Figure 5. Colonies of isolated bacteria, including Enterobacter nimipressuralis

Table 6. Number of bacteria isolated from the affected tissues *Betula pendula*

Tissues	Strains, the number of CFU			
	Enterobacter nimipressuralis	*Xanthomonas campestris*	*Bacillus subtilis*	Others
$Bast_{2-1}$	164	0	54	-
$Bark_{3-1}$	68	0	27	15
$Bark_{3-2}$	19	23	18	39
$Bast_{2-4}$	41	16	21	24
$Cambium_{1-2}$	127	0	31	-
$Bast_{2-6}$	102	0	46	-
$Sapwood_{4-1}$	62	0	41	2
$Bast_{4-1}$	77	0	26	11

CFU – colony-forming units.

spores. Facultative anaerobes grew well on the Ushinsky, Eijkman, Liske, Fermi media with asparagine (they formed strongly pronounced or moderate turbidity, pellicle and sediment). There was no growth in Kohn medium or Czapek medium. On meat–peptone agar (MPA), white, shiny, smooth and round-shaped colonies with somewhat uneven edges formed. In MPB, weak turbidity with traces of pellicle was found. They grew better on PA, where after 40–48 hours of growth after sowing, they formed round colonies up to 4 mm in diameter, the edges of which were elevated, hilly or slightly wavy, and stood out more sharply from the middle. The surface of the colonies was smooth, shiny, white and grey, and translucent. During daylight clarification, a corrugated strip along the edges and radial rays were clearly visible (Patyka and Pasichnyk 2014; Tatarintsev 2014).

Due to the active fermentation of sources of carbon nutrition, all strains fermented (with acid and gas) raffinose, arabinose, mannose, fructose, maltose, lactose, sorbitol and mannitol. Isolates formed gas on manite and salicin, but not on dulcite. They did not absorb inositol. Milk was acidified, which was accompanied by its coagulation. There were no proteinases splitting milk proteins and gelatin, and therefore, no indole, ammonia or hydrogen sulphide was formed. Isolates formed amylases, but not pectinases. They reduced nitrates and absorbed ketoglutaric, citric, formic, acetic, malic, succinic, fumaric and lactic organic acids. A certain variability of isolated strains in the assimilation of certain carbohydrates and alcohols can be explained by the influence of environmental factors on their biochemical properties. An ecological niche affects the antigenic composition of bacteria; therefore, one should expect such an effect on other properties (Khodaygan et al. 2012).

The only reliable way to differentiate pathogens from saprotrophs is their pathogenicity or ability to infect living cells. As a result of artificial inoculation of model trees, *E. nimipressuralis* is experimentally proven to be the primary causative agent of bacterial wetwood in silver birch stands (Fig. 5). *X. campestris* is

found to be mainly concomitant, with only one variant of *X. campestris* showing weak pathogenic properties. The leaves and shoots of *B. pendula* are not found to be sensitive to *E. nimipressuralis*.

Mycobiota in the pathogenesis of bacterial wetwood was represented by the fungi *Rhizopus microsporus* Tiegh., *Mucor mucedo* Lin., *Penicillium aurantiogriseum* Dier., *Penicillium purpurogenum* Stoll, *Acremonium strictum* W. Gams and turned out to be an accompanying microflora. Micromycetes isolated from the pathology of bacterial dropsy will be used in our study of interactions in "bacterium–micromycete" and "micromycete–micromycete" systems.

Discussion

Owing to the continuous evolutionary adaptation of plant biomes to intensifying environmental changes, catastrophic disturbances of even the most resistant plant species occur. One of the manifestations of such disturbances in recent years is an increase in the number and distribution of phytopathogenic organisms that have a detrimental effect on forest biodiversity. Bacteria are presently one of the most aggressive pathogens causing both epiphytotic and panphytotic bacteriosis, including in forest biomes.

Bacterial wetwood is the vascular parenchymatous bacteriosis of birch, characterised by complex patterns of propagation and significant phenotypic and modification variability. The causative agent of bacterial wetwood of silver birch is *E. nimipressuralis*, the pathogenicity of which was proved in the experiment. Its activity is directly related to the redistribution of nutrients in violation of the physiological state of the plant. The pathogen overwhelms plant defence mechanisms by injecting directly effector agents into plant cells to suppress a host response. Virulence involves the production of cell-degrading toxins, enzymes or plant hormones, often under the control of quorum-sensing mechanisms (Van der Wolf and De Boer 2015).

Because of its spread and variability, bacterial wetwood of *B. pendula* poses an urgent and complex challenge for forest managers and plant pathologists alike. Efficient control of bacterial wetwood is achieved mainly by proper planning and prevention. Direct methods of bacterial wetwood control in natural birch stands, such as chemical spraying, sanitary felling, pruning, soil fumigation or fertilisation, often are not economical or practical (Ward and Pong 1980; Agriculture and Biosciences International 1999).

Our research demonstrated that tree stress prevention, proper site selection based on the hydrological regime and soil quality, improved timing of sanitary cuttings and timber harvests, optimised rotation age and enhancing plant biodiversity make it possible to significantly alleviate many problems associated with bacterial wetwood. When planning for soil protection and reforestation projects, it is critical to account for present and projected climatic and environmental conditions. A systems approach to integrated pest management is the key to effective control of bacterial wetwood in *B. pendula* stands.

Proper identification of causative agents, their pathogenic properties and antagonistic relationships between the components provides a solid foundation for developing effective biochemical control methods of bacterial wetwood. More research is needed to assess the epiphytotic spread of bacterial wetwood across different birch biomes and its variability on the landscape scale (Alizadeh et al. 2017). The methods of early detection, including modern non-invasive techniques such as nuclear magnetic resonance and electrical resistance, should be further investigated (Goh et al. 2017).

