

Bacterial wetwood of *Ulmus glabra* Huds. in the relict coenopopulation of the Pokutsky Carpathians (Ukraine)

Ivanna Kulbanska¹, Anatoliy Goychuk¹, Myroslava Soroka², Petro Plikhtyak³, Maryna Shvets⁴ ✉, Lidiya Pasichnyk⁵

¹ National University of Life and Environmental Sciences of Ukraine, General Rodimtsev 19, 03041, Kyiv, Ukraine

² National Forestry University of Ukraine, Generala Chuprynky 103, Lviv 79057, Ukraine

³ Kutske Forestry State Enterprise, vul. Sichovykh Striltsiv 1, village Yabluniv, 78621, Kosiv district, Ivano-Frankivsk region, Ukraine

⁴ Polissia National University, Staryi Boulevard 7, Zhytomyr, 10008, Ukraine

⁵ Institute of Microbiology and Virology named after D.K. Zabolotny NAS of Ukraine, Kyiv, 03143, Ukraine

ABSTRACT

In recent years, many abiotic and biotic factors have negatively impacted the health status of *Ulmus glabra* trees. Based on the syntaxonomic analysis of vegetation and a set of laboratory diagnostic studies, an attempt was made to isolate the catalysing factors and identify the causative agents of pathogenic processes that lead to the weakening and death of individuals of the *U. glabra* coenopopulation. The aim of the research was to evaluate the current health condition of trees of the relict coenopopulation of *U. glabra*, identify the factors of their weakening and dieback, study the pathogenesis and aetiology of the disease and experimentally confirm its causative agent.

Classical geobotanical and silvicultural, special microbiological (in particular, bacteriological analysis), mycological, and statistical research methods were used in the work.

Based on many years of research in the Pokutsky Carpathians, typical symptoms and signs of bacterial infection of *U. glabra* individuals resistant to Dutch elm disease were identified and described. It has been found that the current threat to old (100 or more years) *U. glabra* trees is a systemic vascular parenchymal disease known as bacterial wetwood. It has been studied that the drying of trees develops from the upper part of the crowns, and later, characteristic longitudinal cracks are formed on the trunks. In the active phase of the disease, there is a fountain-like release of exudate, and wet rot with a typical sour smell of fermentation appears. It was investigated and experimentally confirmed that, based on the signs of infection and the set of main characteristics, isolates of bacteria from the wood of diseased trees of *U. glabra* belong to the causative agent of the disease – the phytopathogenic bacterium – polybiotroph *Lelliottia nimipressuralis* (Carter 1945) Brady et al. 2013.

KEY WORDS

tree health, symptoms of infection, identification of the pathogen, elm trees, bacteriosis

INTRODUCTION

The species of genus *Ulmus* L. are very ancient inhabitants of Earth: their pollen was found in the Miocene deposits of most European regions (Łańcucka-Środoniowa 1963). In the middle Holocene, the species of the genus *Ulmus* became permanent elements of the European non-native forests, spreading from Western Europe to the Urals from the refugia of the Portuguese mainland forests (Monteiro-Henriques et al. 2010). In the Carpathians, species of the genus *Ulmus* appeared during the Atlantic phase of the Holocene (Firbas 1949). In the 20s and 70s of the XX century, elms were practically eliminated from the broad-leaved forests of Europe due to two epidemics caused by the hypervirulent phytopathogenic fungi *Ophiostoma ulmi* (Buisman) Melin & Nannf (1934) (Syn. *Graphium ulmi* M.V. Shwarz (1922), *Ceratocystis ulmi* (Buisman) C. Moreau (1952), *Ceratostomella ulmi* Buisman (1932)) (Index Fungorum, 2023) and *Ophiostoma novo-ulmi* Brasier (1991) and *O. himal-ulmi* Brasier & Mehrotra (1995) (Caudullo and De Rigo 2016). This disease of elms was called either Dutch because of its widespread distribution in Holland or graphiosis after the old name of its causative agent *Graphium ulmi* M.V. Schwarz (Jernelöv and Jernelöv 2017; Thomas et al. 2018). The modern name of this disease Dutch elm disease (DED) accurately describes its typical symptoms: infected elm trees, preventing the progress of the phytopathogen along the upward (xylem) flow of substances in the trunk, clogging the vessels with gums, which eventually leads to a lack of water, wilting and dieback of the trees (Thomas et al. 2018; Bernier 2022). Agents of DED spread are elm bark beetles (Coleoptera, Curculionidae: Scolytinae) (Santini and Faccoli 2015; Jürisoo et al. 2021) and xylobiont nematodes (Polyanina et al. 2019). In addition to the causative agents of mycoses, other phytopathogens – phytoplasmas (Flower et al. 2018) and phytopathogenic bacteria – also affect the health condition of weakened elm trees (Alizadeh 2017; Ali et al. 2020). In particular, the bacterium *Xylella fastidiosa* Wells et al. causes bacterial burn of elm leaves (Ali et al. 2020). Large-scale studies are underway on potential biological control of DED spread (Büchel et al. 2016; Martín et al. 2019; Scheffer and Strobel 2020) and searching for resistant elm individuals (Domínguez et al. 2022). It has been researched that almost all European elm species are susceptible to infection, but only *Ulmus*

glabra Huds. (IPNI ID: 856863-1, GBIF ID: 5361866, The PlantList ID: kew-2448690) is the most resistant to DED (Gravendeel et al. 2009).

Considering the features of the pathogenesis and the rapid spread of DED, the test use of a Geographic Information System (GIS) for the epidemiological examination of elm diseases, in particular, and the diagnosis and forecasting of the spread of bacterial wetwood has begun (Alizadeh et al. 2017), for which the symptoms are formation of cracks and wounds on the branches and trunks of elms, leakage of bacterial fluid from the affected organs in the spring and a strong smell of fermentation (Alizadeh 2017). Bacterial wetwood is one of the most common and dangerous diseases of forest trees, in particular, *Quercus robur* L. (Kulbanska et al. 2023; Alizadeh 2017; Alizadeh et al. 2017), *Betula pendula* Roth (Goychuk et al. 2020) and *Abies alba* Mill. (Kulbanska et al. 2022). According to the results of biochemical and molecular tests, the causative agents of this pathology were identified, namely, representatives of several genera of bacteria: *Clostridium*, *Bacillus*, *Enterobacter*, *Klebsiella* and *Pseudomonas* (Alizadeh 2017). In particular, some species were identified: *Enterobacter nimipressuralis* (Carter 1945) Brenner et al. 1988 (Khodaygan et al. 2012), *Brevundimonas bullata* (Gray and Thornton 1928) Kang et al. 2009, *Paracoccus alcaliphilus* Urakami et al. 1989, *P. marcusii* Harker et al. 1998 and *Luteimonas aestuarii* Roh et al. 2009 (Alizadeh et al. 2017).

