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BACTERIAL AND FUNGAL INTERACTIONS IN THE TUBERCULOSIS PATHOLOGY OF COMMON ASH IN UKRAINE

INTERAKCJE BAKTERII I GRZYBÓW W PATOLOGII GRUŹLICY JESIONU ZWYCZAJNEGO W UKRAINIE

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Slowa kluczowe: *Fraxinus excelsior*, *antagonizm, bacterie, grzyby*, *Pseudomonas syringae* pv. *savastanoi*, *patologia*

Abstract. The present study is focused on the recent epiphytotic dieback of many forest species both in Ukraine and other countries of the world. In the research on the phenomenon, there has been little concern regarding phytopathogenic bacteria, which have high reproduction energy and can penetrate the plant and cause a pathological process as vital obligates. One of the most common and harmful diseases of common ash in Ukraine is tuberculosis. The causative agent of the disease is the phytopathogenic bacterium *Pseudomonas syringae* subsp. *savastanoi* (ex Smith 1908) Janse 1982. This species affects both trunks, branches and shoots, and inflorescences of a common ash. The bacteria *Pseudomonas* sp., *Pseudomonas fluorescens* Mig., *Pseudomonas syringae* Van., *Erwinia herbicola* Eh., *Xanthomonas* sp. were isolated from tuberculous samples as a concomitant myco- and microbiota and micromycetes *Cladosporium cladosporioides* (Fresen.) G.A. de Vries., *Alternaria botrytis* (Preuss) Woudenb. & Crous, taxon *Mycelia sterilia* (dark) and *Mycelia sterilia* (orange), *Fusarium heterosporum* Lin., *Fusarium* sp., *Cylindrocarpon didymum* (Harting) Wollenw, etc. The mechanism of systemic relationships of the components of myco- and microbiota of tuberculous pathology of the common ash in the regulation (self-regulation) of pathogenicity and aggressiveness of vital obligates has been investigated. The study is focused on the prospects and expediency of using the antagonistic properties of myco- and microorganisms and biological products based on them for the prevention and protection of tree plantations from bacterial pathogens. It is shown that the pathology of the common ash is a multifaceted phenomenon with interrelated processes of an infectious and non-infectious nature. Plant diseases caused by pathogens have been increasing in number and severity over the last few decades in response to increases in human mobility, climate change, land-use intensification, urbanization and the creation of new habitat conditions (Anderson et al. 2004, Aukema et al. 2010, Meentemeyer et al. 2012). The need to distinguish between the etiology and pathogenesis of this negative phenomenon is indicated, that is, not to mix the factors that lead to the weakening of the ordinary ash (factors catalyzing the disease) and the factors that cause its epiphytotic dieback.

INTRODUCTION

Mico- and microorganisms are an integral part of the forest biocenosis, which determines its depth, which is directly involved in all stages of growth and development of woody plants and the utilization of mortmass and detritus (Borkar and Yumlembam 2016). Among the systematic and functional groups of myco- and microbiota, a special place is occupied by phytopathogenic endophytes of various trophic specializations, capable of causing significant ecological, economic and social damage under certain conditions, which is confirmed by deep pathology with massive (epiphytotic) dieback out of many species of woody plants (Scots pine, European spruce, silver birch, common ash, in somewhat smaller volumes common oak, white fir, aspen, common hornbeam) both in Ukraine and abroad (Cherpakov 2012; Goychuk et al. 2020a,b, 2021).

In particular, the degradation and massive dieback of tree plantations has reached a global level and has been noted practically throughout the entire range of many ash species, including the common ash, in Europe, the USA and Asia (EPPO 2020).

The ash dieback was first discovered and described in Poland in the 1990s (Kowalski and Holdenrieder 2009a), later in Germany (Heydek et al. 2005), Sweden (Barklund 2005), Norway (Talgø et al. 2009), the Czech Republic, Slovakia and Finland (Jankovský and Holdenrieder 2009), Austria (Halmschlager and Kirisits 2008) and Hungary (Kirisits et al. 2010). In 2008, the disease spread to France (Husson et al. 2011), in 2009 it was in Italy and Greece (Ogris et al. 2009), which caused the trees to dieback. The latest reports of the noted pathology of *Fraxinus excelsior* L. were received from Belgium (Chandelier et al. 2011), the Netherlands, England and Ireland (Vasaitis and Enderle 2017). In Ukraine, the pathological dieback of the ash has been reported since 2014 (Davydenko et al. 2013, Matsiakh and Kramarets 2014). The ash is currently dying in 30 European countries (Vasaitis and Enderle 2017).

