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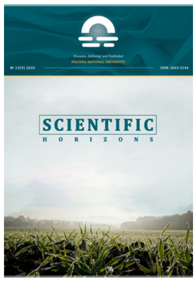

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Tuberculosis Pathology of *Fraxinus Excelsior* L. in Ukraine: Symptomatology, Etiology, Pathogenesis

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Abstract. Given the forestry, the ecological and economic value of forests with the participation of *Fraxinus excelsior* and taking into account the intensive deterioration of their phytosanitary status in modern conditions, a comprehensive study of symptoms, causes, and pathogenesis of ash tuberculosis with further development of effective protection measures, including using biological products based on *Bacillus sp*. and other myco- and microorganisms, is a particularly relevant area of research. The aim of the research is to identify negative abiotic and biotic factors in the pathology of *F. excelsior*. In the process of research general scientific and special research methods were used (microbiological, mycological, phytopathological, entomological, and silvicultural-ecological methods). It is emphasized that the pathology of the common ash is a multifaceted phenomenon with interrelated processes of infectious and non-infectious nature, which in recent years has led to epiphytic dieback and now has a tendency to increase. It has been established that tuberculosis of *F. excelsior* is the most common and harmful disease within the study region and causes more economic than environmental damage. Bacteria of the genera *Pseudomonas sp., Erwinia sp.,* and *Xanthomonas sp.,* as well as micromycetes, which mainly take the place of the concomitant mycobiota. There are five stages (phases) of the development of tuberculosis pathology, which differ significantly in symptoms. The species composition of harmful insects has been identified. It is shown that hydrothermal stress is a catalyst for the epiphytic dieback of common ash. Direct dependence of the spread of tuberculosis on the share of ash in the stands of different age groups was revealed. In the study area, tuberculosis reaches epiphytotics on *F. excelsior*, especially at a young age. The expediency of distinguishing the causes and pathological processes associated with ash tuberculosis is indicated, so as not to confuse the disease-catalyzing factors and the factors that lead to the complete degradation of ash stands

Keywords: common ash, ash tuberculosis, antagonism, harmfulness of the disease, pathogen, symptomatology, pathogenesis

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INTRODUCTION

It is established that a peculiar trigger of the pathological process and genesis of the disease is a set of factors of synoptic nature and as a consequence of dysfunction of immune structures, with obvious manifestation and implementation of ecological and trophic niches of phytopathogens of fungal and bacterial etiology. According to the authors' research, the formation of neoplasms instead of ordinary inflorescences on common ash trees is associated exclusively with bacterial etiology, including the causative agent of ash tuberculosis – *Pseudomonas syringae* pv. *savastanoi*, and is one of the phases (stages) of pathogenesis [1; 2].

Phytoparasitic nematodes have a harmful effect on trees of the genus Fraxinus. Most often noted by researchers on *Fraxinus americana* – *Meloidogyne* sp. *Aphelenchoides* sp., *Criconema* sp., *Criconemoides* sp., *C. beljaevae*, *C. macrodorum*, *Ditylenchus* sp., *Gracilacus audriellus*, *Helicotylenchus* sp., H. playturus, *Hemicycliophora* sp., *H. gigas*, *Hoplolaimus* sp., *Longidorus* sp, L. *elongatus*, *Meloidogyne* sp., *M. ovalis*, sp': *Paratylenchus* sp., *Pratylenchus* sp., *P. crenatus*, *Rotylenchus* sp., *Trichodorus* sp., *T. aequalis*, *Tylenchorhynchus* sp., *Xiphinema sp*., *X. americanum, X. Chambersi*; on *Fraxinus excelsior* – *Helicotylenchus paxilli*, *H. varicaudatus*, *Pratylenchus penetrans*; on *Fraxinus mandschurica* – *Meloidogyne* sp.; on *Fraxinus nigra* – *Meloidogyne* sp.; on *Fraxinus pensylvanica* – *Criconemoides curvatum, Xiphinema americanum*; on *Fraxinus syriaca* – *Meloidogyne javanica*; on *Fraxinus velutina* – *Meloidogyne* sp. Modern studies of the nematocomplex of ash trees indicate a particular danger from the species *Bursaphelenchus crenati* [3].

A special place in the pathological process of dieback of common ash should be given to phytoplasmas, as the least studied phytopathogens. Periodically in the scientific literature, there are reports of the viral origin of the pathogenesis of common ash. At the same time, today the world community knows only about the mosaic disease of trees of the genus *Fraxinus*, the causative agent of which is *Tobacco mosaic virus* (TMV). Thus, the pathology of *Fraxinus excelsior* L. is associated with various factors – micromycetes [4; 5], bacteria [6; 7], nematodes [3], mycoplasmas, harmful entomofauna [8], also with unfavorable climatic (synoptic) and soil-hydrological factors, this indicates that the pathology of *F. excelsior* is a multifaceted phenomenon in which the processes of an infectious and non-infectious nature are systemically interrelated, which significantly complicates the diagnosis of its root causes.

Now in Ukraine, there is a difficult situation with the phytosanitary state of common ash, which requires an urgent solution [1; 6]. A characteristic feature, in this case, is the consistent geographical deterioration of the state of tree stands subordinate to the State Forest Resources Agency of Ukraine, as well as ash trees in forest parks, field protection belts, in plantings of settlements. The visual manifestation of the consequences of pathology is systemically interrelated with the hydrothermal indicators of the current year, the physiological state of trees, and the presence of phytophagous insects.

Of particular concern is ash tuberculosis (*pathogen Pseudomonas syringae pv. savastanoi*), which systemically affects trunks, shoots, and inflorescences [2; 7]. In particular, under the action of the pathogen, numerous wounds, voids, caverns, rotten areas, etc. are formed in the affected plants, not only impairing the physiological processes of trees and devaluing wood but significantly threatens the formation the seed of this valuable woody plant due to damage to the generative organs.