Due to the complex impact of abiotic (Shvydenko et al. 2018) and biotic environmental factors, the Dutch disease pandemic has led to a decrease in the population density of *U. glabra*, a transformation in gene flow and pollination patterns, which poses a serious threat to its existence (Devetaković et al. 2019). That is why *U. glabra* received the status of Noble Hardwoods (uncommon valuable forest species) and is protected by the European Program of Forest Genetic Resources (EUFORGEN) and requires preservation of the evolutionary potential *in situ* through the cooperation of all countries within the range of its distribution (Aravanopoulos et al. 2015).

Ukraine joined the conservation programmes of the European population of *U. glabra*, giving it the status of a species with small populations and creating the only genetic reserve with an area of 2.5 ha on the territory of the Carpathian National Nature Park. Agricultural techniques have been developed to create artificial elm stands, including the use of local seed material and the

natural regeneration of elm trees over 70 years old from DED lesions that have shown resistance to the disease (Debryniyuk and Skol'sky 2012). The search for resistant age-old generative individuals of *U. glabra* is an extremely important scientific task that can be used in practical forestry to restore elm forests. After all, it is with the help of phytopathogen-resistant woody plants that European countries, in particular, Germany, are restoring lost elm forests and park stands. For example, single trees of *U. glabra* surviving the Dutch disease were found in the floodplain forests of Roztochchya (Pokutsky Carpathians) (Soroka 2008). A coenopopulation of this species is being studied, some trees of which are more than 100 years old. The *U. glabra* coenopopulation in the Pokutsky Carpathians is relict in nature, as it is a remnant of the wet forests of the *Alno-Ulmion* Br.-Bl union. et R. Tx. 1943, which, as a result of dynamic changes in vegetation and a drop in the groundwater level, are gradually transformed into forests of *Tilio platyphyllis*–*Acerion pseudoplatani* Klika 1955.

The examined coenopopulation does not belong to the complex of beech forests, as traditionally believed, but is an independent element of groups of rare mountain vegetation.

Although in recent decades the intensity of the spread of Dutch disease in the forests of Ukraine has decreased (Debryniyuk and Skol'sky 2012), author's research recorded a new wave of weakening and dieback of age-old generative individuals of *U. glabra* in the Pokutsky Carpathians due to the spread of another disease of bacterial aetiology.

If in the past the death of *U. glabra* individuals was mainly caused by mycoses, at the present stage, even trees resistant to mycoses are dying from bacterial infections. Therefore, special attention was needed to study the primary symptoms of infection and the features of pathogenesis of bacterial wetwood of *U. glabra* for early diagnosis and identification of individual plants with biotic resistance to it.

METHODS

The aim of the research was to evaluate the current phytosanitary state of trees of the relict coenopopulation of *U. glabra*, identify the factors of their weakening and dieback, study the pathogenesis and aetiology of the

disease and experimentally confirm its causative agent. The object of the research was old *U. glabra* trees. The subject of the study was the pathogenesis of bacterial wetwood as a leading factor in weakening and dieback of *U. glabra* trees.

The research was carried out during 2019–2023 in the forests of the 'Kutske Forestry' branch of the State Specialised Enterprise 'Forests of Ukraine'. According to the geobotanical zoning of Ukraine, the study area belongs to the district of beech forests of the Ukrainian Carpathians, the subdistrict of dark coniferous–beech water-divided forests of the region of Pokuttia-Bukovina spruce-fir-beech and spruce-beech-fir forests (Golubec 2003).

Based on a 5-year assessment of tree health condition, detailed field surveys and the collection of biotic material, with further laboratory analysis, the main threats that could lead to death of the old *U. glabra* trees were identified.

During the survey of the forests of the branch of 'Kutske Forestry' in the forest fund of the Kosivske Forestry (compartment 31, subcompartment 9) in the gorge of a forest stream on the slope of the southern exposure with a steepness of 5°, a coenopopulation of old *U. glabra* trees was found on an area of about 1 ha. The find is unique considering the epiphytotics of the Dutch disease in the last century, which caused the mass dieback of elms in the forests of Europe. The identified coenopopulation consists of 15 *U. glabra* trees about 100 years old and even includes many juvenile and virginal individuals, which can be a reserve for creating genetic stands.

Health condition of trees was estimated following the Sanitary Forest Regulations (Sanitary Forests Regulations in Ukraine 2016) in six categories – I (healthy), II (weakened), III (very weakened), IV (drying), V (recently died) and VI (dead over a year ago). Crown defoliation was assessed visually in percentage: 0 – absent; 1 point – up to 10%; 2 points – 11%–50%; 3 points – 51%–75%; 4 points – more than 75%. The presence of epicormic shoots was assessed by points: 0 – absent; 1 – single; 2 – multiple; 3 – completely covered trunk.

To evaluate the biodiversity of the relict elm coenopopulation, an inventory of the flora of higher plants, phytosociological descriptions for biotope identification and sampling of fruiting body samples of xylodestructors and litter macromycetes were carried out.

Vegetation research was carried out on the basis of ecological and floristic classification using the method