There are reports from different European countries of similar and different etiology and symptoms of tuberculous pathology. Based on scientific research so far many possible pathogens have been put forward, such as fungi (Przybył 2002, Lygis et al. 2005, Kowalski and Holdenrieder 2009b, Langer 2017) (in particular, *Hymenoscyphus fraxineus* Bar., which was identified in Ukraine (Davydenko et al. 2013), bacteria (Cherpakov 2012, Goychuk et al. 2021), nematodes (Ruehle 1967, Ryss and Polyanina 2018), mycoplasmas (Bricker and Stutz 2004), representatives of harmful entomofauna (Korda et al. 2019), as well as the influence of climatic and soil-hydrological indicators (Goberville et al. 2016). However, after analyzing the above, in our opinion, no consensus has been reached yet.

The aim of the study is to investigate the elements of antagonistic relations between components of different systematic and functional groups of myco- and microorganisms in the ash pathology.

MATERIALS AND METHODS

Sampling for the study of the peculiarities of ash tuberculous has been collected in fresh oak forests of Western Podillia of Ukraine, which are optimal for the growth of highly productive deciduous forests with the participation of *Fraxinus excelsior*, in particular, in natural conditions on the territories of state forestry enterprises (Chortkivske forestry enterprise, Ternopilske forestry enterprise, Buchatske forestry enterprise). *Fraxinus excelsior* usually grows in mixed stands with *Acer pseudoplatanus* L., *A. platanoides* L., *Quercus robur* L., *Tilia cordata* Mill., *Alnus glutinosa* (L.) Gaerth., *Prunus avium* L., *Carpinus avium* L. and *Ulmus campestris* L., *U. glabra* Mill. However, there are also mono-cultures of the ash, which are also characterized by high productivity. The vast majority of stands with *Fraxinus excelsior* have a stand density of 0.7–0.8. The age groups are dominated by middle-age and pre-matured stands, which account for about 58 % of the area of stands.

The samples were taken from symptomatic trees at different levels of intensity different age groups (young, by middle-age and matured stands) and from different parts of the trunk in transverse and longitudinal sections – from the bark, from the affected bast part, on the border of the healthy and affected wood as well as from the visually healthy tissue.

The prevalence of the disease is the number of the diseased plants or plant organs, expressed as a percentage. The calculations are performed according to the formula (1) (Goychuk et al. 2004):

$$P = n/N \cdot 100, \tag{1}$$

where: P is the prevalence of the disease, %; n is the number of the diseased plants in the test area, pcs.; N is the total number of plants in the test area, pcs.

During the research, reconnaissance and detailed methods of forest pathology were used, as well as methods according to the generally accepted methods (Patyka et al. 2017), due to which the pathogenic, anatomical-morphological, cultural and antagonistic properties of *Pseudomonas syringae* pv. *savastanoi* with the associated bacteria and micromycetes in the tuberculous pathology of the ash tree have been studied (Caballo-Ponce et al. 2017, Goychuk et al. 2020b).

The identification of micromycete cultures was performed on a set of cultural and morphological characteristics. In particular, the mycological analysis of the affected wood samples was performed by the method of accumulation and inoculation of dense nutrient medium Chapek. The microbiological inoculations were cultured at a temperature of 26±2 °C. The identification of micromycete species was performed for 7–14 days using an Axiostar Plus light microscope, using the determinants of domestic and foreign authors (Sutton 2001, Schoch 2020). To determine the position of the genus of fungi in the structure of dominance in the substrates for cultivation, we used the criteria of population density and frequency of micromycetes (Sutton 2001, Schoch 2020).

The inoculation of the common ash has been done by injection into the trunks of a suspension of a daily culture of microorganisms (8.6–9.97 CFU·ml^{-1}) and by introducing a pure bacterial culture under the bark (14.1–21.27 CFU·ml^{-1}) (both *in vivo* and *in vitro*), we confirmed the bacterial etiology of tuberculous pathology (Figure 3) and isolated the pathogen – the phytopathogenic bacterium *Pseudomonas syringae* pv. *savastanoi*. In our research circadian rhythms of plant resistance (Gvozdyak 2005) to bacteriosis pathogens were taken into account.

The pathogenic properties of bacterial isolates were detected by the artificial infection of cut shoots, leaves and single-winged *F. excelsior* in both laboratory and natural conditions. Leaf leaves and stems of *Phaseolus vulgaris* L., as well as leaves of tobacco (*Nicotiana tabacum* L.) and kalanchoe (*Kalanchoe laciniata* L.) were used as indicator plants. To infect the plants, an aqueous suspension was prepared from the cells of bacterial isolates that had grown on the potato agar for 1–3 days. The titer of bacterial cells was set according to the turbidity standard with a titer of 108 cells·ml^{-1}. Given the circadian rhythms of plant resistance to bacterial pathogens, infection was carried out from 9 to 12 o'clock. Artificial infection was performed using a syringe or a triple needle, injecting the suspension into the intercellular part of the leaf, ash-key, shoots, apical bud and under the bark of healthy *Fraxinus excelsior* plants, followed by a light injection in three places. The frequency of experiments is threefold. Each infected organ had the symbols of the isolates. The development of the infectious process was periodically monitored.