To date, infectious diseases of forest tree plants are associated mainly with an external infection that penetrates plants through biological or mechanical carriers, as well as by contact. Recent studies, including in Ukraine, experimentally confirmed the presence of phytopathogenic endophytic bacteria in automicrobiota of healthy plants. Such bacteria are proposed to be called vital obligates (from the Latin *vitalis* meaning vital and *obligatia* meaning obligatory) (Gvozdyak et al. 2011).

The presence of the causative agent of bacterial wetwood in minor quantities in healthy plants requires a different approach to understanding the concept of the incubation period or the period between infection and the appearance of the first symptoms of the disease. Under the classical definition, the duration of this period depends on the type of pathogen, sensitivity to the pathogen of a tree plant, infectious load, etc., and may continue from a few days to several years. As vital obligates are constantly present in healthy plants in a state of incubation, there is a need to distinguish between ex-

ternal and internal infections or between potential and actual incubation periods.

Vital obligates accompany plants from generation to generation and perform a wide range of biocontrol, regulatory, protective and possibly other unknown functions. However, in the case of a violation of systemic interactions and metabolic processes under the influence of various (often not fully elucidated) factors, which are the basis of any pathological process, vital obligates cause infectious pathologies of plants, including epiphytotic, without the participation of external infection.

The seasonal dynamics of the resistance of silver birch and other woody plants is characterised by a genetically determined circadian structure. It lies in the fact that physiological processes are characterised by the alternating amplitude of oscillations of the maximum stability of the stands with a gradual extinction of this process. This means that in the summertime, no manifestations of pathogenesis are observed, despite the presence of a large population of *Tremex* spp. and other xylophagous insects and their frequent contacts with the plant. Thus, the process of transmission of the pathogen is also subject to the circadian rhythm of the processes of resistance and the extinction of the immune reactions of silver birch.

Xylophagous insects are not only the determining factor in the preservation and accumulation of infection, but also one of the vectors of the spatial distribution of bacterial wetwood in physiologically weakened *B. pendula* stands. Their role in spreading the infection, however, may only concern weakened stands and individual trees. Externally healthy trees exhibit pronounced resistance to xylophagous insects. Their appearance on the trunks of birch, as well as other woody plants, testifies to the so-called "clinical death" of the plant, which may serve as a biological indicator of the physiological state of stands.

The regulatory factor of xylophagous insects is exclusively phytohormones of a woody plant, which closely correlate with its general physiological state. Physiologically healthy trees demonstrated a strong tendency for implementing survival tactics. For weakened trees, adaptive efforts were directed to the implementation of breeding tactics. This indicates a potential ecological and trophic association of the causative agent of bacterial wetwood with xylophagous insects.

Therefore, in the epiphytotic pathologies of forest woody plants, including *B. pendula*, it is extremely important to assess biotic and abiotic factors, including hydrothermal stress, as catalysts for pathology. The rate at which infection spreads is largely associated with two synoptic anomalies: unusually high average air temperatures of the summer months and abnormal disruption of hydrological regime. Repetition of these anomalies at short intervals weakens the stands and prevents them from recovering stability (Cherpakov 2012; Sagitov et al. 2005). It is proposed to introduce a "pre-disease" phase in the phases of the infectious process – a phase during which the physiological (metabolic) processes in plants are violated.

Different sensitivities of different birch sample groups to bacterial wetwood, even in its foci, emphasise not only the plasticity of the species, but also the broad species, and form diversity of the genus *Betula*. To a certain extent, our research is consistent with the experimental data of other authors on the resistance of varieties of birch pathogens. This issue is extremely important in the context of the formation of biologically stable stands with the participation of birch.

Elements of the antagonistic relationship between the components of various systematic and functional groups of mycoorganisms and microorganisms indicate the possibility and necessity of using this phenomenon in the context of the mechanisms of positive and negative feedback to develop means and methods for the biological protection of forest trees (including *B. pendula*) from causative agents of infectious diseases, including bacteriosis (Drozda and Goychuk 2018a, b).

Our research demonstrated the presence of such antagonistic relationships between the components of the "bacterium–bacterium" system, particularly the inhibitory effect of *B. subtilis* on *E. nimipressuralis*. In combination with serology, cell wall analysis and deoxyribonucleic acid homology studies, this may be a promising direction for biological control of pathogenic microbiota of bacterial wetwood (Goychuk and Shvets 2017).

Conclusion

This research has provided new insight into certain aspects of etiology, symptoms and pathogenesis of the bacterial wetwood in *B. pendula*. *E. nimipressuralis* has

been experimentally determined to be a causative agent of the disease. The role and place of microorganisms associated with the causative agent of the disease, in combination with meteorological conditions and harmful entomofauna have also been clarified. With the help of statistical modelling, definite relationships between the spread of bacterial wetwood in birch stands of different ages, forest stand composition, relative density of stocking and in different forest conditions have been established.

Our research demonstrated that moisture supply and soil richness are among the catalytic factors of pathology. The role and place of vital obligates and harmful entomofauna as a vector for the spread of infection have been investigated. Recommendations on the future research of bacterial wetwood have been provided, as well as suggestions on improving the management practices to better counter the spread of the disease.

Future microbiological research of bacterial wetwood should be coordinated with studies of its other possible causes, such as water and oxygen stress, fungal pathogens, tree damage due to forest management and harvesting operations, etc. Special attention should be paid to the ecological and trophic associations of the pathogen with different populations of xylophagous insects, their correlation with meteorological conditions and potential roles as indicators of the physiological state of birch stands. Systemic interaction of the causative agent with other microorganisms is also of interest as a topic of future research, including fungi, pectolytic and phenyl oxidising bacteria.

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Conflict of interest

The authors declare no conflict of interest pertaining to this study.

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