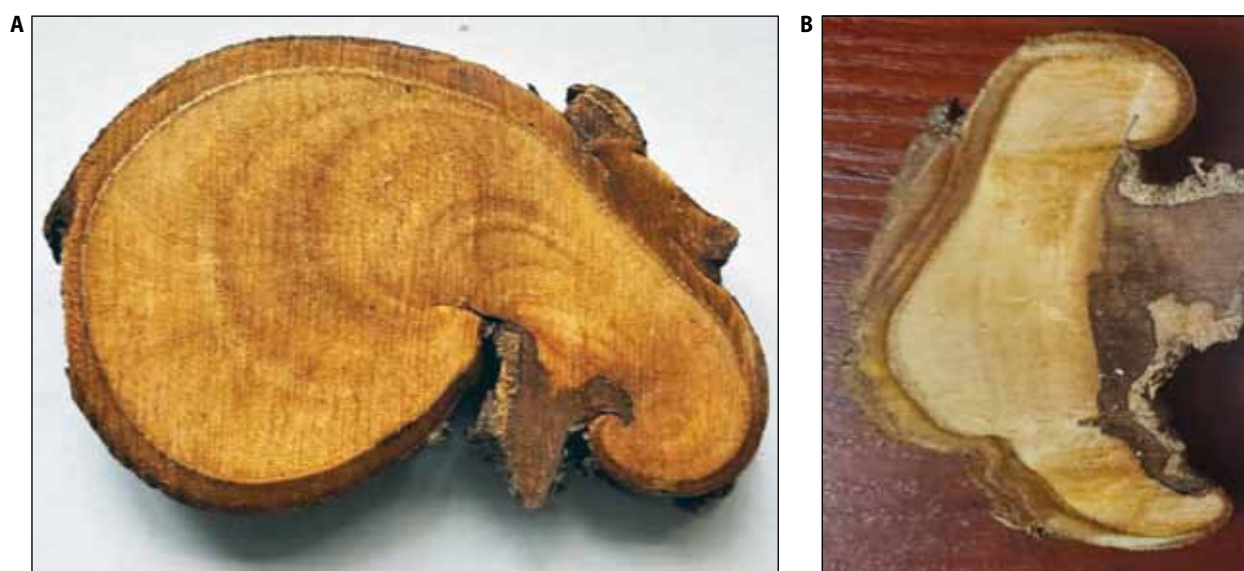


Figure 1. Samples of *U. glabra* wood with typical browning of infected tissues: sample 1 (A) and sample 2 (B)

of J. Braun-Blanquet (1964). The structure and names of syntaxons were provided by W. Matuszkiewicz (2023).

Latin names of species are given according to electronic databases: higher plants – according to World Flora Online (<http://www.worldfloraonline.org/>), mycobacteria – according to Index Fungorum (<http://www.indexfungorum.org>), microbiota – by: List of Prokaryotic names with Standing in Nomenclature (<http://www.bacterio.cict.fr/e/erwinia.html>).

Wood samples of *U. glabra* affected by bacteriosis were used for bacteriological research. The material for analysis was selected at the border of externally healthy and affected wood with signs of browning (Fig. 1).

Bacteriological analysis was carried out *in vitro* at the laboratory of the Department of Phytopathogenic Bacteria of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine (IMV NASU). Their diagnosis was carried out by comparing their characteristics with the characteristics of the collection strain *Lelliottia nimipressuralis* 8791 from the collection of phytopathogenic bacteria (Bergey 2005), namely, by homogenisation of plant material followed by sowing on agarised nutrient media in Petri dishes and growing for 4–5 days at +28 °C under thermostat. The isolated bacteria were tested for the presence of oxidase and protopectinase, as well as stained by Gram staining. Isolates of oxidase-negative bacteria

were identified by their anatomical–morphological and physiological–biochemical characteristics (Kovak’s test, Hugh–Leifson’s test, acid formation from carbohydrates, liquefaction of gelatin, formation of reducing sugars from sucrose, growth in 5% NaCl solution, etc.). Identification of bacterial isolates was also according to generally accepted methods (Patyka et al. 2017) and using the API 20E test system and the NEFERMtest24 test system MikroLaTEST®, Erba Lachema. The results were compared with the characteristics of the collection strain *Lelliottia nimipressuralis* 8791 and the characteristics of the bacteria given in Bergey’s Manual of Systematic Bacteriology (2006). The phytopathogenic properties of the isolates were tested by artificial infection with a bacterial suspension at a titre of 10^8 – 10^9 cfu ml⁻¹ *in vivo* and *in vitro* conditions of vegetative organs of *U. glabra* and test and indicator plants (*Nicotiana tabacum* L., *Phaseolus vulgaris* L., *Kalanchoe laciniata* L.).

RESULTS

Detailed forestry and phytosociological studies have shown that a unique natural complex of mountain riparian forests has formed here, the taxa of which belong to three forest associations, rare on a European scale, which, due to climate changes, human economic activ-

ity and accompanying pathogenic processes in forests, have fallen into the zone of extinction risk. These are forest associations of the class *Querc-Fagetum* Br.-Bl. et Vlieg. 1937 – *Stellario nemorum-Alnetum glutinosae* Lohm., *Ulmus glabrae-Aceretum pseudoplatani* Issler 1926 and in the highest part of the slope – *Dentario glandulosae-Fagetum* W. Mat. 1964 et Guzikowa et Kornaś 1969 in three variants – *Dentario glandulosae-Fagetum* 1969 *Picea abies*, *Dentario glandulosae-Fagetum* 1969 *Abies alba* and *Dentario glandulosae-Fagetum* 1969 typicum. In the direction from the water flow upwards, they form a succession of hygrophilous vegetation in the altitudinal gradient of ravine forests. Syntaxon, within the phytocoenoses of which the *U. glabra* coenopopulation is registered, is diagnosed as a fragment of the *Ulmus glabrae-Aceretum pseudoplatani* association based on general phytocoenotic features. Individuals of *U. glabra* were preserved only on the first terrace of the riparian part of the water stream, forming a strip that follows *Stellario nemorum-Alnetum glutinosae*. In this biotope, the most important edificers are individuals of *U. glabra*, among which 15 trees are old. General appearance of old *U. glabra* trees are shown in the figure (Fig. 2).

The assessment took into account the category of sanitary condition, the degree of defoliation and the

presence/absence of epicormic shoots and vegetative buds (Tab. 1).

In recent years, the existence of this relict elm population, which has survived the epiphytotic Dutch disease, is threatened by a number of biotic factors, among which a symptom of a bacteriosis, a dangerous infectious disease, has been diagnosed based on typical macroscopic signs (Soroka 2008). In the lower part of the trunk, typical signs of damage by bacterial wetwood. The causative agent of wetwood is the phytopathogenic bacterium-polybiotroph *Lelliottia nimipressuralis* (Carter 1945) Brady et al. 2013 (confirmed experimentally).

Several associated phytopathogens of *U. glabra* were also identified. Thus, the assimilation organs are negatively affected by the spread of the fungi *Taphrina ulmi* (Fuckel) Johanson 1886 causes deformation, in particular, wrinkling and curling, as well as chlorotic leaves, *Septogloeum ulmicolum* (Biv.) Elenkin & Ohl 1912 and *Septoria ulmi* Ellis & Everh. 1897 cause leaf spotting, forming large 0.5–1.0 cm in diameter, rounded, dark brown spots with a light border. Withering of individual branches is caused by *Nectria cinnabarina* (Tode) Fr. 1849; as a result, water exchange is disturbed, leading to accelerated dieback of branches and leaves.