The purpose of this study is to identify negative abiotic and biotic factors in the pathology of *F. excelsior*, to determine the microbiota on infected shoots, leaves, and buds of dieback stands, to establish the dependence of the spread of tuberculosis on the proportion of ash in the composition of stands of different age groups.

THEORETICAL OVERVIEW

The implementation of their life strategies depends on competitiveness and aggression. Nematodes and mycoplasmas are also involved in the pathological process. It is important, according to the authors, that the possible mediating role of populations of arthropods, mainly xylophages, and their participation in the complex circulation process of pathogens are discussed in a certain way. Available primary sources do not make it possible to trace the process of transformation of populations of native pathogenic microbiota or attack of aggressive strains. It is important that in modern pathology of woody plants the problem of sanogenesis is practically not discussed. Recent reports of deteriorating deterioration of the sanitary condition and the dieback of *Fraxinus excelsior* in more than 30 European countries have spread and alarmed scientists and practitioners of the forestry industry, and have given rise to controversy over its causes.

The mass dieback of *Fraxinus excelsior* was first recorded in the early 1990s in northeastern Poland and Lithuania (according to the latest data, today the disease affects more than 30 thousand hectares or 60% of the entire area of ash stands) [9; 10]. The disease then spread north to Latvia and Estonia [11]. In 2002, this disease was first reported in Germany and Sweden (in 2010, common ash was included in the Red Book of Sweden) [12], in 2004 in the Czech Republic, Slovakia, Finland, and Denmark, and 2005 in Austria. Subsequently, in 2007, ash dieback spread to Hungary, Slovenia, and Norway. In 2008, the disease reached France, in 2009 – Italy and Greece, where it caused massive deaths of trees. Recent reports on the noted pathology of *F. excelsior* were received from Belgium, the Netherlands, England, and Ireland [13]. Ash trees are now dieback in 30 European countries. Programs of countries where signs of ash dieback have been identified, aimed at identifying the origin of the pathogen, assessing its impact on forests, developing methods for diagnosing and conducting forestry in affected forests, including in the direction of the selection of ash for resistance to pathogens.

Now the degradation and mass dieback of ash stands has reached a global level and is noted practically throughout the range of many ash species, including *Fraxinus excelsior*. From different parts of the planet, there are reports of similar symptoms of pathology. Based on research, several possible reasons have been put forward.

In the etiology, the deterioration of the sanitary condition of trees of the genus *Fraxinus* prevails "mycological" point of view. In particular, the causative agent of "ash dieback", which is considered the main pathology of ash, is called the anamorphic fungus *Chalara fraxinea* Kowalski. In 2009, it was discovered that this is the asexual stage of the teleomorphic species (new to Europe) *Hymenosyphus pseudoalbidus* Quel. [9]. Recently, the name of the fungus has been clarified *Hymenoscyphus fraxineus* Baral et al. and genetic studies of the genomes of *H. pseudoalbidus* and *H. albidus* by molecular methods were carried out [5; 14]. It is worth noting that in the oak forests (fresh) of the Western Podillya of Ukraine the authors found identified typical symptoms of the disease known as "ash dieback" ("fatal disease" of ash, "peripheral death", "pathogenic dieback of ash") [15]. At the same time, from the pathology of the "ash dieback" type, identified several species of anamorphic fungi and bacteria, in particular *Pseudomonas syringae* pv. *savastanoi, Erwinia horticola* and *Xanthomonas* sp., however, H. *pseudoalbidus* was not isolated [7]. The role of basidiomycetes of the genus *Armillaria*, in particular *Armillaria cepistipes*, in the phenomenon dieback of an ash-trees with a root system is also known [4]. Of course, but not decisive, is the role of other species of fungal organisms, in particular *Alternaria* sp, *Epicoccum* sp, *Phytophthora* sp., in the dieback of trees of the genus *Fraxinus*.

Relatively small diversity of genera *Pseudomonas, Xanthomonas, Erwinia, Agrobacterium, Brenneria, Xylella, Rhizobium, Azotobacter, Corynebacterium, Bacillus, Clostridium, Enterobacter,* but a significant role in the pathological processes of forest woody plants, including, and common ash, have phytopathogenic bacteria. Among the infectious diseases of shoots, inflorescences and trunks of *Fraxinus excelsior,* the most common and most harmful is the disease of bacterial origin – tuberculosis, the causative agent of which is the phytopathogenic bacterium *Pseudomonas syringae* pv. *savastanoi* [16]. Scientists are constantly researching to better study the virulence and aggressiveness of the pathogen. Thus, in Italy, mutants of the bacteria *P. syringae* sp. *savastanoi* (for various manifestations of pathogenicity and hypersensitivity reactions) were isolated and characterized [11]. In Japan, *Pseudomonas syringae* strains are classified into five groups by comparing DNA homology. Currently, the aggressiveness of the pathogen is associated with the formation of phytohormones indole acetic acid and cytokinins. Studies of microbial-plant relationships are underway, in particular in the induction of demutation processes in the phytocenosis and increase the immune protection of plants [17].

Invasive insect species, in particular *Agrilus planipennis* F., are dangerous for deciduous tree species, especially for common ash, because they are capable of attacking and leading to the rapid death of absolutely healthy trees [18].

Many authors argue that the cause of atypical clusters (galls, growths, neoplasms) is the insects *Eriophyes fraxinivorus* Felt (synonym for *Aceria fraxiniflora* Felt) [8; 19]. The authors analyzed the phytochemical composition of galls, which are formed on inflorescences of ash species (*Fraxinus angustifolia, F. excelsior, F. ornus*). However, it is not established and has not confirmed the causes of this phenomenon [18].

MATERIALS AND METHODS

Due to biological characteristics and wide ecological amplitude, in particular, significant shade tolerance in the first years of life, common ash acts as an integral component (as an accompanying woody plant) in many forest associations, while forming high-productive stands together with *Quercus robur* L., *Carpinus betulus* L., and other forest woody plants. At the same time, unique natural ash forests, which are mainly concentrated on rich loamy and viscous soils, have survived only in the Western Podillya of Ukraine, the climatic conditions of which are most favorable for the growth and development of this tree species.

The main algorithm for detecting and studying the pathology of *Fraxinus excelsior* included certain stages: exploration and forest pathological detailed examinations by general forestry and phytopathological methods; detailed examination and research of the affected organs, isolation of pathogens in pure cultures; verification of the properties of pathogens and their accurate identification; study of antagonistic relationships in the systems "bacterium-micromycetes" and "bacterium-bacterium". The influence of meteorological factors as catalysts of the disease on *F. excelsior* and harmful insects has been studied, given the trophic links between insects (as vectors) and pathogens.

Reconnaissance and detailed forest pathological inspections of stands with the participation of *Fraxinus excelsior* were carried out with the establishment of 24 trial areas (2020-2021 years) in the state enterprises "Chortkivske forestry", "Ternopilske forestry", "Buchatske forestry" according to Standard of organization of Ukraine (SOU 02.02-37-476:2006). "Trial plots forest inventory. Method of conducting" (2007). 17 model trees were cut down. 240 samples were taken for myco- and microbiological studies; 110 isolates of micromycetes and bacteria were isolated, including 37 strains of phytopathogenic bacteria; the study of anatomical-morphological and physiological-biochemical characteristics of 120 strains of bacteria; 11 species of insects of harmful entomofauna were found. The density index for each species of entomofauna is set between the percentage of populated trees and the average number of individuals per tree.

The number of microorganisms, depending on functional and other characteristics, was tested by their growth on special nutrient media (potato agar, meat peptone agar, meat peptone broth, malt extract of agar, Czapek medium, etc.). The pathogenic properties of the isolates were shown in laboratory and field conditions by artificial infection of vegetative and generative organs of *F. excelsior* and indicator plants (*Phaseolus vulgaris* L., *Nicotiana tabacum* L., *Kalanchoe laciniata* L.) bacterial suspension with a titer of 108-109 cells×ml^{-1} (according to the turbidity standard). Control – sterile tap water. The placement and size of bacterial cells, Gram staining, the morphology of colonies of microorganisms, their biological, biochemical, and cultural properties were studied using the different methods [20]. To determine the ability of bacterial isolates to ferment various sources of hydrocarbons, the mineral Omelyansky medium was used. Various organic compounds were used as sources of carbohydrates, in particular: lactose, xylose, rhamnose, trehalose, raffinose, L-arabinose, maltose, sorbitol, salicin, sucrose, galactose, fructose, glycerin, mannitol, citrate, etc.

The enzymatic or oxidative pathway of glucose assimilation was determined by the growth of microorganisms on Omelyanskiy's medium under anaerobic conditions under a 1 cm layer of vaseline oil. The indicator was an aqueous solution of bromothymol blue. Milk and gelatin were used to detect proteolytic enzymes in bacteria. The names of bacteria and micromycetes are given according to the atlases-determinants of bacteria and fungi and other specialized literature [20]. The species of phytophages were determined according to the "Atlas of insects of Ukraine", "Keys to the insects of the European USSR" and other determinants. Calculations and statistical data processing were carried out using *Microsoft Excel* computer programs.

RESULTS AND DISCUSSION

Forest pathological examination, symptoms and diagnosis of the disease

Ash tuberculosis is one of the most dangerous diseases of common ash, which in Ukraine has reached epiphytotics, especially on overgrowth origin trees. In modern scientific literature, this disease is associated with bacterial cancer of ash. Cancer, as one of the types of diseases, characterized by overgrowth of individual parts of the plant as a result of hyperplasia or hypertrophy, or both at the same time, which leads to the formation of tumors. Now, cancer is identified with ulcers of various shapes, highly healing or non-healing wounds, including non-infectious ("frosty cancer"), etc. *P. syringae* pv. *savastanoi* causes a typical tuberculosis disease, because tuberculosis is a tumor with voids or other defects, often filled with bacterial mucus, especially in the initial stages of pathology. These are the symptoms and inherent in tuberculosis. In the literature, the symptoms of tuberculosis are described with the final manifestation of the pathological process. At the same time, certain symptomatic differences are inherent in various stage of the manifestation of the disease, which made it possible, with certain conventions, to distinguish several stages (phases) of pathology. According to the authors, this makes it possible to diagnose the affected tree in time at any stages of the disease, with the subsequent development of appropriate protective measures.

The infection of common ash begins at the age of 2-3 years at the corresponding height of the trunk. Infection can be both exogenous and endogenous. The primary symptoms of tuberculosis (the so-called "scab") appear on young trunks with a smooth (primary) grayish-green bark and are characterized by slight local swelling of the upper layer of cells, the appearance of microcracks, and small elliptical soft tumors filled with an odorless gray sticky bacterial mass.