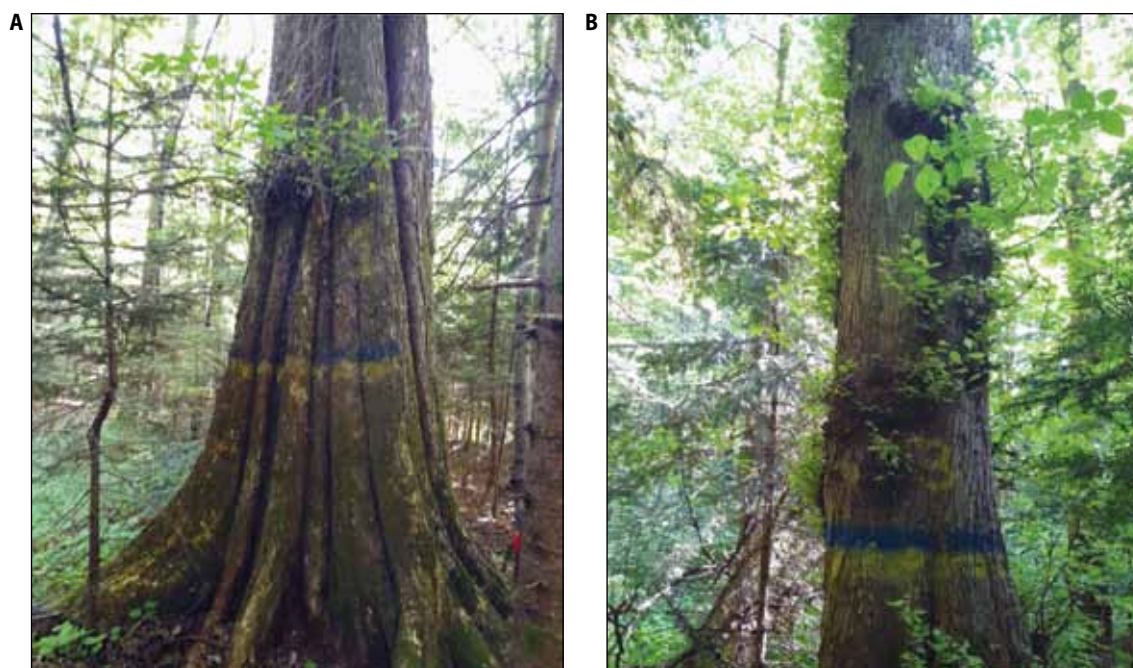


Figure 2. General appearance of old *U. glabra* trees: accounting tree No 1 (A) and accounting tree No 3 (B)

Table 1. Characteristics of the health condition of old *U. glabra* trees and causes of their weakening

Numbers of trees	Forest measurement parameters		Category of health condition	Defoliation, points	Epicormic shoots and vegetative buds, points	Causes of weakening
	diameter, cm	height, m				
1	52	30	I	0	0	–
2	52	29	III	2	2	Bacterial wetwood
3	32	27	I	0	0	–
4	40	28	II	1	1	Dieback
5	90	32	II	2	2	Bacterial wetwood
6	24	20	I	1	0	–
7	28	22	I	0	0	–
8	24	18	II	1	1	Leaf deformation, brown leaf spot
9	28	20	I	0	0	–
10	48	28	I	1	0	–
11	86	33	III	2	3	Bacterial wetwood, <i>Ceriporus squamosus</i>
12	56	30	I	0	0	–
13	36	28	II	1	1	Leaf deformation
14	68	32	II	1	1	<i>Ceriporus squamosus</i>
15	56	31	I	0	0	–

Single fruiting bodies of *Ceriporus squamosus* (Huds.) Quél., 1886 were recorded on the trunk. Even though *Ceriporus squamosus* is an edible mushroom of category 4 when young, it is a typical parasite that penetrates through mechanical damage to the trunk or branches of trees and causes white, finely fissured central rot. In the biotope of the *Ulmo glabrae-Aceretum pseudoplatani* coenosis, we described an accompanying non-pathogenic mycobiota that forms consortial relationships of various orders with elm trees.

Among epiphytic lichen biota, on the bark of the trunks, *Flavoparmelia caperata* (L.) Hale, *Hypogymnia physodes* (L.) Nyl., *Parmelia sulcata* Taylor and *Lecanora carpinea* (L.) Vain. were identified. Lichens are rare in these forests, as the bark of old elms does not allow them to grow. It is known (Asplund and Wardle 2017) that lichens almost do not harm trees, as they rarely penetrate to the living cells of the phellogen. However, by inhabiting the bark, they complicate gas exchange, delay the flow of precipitation and thereby promote the development of phytopathogenic myco- and microorganisms, as well as harmful insects. In recent years, lichens have been proposed to be used as indicators of the physiological condi-

tion of trees, in particular, focusing on their presence or absence, as well as the density of the substrate. Among the humus saprotrophs, large ‘witch rings’ of the fruiting bodies of *Megacollybia platyphylla* (Pers.) Kotl. & Pouzar, 1972 are most often found here and single specimens of *Strobilomyces strobilaceus* (Scop.) Berk, 1851 entered the Red Book of Ukraine (Didukh 2009).

A visual examination of old *U. glabra* trees revealed typical macroscopic symptoms of bacterial wetwood, which was confirmed during experimental research at the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine (IMV NASU). The registered signs are identical to the typical manifestations of bacteriosis of this type described in the special scientific literature (Meshkova et al. 2022), which confirms persistent signs of infection with bacterial wetwood regardless of the region of research and the type of woody plant.

Based on research and long-term observations in the elm forests of the Pokutsky Carpathians, the typical symptoms of *U. glabra* bacterial wetwood have been identified, which include the following morphological and anatomical signs and changes in the structures of infected trees:

Primary symptoms:

- sparse crown;
- accelerated wilting of leaves and shoots;
- change in colour (yellowing) and premature fall of the assimilation organs on individual shoots or in general the entire crown of the tree;
- wet basal part of the trunk and
- longitudinal cracks on the bark are a typical diagnostic sign of bacterial wetwood, which indicates the transition of the disease into the active phase.