A narrow, shallow, oblong, straight, or tortuous crack forms in the center of the bulge. Exudate, released through cracks, upon drying forms a thick or thin gray membrane, which remains on the surface of the periderm for a long time. The rhytidoma of the affected trees in the places of the pathological process becomes dark gray, gradually dies off in small pieces, and disappears. Along the perimeter of the lesion, the bast part of the trunk turns slightly yellow or reddens. On a cut of the primary bark, a narrow, colored, winding strip is always visible. Over time, the affected primary bark dries up, hardens, and cracks, but the wood is usually not exposed. The site of the lesion seems to be overgrown, but complete overgrowth does not occur. Therefore, local necrosis sinks into the trunk, which leads to the formation of black, dark brown stripes of various thickness in it, large or smaller voids, rotten areas, in the spring-summer period are sometimes filled with a sticky bacterial exudate. Over time, under the influence of factors favorable for the pathogen, new lesions are formed (the so-called "spread" of the disease) along the length and perimeter of the *F. excelsior trunk*. New foci of tuberculosis can appear in different places of the tree without a certain relationship and sequence (Fig. 1a).

In "real tuberculosis", typical tuberculosis formations are formed with a subsequent increase in their size both along the length and along the perimeter of the trunk. Under the influence of the pathogen, a certain deformation of it occurs in the trunk. Necrosis at the initial stage of the disease, especially on young ash trees and shoots, small, ranging in size from 1 to 2-3 cm, but grow over time, often merge, forming a straight or winding strip of dead sapwood, time up to 0.5 m and more. The depth of placement of various flaws in the wood usually depends on the period of infection of the tree: the earlier the tree is infected, the deeper the wounds are formed in the trunk. As the tree grows, the number of defects increases in proportion to new lesions (Fig. 1b).

In addition to the trunks and branches, the causative agent of tuberculosis also affects the inflorescences of common ash, which can potentially threaten the seed recovery of this valuable woody plant ("deformation of the generative organs"). Flowers affected by the causative agent of tuberculosis usually do not form single ash-keys, but accumulate around the undeveloped apical bud and form small (1-2 mm in diameter), first light pink, purple, and then dark brown tuberculous accumulation of rather large sizes (sometimes up to 10 cm), resembling bunches of grapes and remain on the tree until spring (summer) the next year (Fig. 1c).

a) b) c)

Figure 1. *Stages of tuberculosis F. excelsior: a – "actual tuberculosis", b – "deformation of generative organs", c – "wood defects"*

Based on the analysis of the phytochemical composition of galls that form on the inflorescences of European ash species (*F. angustifolia, F. excelsior*, and *F. ornus*), it is argued that the cause of their occurrence is the insects *Eriophyes fraxinivorus* Felt (synonym for *Aceria fraxiniflora* Felt) [8], however, so far such statements have not been properly confirmed [18]. Galls, instead of the usual inflorescences on common ash trees, have a bacterial etiology associated with *Pseudomonas syringae* pv. *savastanoi* – the causative agent of ash tuberculosis [7]. In particular, the authors found inflorescences affected by the causative agent of tuberculosis both on trees with tuberculous pathology and on externally healthy ones, which indirectly testifies to *Ps. syringae* pv. *savastanoi* as a vital obligate. The number of lesions on a tree depends on the degree of damage and the age of the tree, but mainly on one affected tree, dozens and even hundreds of foci of tuberculosis can be counted. In this case, up to 60 or more lesions can form on one running meter of the trunk. Trunks on which single tuberculous lesions are formed are rare. That is, if a tree is infected with a bacteriosis causative agent, then the disease progresses rapidly, often affecting the entire trunk and branches. Ash tuberculosis is a chronic disease.

In an infected tree, wounds form across the entire thickness of the trunk at different heights. Sometimes there is a rupture of annual rings under the influence of myco- and microbiota, necrotic areas spread through several annual rings. If there are voids in the longitudinal and transverse sections, it is possible to establish at what age the tree was infected in a given section of the trunk (Fig. 1). The causative agents of common or graded cancers, mainly *Nectria galligena* Bres, are usually involved in the formation of open ulcers. or *Endoxylina stellulata* Rom. (anamorph *Libertella fraxini* Ogan.). And then the disease proceeds with symptoms characteristic of these pathogens (ulcers are formed) (Fig. 2).

Figure 2. *Change of typical color and formation of open wounds (ulcers)*

Rotten areas on the ash tree trunk are formed exclusively with mixed infection with the formation of open ulcers with the participation of wood-destroying and wood-coloring fungi, in particular *Ascomycota, Basidiomycota* and *Deuteromycota*.

Tuberculosis causes more economic damage than environmental damage. Affected trees of older age groups die off relatively rarely, but as a result of a characteristic pathological process, wood is devalued. Affected trunks are usually retracted into firewood. The most affected by the causative agent of tuberculosis are sprouting ash trees. Taking into account the biology of the pathogen and the pathogenesis of the disease, ash trees, which at a young age have at least a single, insignificant damage to the trunks or branches, should be cut down and disposed of, since under the conditions of Ukraine, against the currently existing infectious background, to grow ash trees with high quality wood age ripeness is problematic, and partly impossible (for the reasons noted above).

Alone on ash trees in the fresh oak forests of Western Podillya of Ukraine "ash dieback" was found, which is considered the main pathology of ash in Eastern Europe. Symptoms of the disease appear at any age of the plant, but young plants of *Fraxinus excelsior* are especially sensitive to damage. In affected plants, there is a gradual (sometimes sudden) dying off of young plants or individual shoots of the crown due to the formation of local necrotic areas on the shoot (trunk). The leaves above the lesion site wither (starting from the top), and by the end of summer they turn black (like those burnt by fire) and do not fall off for a long time.