The active phase of the disease

- Release of bacterial exudate from cracks, which is active in April–May, and later it dries on the surface of the bark, forming visible dark wetting spots, which flow down the trunk from the site of infection and later spread over the soil surface. Exudate is a liquid of viscous consistency, brownish-brown in colour, which is a mixture of bacterial cells and the sap of a woody plant, which has a typical unpleasant smell of fatty acid fermentation and is accompanied by the formation of bubbles (Fig. 3A).
- At the base of dieback branches, black wetting spots and ulcers form on the bark, sometimes depressed

wounds of various shapes and sizes. Over time, the bark around these formations dies. When removing the surface layer of the periderm and a part of the primary bark, you can see brown or dark brown necrotic areas, sometimes with a purple tint, extending from the site of primary infection up and down for a length of 0.3–0.8 m and more (Fig. 3B).

- Massive formation of epicormic shoots on the trunks. Epicormic shoots are shortened, have chlorotic, pale or yellowish underdeveloped leaves and form very characteristic ‘witches’ brooms’ (Fig. 3C).
- A pathological or fault star-shaped core is visualised on the cross section of the trunk. The wood of this pathological core is moist, with a sharp sour smell.

Secondary changes in the late stages of the disease

- At the final stage of bacterial wetwood, the pathogenesis is accompanied and enhanced by xylophages and wood-staining fungi. Under such conditions, blackening of the annual rings on the cross section of the trunk, deformation and curvature of the branches and tops are observed.
- The trees show dieback of the crown, damage to the roots and rapid dieback.

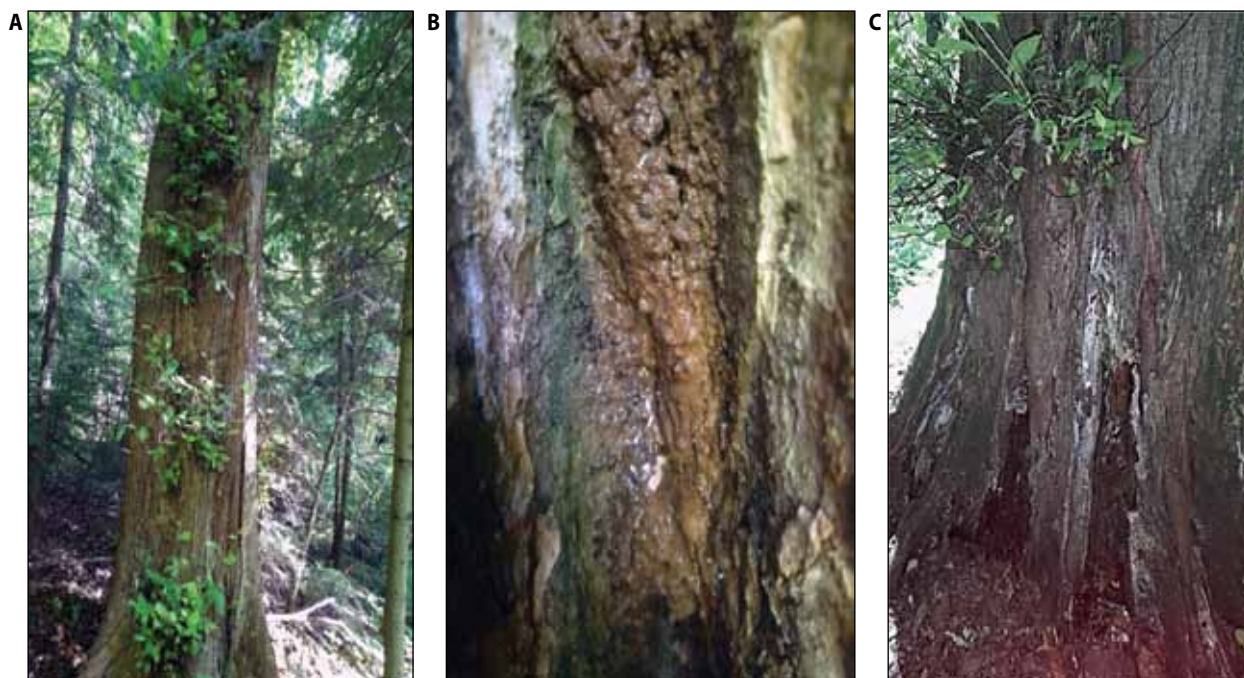


Figure 3. Macro signs in the trunk of *U. glabra* infected with bacterial wetwood: A – leakage of bacterial exudate, B – longitudinal ulcers in the lower part of the trunk, C – mass formation of ‘witches’ brooms’

Based on the results of bacteriological analysis, isolates of oxidase-negative bacteria in grey colonies were isolated from the affected wood of *U. glabra* (Tab. 2).

Table 2. Characterisation of bacteria isolated from *U. glabra* wood samples against collection strain and identifier

Tests	Tested bacteria	<i>Lelliottia nimipressuralis</i>	
		collection strain 8791	Bergey, 2005
Gram stain	–	–	–
Bacterial motility	+	+	+
Yellow pigment	–	–	–
Oxidase	–	–	–
Nitrate reduction	+	+	+
Protopectinase	–	–	–
Use of glucose (anaerobic)	+	+	+

Isolates from elm wood, as well as the collection strain *Lelliottia nimipressuralis* 8791, were found to be motile Gram-negative rods with a ‘bacillus’ cell morphology. The studied bacteria are facultative anaerobes, use glucose both in aerobic and anaerobic conditions and reduce nitrates (Fig. 4).

Bacterial isolates do not produce gelatinase and protopectinase and give a positive Voges–Proskauer reaction. Bacteria use glucose, arabinose, sucrose, meli-

biose and amygdalin as the only source of carbon nutrition (Tab. 3). They do not use inositol and sorbitol and do not form indole and hydrogen sulphide. They also lack arginine hydrolase and lysine decarboxylase, but β -galactosidase and ornithine decarboxylase are present.