Etiology and the dynamics of the development artificial infection of the organs of Fraxinus excelsior

Anatomical, morphological, cultural, and physiological-biochemical studies carried out in the Department of Phytopathogenic Bacteria D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine made it possible to establish that the microbiota of tuberculous pathology of *F. excelsior* is made up of bacteria of the genera *Pseudomonas, Erwinia, Xanthomonas*, in particular *Pseudomonas* sp., *P. syringae* pv. *savastanoi*, *P. fluorescens*, *P. syringae*, *P. agglomerans* (synonyms *Enterobacter herbicola*, *E. agglomerans, Erwinia herbicola*), *E. horticola*, *Xanthomonas* sp., as well as the spore-bearing bacteria *Bacillius* sp., which accompanied the tuberculous pathology of *F. excelsior* at all its stages. The average values of the content of bacteria isolated from the vegetative and generative organs of *F. excelsior* ranged from 1 to 168 colony-forming units (CFU). The largest number (116, 168 CFU) of bacteria was obtained during the isolation of *Ps. syringae* pv. *savastanoi*. At the same time, for bacteria, primarily phytopathogenic, it is not so much their quantity that is important as their presence. Under favorable conditions for phytopathogenic bacteria, they can very quickly fill an ecological niche to a threshold concentration, thereby causing even epiphytotics, to a certain extent, which is observed during the mass drying of many species of both coniferous and deciduous woody plants [7].

The only reliable way to separate pathogens from saprotrophs is pathogenicity, that is, the ability of a microorganism to infect living cells. In vivo testing of the pathogenicity of the isolates isolated from the tuberculosis pathology of ash was carried out by injecting a suspension of a daily culture of microorganisms (8.6-9.97 CFU×ml^{-1}) into the trunks and by introducing a pure bacterial culture under the bark (14.1-21, 27 CFU×ml^{-1}) and a mixture of the "Victant" preparation based on *Bacillus* sp. with a collection strain *Pseudomonas syringae* pv. *savastanoi* working solution with a titer of 1×107 CFU×ml^{-1} (Fig. 3) into mechanical damage to the section of the trunk (cut) of a bacterial loop previously sterilized over the flame of an alcohol lamp. Control – sterile tap water. When carrying out the above studies, the circadian rhythms of plant resistance to bacteriosis pathogens were taken into account.

a) b) c) d)

Figure 3*. Natural tuberculosis lesions (a), artificial lesions of common ash shoots by the Pseudomonas syringae pv. savastanoi (strain Ps) (b), control (c) and a mixture of Victant-strain Ps (d)*

Establishing the true causative agent of the disease is significantly complicated by the wide systemic interaction of microorganisms with all living components of the biogeocenosis against the background of constant changes in environmental conditions, ecological plasticity, and variability of phytopathogenic bacteria. Observation of the course of tuberculous pathology with artificial lesions of *Ps. syringae* pv. *savastanoi* continued for a year from the moment of infection, constantly noting the dynamics and progress of this phenomenon. The dynamics of the development of artificial infection of the branches of *Fraxinus excelsior* shows that the first signs of damage manifested themselves in the cracking of the bark at the injection site of the bacterial suspension already on the 15th day of the experiment.

After 10 days, individual cracks merged into one continuous wound, its size increased, and the destruction of not only the surface bark but also the primary bark and bast became noticeable. Three months after the lesion, the upper layer of the bark separated, there was "exposure" of the integumentary tissues and scarring of individual layers. The process of development of the affected area began to fade, but in the spring of the next year (9 months after the defeat) the process of the development of the disease resumed, it acquired a form typical for tuberculosis. Internal cracks deepened and increased in size. A year later, symptoms of tuberculosis clearly appeared in the affected areas.

An interesting fact is that from the affected *Ps. syringae* pv. *savastanoi* of common ash trees isolated *Xanthomonas* sp. (Strain K_5), which revealed pathogenic properties in the experiment. Now in the literature, these bacteria are not marked as pathogens for *F. excelsior* (there is only information about *X. juglandis* as the causative agent of bacteriosis *Juglans regia* L. At the same time, the sensitivity of *F. excelsior* to *Xanthomonas* sp. in case of artificial infection, it testifies, on the one hand, to the expansion of specialization, and on the other, to the insufficient study of the bacterial pathology of forest woody plants. Now *Xanthomonas* sp. isolated from many agricultural plants, where it causes numerous types of diseases – from necrosis to burns [20].

In general, the expected susceptibility of ash to *Erwinia hoticola* (strain K_8), which was isolated from the bark of tuberculosis-affected trees. This bacterial species was first isolated from *Fagus sylvatica* in 1972, where it caused a rather harmful disease known as black bacteriosis in woody plants. Note that this disease resembles the "fatal disease" of ash in symptoms (now this disease, as have already noted, is associated with micromycetes, in particular with *Chalara fraxinea*). In the course of analysis of the mycobiota of common ash branches affected by tuberculosis pathology in the entire research region (including identified only to the level of the genus *Fusarium* sp. and *Phoma* sp.), 7 genera and 10 species of micromycetes were isolated, in particular, at different stages of tuberculous pathology *Fraxinus excelsior*, isolated *Acremonium strictum*, *Cladosporium cladosporiodes*, C*ylindrocarpon didymum, Fusarium* sp., *F. sporotrichiella, F. heterosporum, Phoma* sp., *Ulocladium botrytis*, etc. Usually, samples of the affected tissues had a mixed infection, which consider which part of the concomitant mycobiota, however, as the cause of the mass dieback of ash trees in the study region.

The authors isolated anamorphic micromycetes, in particular, *Fusarium* sp. and bacteria *Pseudomonas syringae* pv. *savastanoi*, *Erwinia horticola* and *Xanthomonas* sp. Artificial infection of the ash organs with micromycetes did not lead to symptoms similar to "ash dieback", and infection with *P. syringae* pv. *savastanoi* caused pathological processes similar to tuberculosis. In other words, all currently known pathogens of necrotic diseases of forest woody plants are directly involved and accelerate the death of woody plants and organs, in particular shoots, at later stages of pathology and are usually secondary, although no less harmful factors.

Studies have established a direct relationship between the prevalence of *Fraxinus excelsior* tuberculosis and its share in stands of various age groups. With a decrease in the proportion of ash in the stand, a decrease in the number of infected trees by *Pseudomonas syringae* pv. *savastanoi*. Thus, in ash stands the prevalence of tuberculosis was the highest for all age groups: young – 79.3%, middle-aged – 47.8%, pre-mature stands – 42.3%. In young, middle-aged, and mature stands with 6-9 units of *Fraxinus excelsior* in the composition, the prevalence of the disease was 41.6%, 33.6%, and 30.7%, which is 1.4-2 times less than in "net" stands. With the share of ash in the stands within 3-5 units, the prevalence of the disease in young, middle-aged and mature stands was 24.2%, 20.5% and 15.8%, respectively.

Pathogenesis (spread of tuberculous pathology in stands of different ages, tree species composition and origin)

At the same time, 17.6% were found in stands with a share of *F. excelsior* in the composition of young, middle-aged, and pre-mature stands; 14.8% and 9.7% of trees affected by the tuberculosis pathogen, respectively, which is 4.5; 3.2 and 4.3 times less than in "net" stands of the respective age groups (Fig. 4).

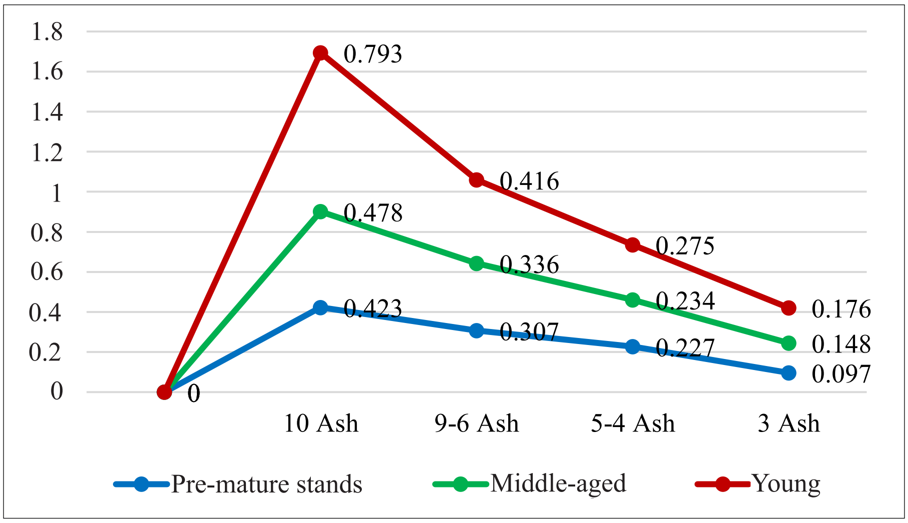

Figure 4*. Distribution of tuberculosis in stands of different ages depending on the share of Fraxinus excelsior in the stand*

The obtained data indicate that the share of common ash in stands within the coenotic optimum (25-30%) during the whole period of stand cultivation is one of the important factors of induction of demutation processes in forest biocenoses and promotes the formation of highly productive, biologically resistant oak-ash stands from the point of view of activation of metabolic processes, and increase of resistance to pathogens of infectious diseases, in particular to ash tuberculosis. As for the decrease in the prevalence of the disease in the stands of older age groups (middle-aged, pre-mature) compared to the young, it is usually directly related to economic activity. In addition, a certain number of affected trees (in young – more, in the middle and middle-aged – less) dies naturally (Fig. 5).

Figure 5*. The spread of tuberculous pathology of ash, depending on the degree of density (a), age (b), composition (c) and origin (d)*

Sprouting ash stands are characterized by reduced resistance to the pathogen of tuberculosis. Both in the study region and in the *F. excelsior* range in general, including in Ukraine, tuberculosis has reached epiphytosis on overgrown plants, especially at a young age (with a significant spread and development of tuberculosis, such plants usually die). Given the pathogenesis of *Pseudomonas* syringae pv. savastanoi have identified three categories of lesions of the trunk: continuous, local, and single (focal or spotted) (Fig. 6).