Table 3. Properties of phytopathogenic bacteria isolated from *U. glabra* wood samples on API 20E strips

Tests	Reaction/enzyme	Tested bacteria	Collection strain <i>Lelliottia nimipressuralis</i> 8791
1	2	3	4
ONPG	β -galactosidase	+	+
ADH	arginine dihydrolase	–	–
LDC	lysine decarboxylase	–	–
ODC	ornithine decarboxylase	+	+
CIT	use of citrate	+	+
H ₂ S	hydrogen sulphide production	–	–
URE	urease enzyme test	–	–
TDA	tryptophan deaminase	–	–
IND	indole test	–	–
VP	voges–proskauer test	+	+
GEL	gelatinase	–	–
GLU	glucose fermentation	+	+
MAN	fermentation of mannose	+	+

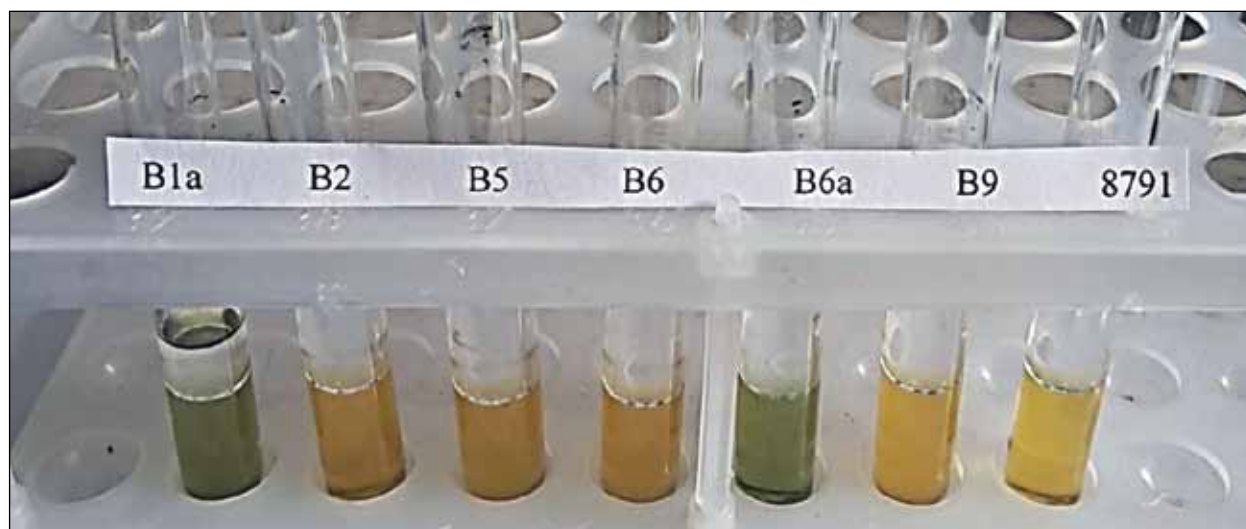


Figure 4. Anaerobic growth of bacterial isolates from samples of infected *U. glabra* wood on Omelyansky medium with glucose (B1a, B2, B5, B6, B6a, B9 – conventional designations of bacterial isolates isolated from elm sample; 8791 – conventional designation of the collection strain)

1	2	3	4
INO	fermentation of inositol	–	–
SOR	fermentation of sorbitol	–	–
SAC	fermentation of sucrose	+	+
MEL	fermentation of melibiose	+	+
AMY	fermentation of amygdalin	+	+
ARA	fermentation of arabinose	+	+

Note: + positive reaction, – negative reaction

Based on the conducted research and experiments, we can state that the lesions of *U. glabra* trees are caused by bacteria, which, based on their morphological, physiological and biochemical properties, are identified as *Lelliottia nimipressuralis* (Carter 1945) Brady et al. 2013 (Brady et al. 2013).

DISCUSSION

A complex of biotopes of rare deciduous vegetation in the mountains of the Pokutsky Carpathians was studied, the specific conditions of which determined the preservation of old *U. glabra* trees, which survived the epiphytity of the DED in the last century and could serve as donors of DED-resistant forms of elm to restore those lost due to DED of tree stands (Soroka 2008). In such conditions, the *U. glabra* coenopopulation has a relic character as a remnant of hygrophilous forests of the Alno-Ulmion Br.-Bl union. et R. Tx. 1943, which, as a result of global climate changes and subsequent vegetation dynamics, are transformed into forests of the union *Tilia platyphylis*–*Acerion pseudoplatani* Klika 1955.

However, based on many years of research, it has been found that *U. glabra* trees resistant to mycosis and Dutch disease, in particular, have been damaged by a systemic vascular-parenchymal disease in recent years, which leads to the weakening and subsequent death of old trees. It has been studied that the drying of trees develops from the upper part of the crowns, and later longitudinal cracks are formed with the peeling of the bark, from which the exudate flows (Alizadeh 2017). In the lower part of the trunks, in deep folds, there are areas of wet rot with a typical sour smell. At this time, changes occur in the morphological structure of the trunk – numerous water shoots are formed and very characteristic ‘witches’ brooms are formed (Bernier 2022). In the anatomical structure, the browning of the xylem, the ap-

pearance of a sharp border between the areas of healthy and infected wood and the formation of a pathological core are noted. It was investigated and experimentally confirmed that the bacterial isolates isolated from the infected elm wood according to the studied characteristics are identical to the collection strain *Lelliottia nimipressuralis* 8791 from the collection of phytopathogenic bacteria of the Institute of Microbiology and Virology and fully correspond to the properties given in the Bacteria Identifier (Bergey 2005).

Thus, on the basis of natural and experimental studies, it was concluded that individuals of *U. glabra*, which survived the epiphytotics of the Dutch disease, in dieback due to infection with bacterial wetwood caused by the aggressive phytopathogenic bacterium-polybiotroph *Lelliottia nimipressuralis* (Carter 1945) Brady et al. 2013. It is also the causative agent of bacterial wetwood with similar symptomatic signs on other forest tree species (Goychuk et al. 2020).

Lelliottia nimipressuralis is the main and most dangerous agent of influence on the condition and viability of old *U. glabra* trees and in the coming years (given the intensity of reproduction and spread of phytopathogenic bacteria), may lead to their dieback. A prerequisite for the mass spread of bacterial wetwood within the relict coenopopulation of *U. glabra* in the conditions of the Pokutsky Carpathians is a decrease in plant resistance and immunity due to impact predictors, in particular, hydrothermal stress and climatic changes (Shvydenko et al. 2018; Zhang et al. 2018), as well as the development of destructive processes caused by less-aggressive infectious agents – pathogens of diseases of the assimilation organs, trunk rots, necrosis, phytophagous insects, etc.