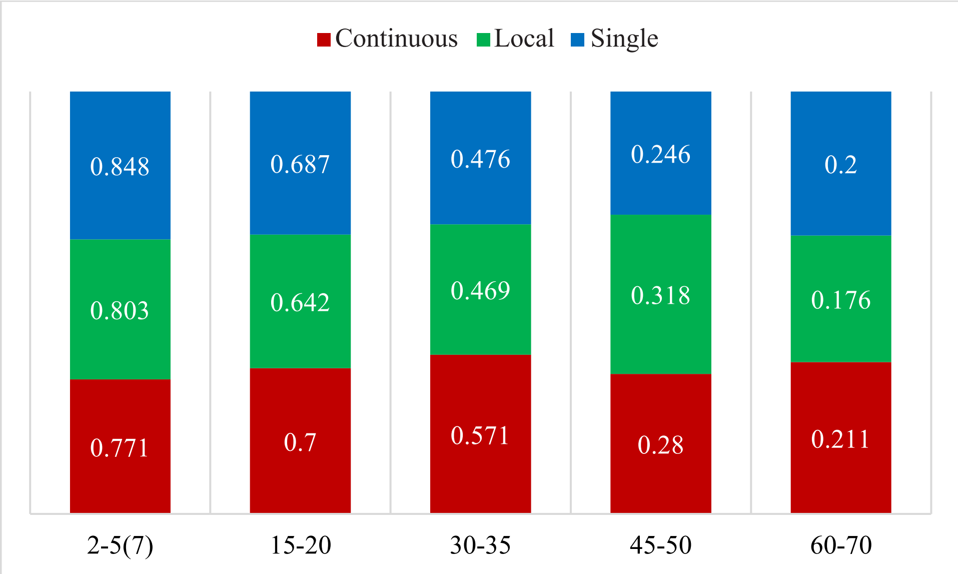

Figure 6*. Distribution of tuberculosis Fraxinus excelsior in sprouting stands*

A decrease in the number of affected *Fraxinus excelsior* trees with age (in 2-5 (7)-year stands 80.7% of tuberculosis-infected plants were found, in 15-20-year-olds 67.6%, in 30-35-year stands 50.5%, at the age of 45-50 – 28.1%, at the age of 60-70 – within 20%) associate with the above factors. However, it is not about the attenuation of the pathological process with age (because the affected *Pseudomonas syringae* pv. *savastanoi* plant does not free itself from infection), but about the death (albeit insignificant) of individual specimens and the removal of diseased trees during cleaning cutting. It should be noted that the affected plants at any age have hidden defectives in the wood (blackening, cracks, rotten areas with a significant spread along the trunk), which devalues it.

Meteorological factors can be catalysts for pathology in stands involving *Fraxinus excelsior*. Based on the calculation of the moisture supply coefficient, have established certain dependencies in the prevalence of tuberculosis on air temperature and precipitation.

Phytophagous insects in the circulation of phytopathogens Fraxinus excelsior L.

In the study area, found 11 species of phytophagous insects of the series *Coleoptera, Hemiptera, Homoptera, Diptera,* and *Lepidoptera*, which are directly or indirectly ecologically and trophically related to the vegetative and reproductive organs of *Fraxinus excelsior*. In particular, *Lignyodes enucleator* Panz. and *Dasyneura fraxini* Kjeff. were identified on the generative organs of common ash. The density of the settlement is in the range of 15-20%. The activity of *Erannis defoliaria* Cl., *Phytagromyza heringi* Hend., *Prociphilus nidificus* Loew., *Psyllopsis fraxini* L., *Prays curtisellus* Don. was noted on the leaves and buds. The density of the colonization is in the range of 5-30%.

Prays curtisellus Don., *Fonscolombea fraxini* Kalt., *Nylesinus crenatus* Fabr., *Hylesinus frakhini* Ranz., *Zeuzera pyrina* L. were found on the branches and trunks. The density of the colonization is in the range of 15-30%. Their pronounced negative impact on producers is usually manifested as a result of stressors. These are, first of all, synoptic anomalies, as well as various anthropogenic factors associated with economic activity.

Analytical analysis of the relationship between dominant species of phytophagous insects, trophically related to the vegetative and generative organs of *Fraxinus excelsior*, indicates the existence of possible ecological and trophic links between carpophagous insects (including *Lignyodes enucleator*) and pathogens of ash tuberculosis as a component. Further research was aimed at a partial experimental substantiation of this phenomenon.

The essence of scientific assumption was based on a detailed analytical foundation of existing literature and own research. Life strategy, biology and ecology *L. enucleator* are completely transformed into a complex biocoenotic structure of deciduous woody plants. A characteristic feature of the species is the lack of cyclical seasonal development. Its number is fully correlated with the dynamics of seed production *Fraxinus excelsior*. Purely formally, the species does not belong to phytophages. Adults and larvae do not cause defoliation, which is why *F. excelsior* and *L. enucleator* trees are not in antagonistic relations.

Imago *Lignyodes enucleator* appears in late May. Initially fed on buds and leaves, during additional feeding damage the young seeds of *Fraxinus excelsior,* laying the ovipositor in it. This period, according to the authors, is considered critical in the genesis of *F. excelsior* and interaction with the pathogen (in particular with the causative agent of tuberculosis). It is at this time that the primary damage to the trees occurs. This is the primary gateway to infection.

The larvae feed on seeds until autumn, in September-October they gnaw small holes in the shell and migrate into the soil for the winter, where they hatch in the spring. The critical period of their development is long (8-9 months – the period of wintering and diapause). As a result, during the spring reactivation their mortality rate did not exceed 32-40%. Viability was largely ensured by such a characteristic feature of the species as the preparation of a specific ecological niche – an area of soil saturated with a special organic secretion that protects against various stressors (entomopathogens and entomophages) – for overwintering phytophages.

It is likely that the primary lesion of *Pseudomonas syringae* pv. *savastanoi* occurs with the participation of *Lignyodes enucleator* populations. Moreover, there is every reason to assume, which is confirmed experimentally, that the causative agent of tuberculosis is transmitted not only by inoculation, but also transovarially. This means that the ovipositor of *L. enucleator* retain the pathogen and that they are the cause of the primary damage to trees.

It should be noted that from the galls left on ash-keys due to damage to their *Lignyodes enucleator,* were isolated bacteria that in the experiment showed pathogenic properties on both *F. excelsior* and indicator plants. Further studies of anatomical-morphological and physiological-biochemical properties of isolates allowed to classify them as gray- and yellow-pigmented species of bacteria, in particular *Xantomonas* sp. and *Pseudomonas* sp. (Fig. 7).

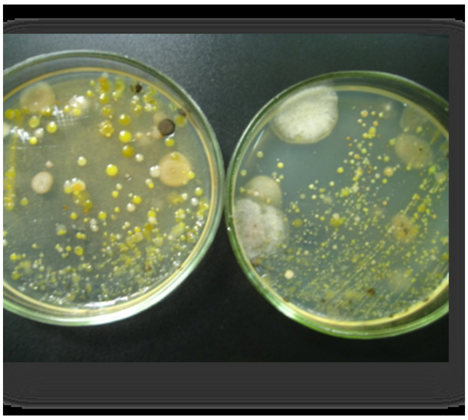

Figure 7. *Galls on the ash-key (left) and bacterial colonies isolated from them (right)*

Therefore, the probability of insect transmission of the bacteriosis pathogen by insects is quite significant. It grows during the ovipositor-period of females *Lignyodes enucleator* in ash-key of *Fraxinus excelsior.* It should also be emphasized that bacterial phytopathogens do not exhibit entomocidal properties in relation to *L. enucleator* and other insects. This property was first experimentally substantiated by us. Of course, further special in-depth studies are needed to determine the role and significance of phytophagous insects in the preservation and circulation of the pathogen inoculum in ecosystems.