CONCLUSIONS

1. In the mountains of the Pokutsky Carpathians, a complex of biotopes of rare deciduous vegetation was discovered, the specific conditions of which determined the preservation of old trees of *Ulmus glabra*, which survived the epiphytotic of the Dutch disease in the last century and could serve as donors of DED-resistant forms of elm.
2. It was established that individuals of *Ulmus glabra* resistant to mycoses, and Dutch disease, in particular, have been damaged by a systemic vascular-pa-

renchymal disease in recent years, which leads to the weakening and death of old trees.

3. It has been investigated that the typical symptoms of the vascular-parenchymal disease of *Ulmus glabra* trees are the formation of longitudinal cracks with exfoliation, from which discharge of exudate with a sour smell occurs. Numerous water shoots and ‘witches’ brooms’ form on the trunk. In the anatomical structure, browning of the xylem and formation of a pathological core are noted.
4. It was experimentally confirmed that the causative agent of the disease identified is an aggressive phytopathogenic bacterium-polybiotroph *Lelliottia nimipressuralis* (Carter 1945) Brady et al. 2013, which causes similar symptoms of damage (typical symptoms of bacterial wetwood) on other species of forest woody plants.
5. Associated phytopathogens of elm trees are *Taphrina ulmi* (Fuckel) Johanson, *Septogloeum ulmicolum* (Biv.) Elenkin & Ohl, *Septoria ulmi* Ellis & Everh, *Nectria cinnabarina* (Tode) Fr. and *Ceriporus squamosus* (Huds.) Quél.
6. In the future, a promising direction of research is the development of specific methods and ways of protecting forest woody plants, in particular, using biological preparations based on *Bacillus* sp. and other myco- and microorganisms with available antagonistic properties to phytopathogens.

REFERENCES

- Ali, E., Hudson, O., Waliullah, S., Ji, P., Williams-Woodward, J., Oliver, J. 2020. First report of bacterial leaf scorch disease of American elm caused by *Xylella fastidiosa* in Georgia, USA. *Plant Disease*, 104 (6). DOI: 10.1094/PDIS-11-19-2367-PDN.
- Alizadeh, M. 2017. Bacterial wetwood disease. *Journal of Plant Chemistry and Ecophysiology*, 2 (1), 1015.
- Alizadeh, M., Khakvar, R., Babai-Ahari, A. 2017. Isolation and characterization of bacterial agents associated of wetwood disease on Elm trees in Iran. *Acta Phytopathologica et Entomologica Hungarica*, 52, 1–11. DOI: 10.1556/038.52.2017.028.
- Alizadeh, M., Moharrami, M., Rasuly Pirouzian, A. 2017. Geographic Information System (GIS) as a tool in the epidemiological assessment of wetwood disease on elm trees in Tabriz city, Iran. *Cer-cetari Agronomice in Moldova*, 50. DOI: 10.1515/cerce-2017-0018.
- Aravanopoulos, F.A. et al. 2015. Development of genetic monitoring methods for genetic conservation units of forest trees in Europe. European Forest Genetic Resources Programme (EUFOR-GEN), Bioversity International, Rome, Italy.
- Asplund, J., Wardle, D.A. 2017. How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews*, 92 (3), 1720–1738. DOI: 10.1111/brv.12305.
- Bernier, L. 2022. Dutch elm disease. In: Forest microbiology. Volume 2: Forest tree health (eds. F.O. Asiegbu, A. Kovalchuk). Elsevier, Amsterdam, The Netherlands, 291–309. DOI: 10.1016/B978-0-323-85042-1.00010-0.
- Brady, C., Cleenwerck, I., Venter, S., Coutinho, T. 2013. Taxonomic evaluation of the genus *Enterobacter* based on multilocus sequence analysis (MLSA): proposal to reclassify *E. nimipressuralis* and *E. amnigenus* into *Lelliottia* gen. nov. as *Lelliottia nimipressuralis* comb. nov. and *Lelliottia amnigena* comb. nov. *Systematic and Applied Microbiology*, 36 (5), 309–319. DOI: 10.1016/j.syapm.2013.03.005.
- Braun-Blanquet, J. 1964. *Plant sociology*. Basics of vegetation science (in German). Springer, Wien, New York.
- Brenner, D.J. et. al. 2005. *Bergey’s Manual of Systematic Bacteriology*. Springer Science, New York, USA. DOI: 10.1111/j.1574-695X.2005.00055.x.
- Büchel, K., Fenning, T., Gershenzon, J., Hilker, M., Meiners, T. 2016. Elm defence against herbivores and pathogens: Morphological, chemical and molecular regulation aspects. *Phytochemistry Reviews*, 15. DOI: 10.1007/s11101-015-9442-0.
- Caudullo, G., De Rigo, D. 2016. *Ulmus*-elms in Europe: distribution, habitat, usage and threats. In: European atlas of forest tree species (eds. J. San-Miguel-Ayanz, D. de Rigo, G. Caudullo, T. Houston Durrant, A. Mauri). Publication Office of the European Union, Luxembourg, 186–188.
- Debryniyuk, Iu.M., Skol’skyy, I.M. 2012. Cultivation characteristics of forest plantations with the presence of *Ulmus* L. family in Western Forest Steppe region of Ukraine (in Ukrainian). *Scientific Works*:

- Forestry Academy of Sciences of Ukraine, 10, 94–103.
- Devetakovic, J., Čortan, D., Maksimović, Z. 2019. Conservation of European white elm and black poplar forest genetic resource: Case study in Serbia. *Advances in Global Change Research*, 165–186. DOI: 10.1007/978-3-319-95267-3_14.
- Didukh, Ya.P. 2009. Red Data Book of Ukraine. Vegetable Kingdom. Globalkonsaltyng, Kyiv.
- Dominguez, J., Macaya-Sanz, D., Gil, L., Martín, J.A. 2022. Excelling the progenitors: Breeding for resistance to Dutch elm disease from moderately resistant and susceptible native stock. *Forest Ecology and Management*, 511, 120113. DOI: 10.1016/j.foreco.2022.120113.
- Firbas, F. 1949. Spät- und nachtszeitliche Waldgeschichte Mitteleuropas nordlich der Alpen. Bd. I. Gustav Fischer, Jena.
- Flower, C., Hayes-Plazolles, N., Rosa, C., Slavicek, J. 2017. First report of 'Candidatus phytoplasma trifolii'-related strain of 16SrVI-A phytoplasma subgroup, associated with elm yellows disease in American elm (*Ulmus americana* L.) in Ohio, USA. *Plant Disease*, 102 (2), 438. DOI: 10.1094/PDIS-08-17-1154-PDN.
- Golubec, M.A. 2003. Geobotanical zoning of the Ukrainian Carpathians is the basis of rational nature utilization (in Ukrainian). *Proceedings of the Scientific Society of them. Shevchenko*, 12, 283–292.
- Goychuk, A., Drozda, V., Shvets, M., Kulbanska, I. 2020. Bacterial wetwood of silver birch (*Betula pendula* Roth): symptomology, etiology and pathogenesis. *Folia Forestalia Polonica, Ser. A – Forestry*, 62 (3), 145–159. DOI: 10.2478/ffp-2020-0015.
- Gravendeel, B., Eurlings, M., Heijerman, T. 2009. Use of DNA barcoding for host plant identification. *Entomologische Berichten*, 69 (2), 30–35.
- Index Fungorum. Available at <http://www.indexfungorum.org> (access on 02 July 2023).
- Jernelöv, A. 2017. Dutch elm disease in Europe and North America. In: The long-term fate of invasive species (ed. A. Jernelöv). Springer International Publishing, Cham, 161–176. DOI: 10.1007/978-3-319-55396-2_12.
- Jürisoo, L., Adamson, K., Padari, A., Drenkhan, R. 2019. Health of elms and Dutch elm disease in Estonia. *European Journal of Plant Pathology*, 154, 823–841. DOI: 10.1007/s10658-019-01707-0.
- Khodaygan, P., Sedaghati, E., Sherafati, F. 2011. Isolation of *Enterobacter nimipressuralis* associated with bacterial wet wood from elm (*Ulmus* spp.) in Rafsanjan. *Iranian Journal of Plant Pathology*, 4 (188), 481–482.
- Kulbanska, I. et al. 2023. Phytopathogenic bacteria associated with bacteriosis of common oak (*Quercus robur* L.) in Ukraine. *Forests*, 14 (1), Article 1. DOI: 10.3390/f14010014.
- Kulbanska, I.M., Plikhtyak, P.P., Shvets, M.V., Soroka, M.I., Goychuk, A.F. 2022. *Lelliottia nimipressuralis* (Carter 1945) Brady et al. 2013 as the causative agent of bacterial wetwood disease of common silver fir (*Abies alba* Mill.). *Folia Forestalia Polonica, Ser. A – Forestry*, 64 (3), 173–183. DOI: 10.2478/ffp-2022-0017.
- Kuznetsova, O.A., Turenko, V.P., Tovstukha, O.V., Davydenko, K.V. 2023. Incidence of diseases and pests of *Ulmus* sp. in forest shelter belts along the Kyiv-Kharkiv highway (in Ukrainian). *Forestry and Forest Melioration*, 143, 102–111. DOI: 10.33220/1026-3365.143.2023.102.
- List of Prokaryotic names with Standing in Nomenclature. Available at <http://www.bacterio.cict.fr/e/erwinia.html> (access on 02 July 2023).
- Łańcucka-Środoniowa, M. 1963. The state of paleobotanical research on the Miocene of southern Poland (in Polish). *Annales Societatis Geologorum Poloniae*, 33 (2), 129–158.
- Martín, J.A., Sobrino-Plata, J., Rodríguez-Calcerada, J., Collada, C., Gil, L. 2019. Breeding and scientific advances in the fight against Dutch elm disease: Will they allow the use of elms in forest restoration? *New Forests*, 50 (2), 183–215. DOI: 10.1007/s11056-018-9640-x.
- Matuszkiewicz, W. 2013. Guide for the determination of plant communities in Poland (in Polish). PWN, Warszawa.
- Meshkova, V.L., Kuznetsova, O.A., Khimenko, N.L. 2022 Occurrence of *Ulmus* L. in the different forest site conditions of eastern Ukraine. *Forestry and Forest Melioration*, 140, 9–11. DOI: 10.33220/1026-3365.140.2022.3.
- Monteiro-Henriques, T., Costa, J.C., Bellu, A., Aguiar, C. 2010. *Fraxino Angustifoliae-Ulmetum* g

- labrae*: An original endemic and extremely localized forest from mainland Portugal. *Braun-Blanquetia*, 46, 323–327.
- Patyka, V.P. et al. 2017. Phytopathogenic bacteria. Methods of research. Monograph. Volume 2 (in Ukrainian). Windruck. Vinnytsia, Ukraine.
- Polyanina, K., Mandelshtam, M., Ryss, A. 2019. Brief review of the associations of Xylobiont nematodes with bark beetles (Coleoptera, Curculionidae: Scolytinae). *Entomological Review*, 99, 598–614. DOI: 10.1134/S0013873819050038.
- Sanitary Forests Regulations in Ukraine. 2016. Resolution of the Cabinet of Ministers of Ukraine No 756 dated 26 October 2016 (in Ukrainian). Available at: <https://zakon.rada.gov.ua/laws/show/756-2016-%D0%BF#n11> (access on 02 October 2023).
- Santini, A., Faccoli, M. 2014. Dutch elm disease and elm bark beetles: A century of association. *iForest – Biogeosciences and Forestry*, 8. DOI: 10.3832/ifor1231-008.
- Scheffer, R.J., Strobel, G.A. 2020. Dutch elm disease, a model tree disease for biological control. In: Bio-control of plant diseases (eds. K.G. Mukerji, K.L. Garg). CRC Press, Boca Raton, 103–119.
- Shvydenko, A.Z., Buksha, I.F., Krakovskaya S.V. 2018. Vulnerability of Ukraine's forests to climate change (in Ukrainian). Nika-Center, Kyiv.
- Soroka, M.I. 2008. Vegetation of the Ukrainian Ros-tochia (in Ukrainian). Svit, Lviv.
- The World Flora Online. Available at <http://www.worldfloraonline.org> (access on 02 July 2023).
- Thomas, P.A., Stone, D., La Porta, N. 2018. Biological flora of the British Isles: *Ulmus glabra*. *Journal of Ecology*, 106 (4), 1724–1766. DOI: 10.1111/1365-2745.12994.
- Zhang, L., Jiang, Yu., Zhao, Sh., Jiao, L., Wen, Ya. 2018. Relationships between tree age and climate sensitivity of radial growth in different drought conditions of Qilian Mountains, Northwestern China. *Forests*, 9 (3), 135–138. DOI: 10.3390/f9030135.