Thus, studies make it possible to come to conclude non-obvious ecological, trophic, and mechanical links between the causative agents of tuberculosis *Fraxinus excelsior*, and phytophages with a specific trophic specialization, in particular from carpophagus, in the accumulation, preservation and transmission of the inoculum *Pseudomonas syringae* pv. *savastanoi* in natural ecosystems.

CONCLUSIONS

The current phytosanitary condition of *Fraxinus excelsior* in the forests of Ukraine is associated with a complex of adverse abiotic and biotic factors in their systemic interaction. Symptoms and features of the pathogenesis of tuberculosis *Fraxinus excelsior* have been studied. There are five stages (phases) of the disease and three categories of trunk damage, which allows you to identify the affected tree in time for each age group.

The microbiota of infected shoots, leaves, and buds of dieback stands of *Fraxinus excelsior* is represented by a complex of pathogenic species of the genera *Pseudomonas* sp., *Erwinia* sp., *Xanthomonas* sp., Which are dispersedly localized in the affected area. The most widespread and harmful component of pathogenic microflora is the causative agent of tuberculosis *F. excelsior*, which identified as *Pseudomonas syringae* pv. *savastanoi*. During artificial infection, determined that *P. syringae* pv. *savastanoi* showed high pathogenic properties on various organs of *F. excelsior* and indicator plants (*Phaseolus vulgaris, Nicotiana tabacum, Kalanchoe laciniata*). *F. excelsior* leaves are not sensitive to the pathogen. From the lesions of *Fraxinus excelsior*, 10 species of micromycetes belonging to anamorphic fungi were isolated. Ulocladium botrytis is characterized by a high colonization rate (57.1%), while *Acremonium strictum, Cylindrocarpon didymum, Fusarium sporotrichiella*, and *F. heterosporum* are characterized by a low one (14.3%).

11 species of phytophagous insects (*Coleoptera, Hemiptera, Diptera, Lepidoptera*) were identified and their ecological and trophic relationships with phytopathogenic bacteria in the accumulation, preservation and transmission of the inoculum *Pseudomonas syringae* pv. *savastanoi* in forest biocenoses. The direct dependence

of the spread of tuberculosis on the proportion of ash in the composition of stands of different age groups was revealed. In the study region, tuberculosis reaches epiphytotics on the ground-ash trees, especially when young.

For the purpose of prevention and to reduce the general infectious background, systematic monitoring should be carried out in stands with the participation of *Fraxinus excelsior*, to observe the cenotic optimum of ash in the composition of forest stands, to prevent thickening, to remove and dispose of young ground-ash tree affected by *Ps. syringae* pv. *savastanoi*, and create favorable conditions for the growth and development of common ash. The use of biological products based on *Bacillus* sp. and other myco- and microorganisms with existing antagonistic properties to phytopathogens.

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Туберкульозна патологія *Fraxinus Excelsior* L. в Україні: симптоматика, етіологія, патогенез

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Анотація. Зважаючи на лісівничу, екологічну та господарську цінність деревостанів за участю *Fraxinus excelsior* та враховуючи інтенсивне погіршення їх фітосанітарного стану останніми роками, комплексне дослідження симптоматики, етиології та патогенезу туберкульозу ясена з подальшою розробкою ефективних заходів захисту, зокрема із використанням біопрепаратів на базі *Bacillus sp.* та інших міко- і мікроорганізмів, є наразі особливо актуальним напрямком досліджень. Мета роботи полягає у виявленні негативних абіотичних й біотичних, зокрема паразитарних, чинників у туберкульозній патології *F. excelsior*. У ході виконання дослідження були використані загальнонаукові та спеціальні (мікробіологічні, мікологічні, фітопатологічні, ентомологічні та лісівничо-екологічні) методи дослідження. Акцентується увага на тому, що патологія ясена звичайного – явище багатогранне зі взаємопов'язаними процесами інфекційного та неінфекційного характеру, яке в останні роки призвело до епіфітотійного всихання і наразі має тенденцію до зростання. Встановлено, що туберкульоз ясена звичайного є найбільш поширеним і шкодочинним захворюванням у межах регіону дослідження і завдає здебільшого економічних, ніж екологічних збитків. З туберкульозної патології ізольовані бактерії родів *Pseudomonas sp., Erwinia sp. та Xanthomonas sp.*, а також мікроміцети, які, головним чином, займають місце супутньої мікобіоти. Виділено п'ять етапів (фаз) розвитку туберкульозної патології, які суттєво різняться за симптоматикою. Ідентифіковано видовий склад шкодочинної ентомофауни. Показано, що гідротермічний стрес є каталізуючим чинником епіфітотійного всихання ясена звичайного. Виявлена пряма залежність поширення туберкульозу від частки ясена у складі насаджень різних вікових груп. У регіоні досліджень туберкульоз досягає епіфітотії на паростевих деревах *Fraxinus excelsior*, особливо молодого віку. Вказується на доцільність розмежування причин і патологічних процесів пов'язаних із туберкульозом ясена, щоб не змішувати каталізуючі хворобу фактори і фактори, які призводять до повної деградації ясеневих деревостанів

Ключові слова: ясен звичайний, туберкульоз ясена, антагонізм, шкодочинність захворювання, збудник, симптоматика, патогенез