In the laboratory of the D. K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine the pieces of affected tissues were homogenized and plated on potato agar (PA). The bacterial cultures were grown in a thermostat at a temperature of 26 °C. The number of microorganisms depending on functional and other traits were tested for their growth on special nutrient media (potato agar, meat-peptone agar, meat-peptone broth, malt extract of agar, a medium of Chapek, etc.) (Patyka et al. 2017). Sterile tap water was used as control treatment. The placement and size of bacterial cells, Gram staining, the morphology of colonies of microorganisms, their biological, biochemical and cultural properties were studied using special methods (Patyka et al. 2017).

To determine the ability of bacterial isolates to ferment various sources of hydrocarbons, the mineral medium of Omelyansky was used (Patyka et al. 2017).

The following organic compounds were used as sources of carbohydrates: lactose, xylose, rhamnose, trehalose, raffinose, L-arabinose, maltose, sorbitol, salicin, sucrose, galactose, fructose, glycerin, mannitol and citrate. The enzymatic or oxidative pathway of glucose uptake was determined by the growth of microorganisms on Omelyansky'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 the bacteria (Patyka et al. 2017).

Interactions between microorganisms were investigated by the method of the delayed antagonism (Patyka et al. 2017). The test cultures were a standard set of bacterial strains from the collection of the Department of Phytopathogenic Bacteria of the D. K. Zabolotny Institute of Microbiology and Virology of the NASU, as well as isolated by us from vegetative and generative organs of the common ash. The degree of antagonistic activity of the strains was determined by the zones of growth retardation of microbiological cultures of the phytopathogenic bacteria. If the growth retardation zones were 0 mm – the culture was considered inactive, from 1 to 10 mm – weakly active, from 11 to 20 mm – moderately active, above 20 mm – highly active. The repetition of experiments is threefold (Patyka et al. 2017).

To initiate the processes related to the activity of natural populations of hyperparasites, which are ecologically and trophically combined with bacterial phytopathogens, we investigated the relationship, in particular antagonistic, in the systems of 'phytopathogenic bacteria-hyperparasites' of tuberculous pathology of the common ash (Patyka et al. 2017). The antibacterial activity of biological products based on *Bacillus* sp. was investigated by titration and on ash *in vivo* (Patyka et al. 2017).

To assess the typicality of the species of micromycetes and to determine their position in the structure of dominance in the biocenosis, we applied the criterion of a spacious frequency of occurrence (Gotelli and Chao 2013). To study the typical diversity of fungi in the biogeocenosis, we used the concept of the seasonal frequency of occurrence of the species (Przybył 2002). To characterize the species composition of fungi in the wood samples under study, the Sorensen-Chekanovsky similarity coefficient was used (Gotelli and Chao 2013).

To determine the antimicrobial activity, we used biological products Victant and P27ant based on aerobic spore-forming bacteria Bacillus sp., which are currently being tested in the field by the staff of the Institute of Microbiology and Virology. Laboratory studies have shown that the drug exhibits antagonistic properties against various species of bacteria of the genus *Xantomonas* and *Pseudomonas* (Patyka et al. 2017).

Latin names of higher plant species are given by: The Plant List (http://www.theplantlist.org), mycobiota – by: Index Fungorum (http://www.indexfungorum.org), microbiota – by: List of Prokaryotic names with Standing in Nomenclature (http://www.bacterio.cict.fr/e/erwinia.html).

RESULTS AND DISCUSSION

Nowadays, practically all systematic groups of myco- and microorganisms (as causative agents of diseases) are known on the common ash – fungi, bacteria, mycoplasma-like organisms (MPO), viruses, viroids, as well as algae, lichens, higher flowering plants, etc. Our research is aimed directly at establishing the species composition and mechanism of systemic relationships of the components of the myco- and microbiota of tuberculous pathology of the common ash to regulate (self-regulate) the pathogenicity and aggressiveness of vital obligate as a permanent (inalienable, obligatory) presentation in a part of the plant organism (Patyka et al. 2017).

The symptomatology of tuberculous pathology of the common ash in the study region is typically infectious and manifests itself on the branches, trunks, and generative organs of the ash. At the same time, instead of a smooth greenish-grey bark, small elliptical soft tumors are formed due to local swelling of the bark and filling the voids with an odorless gray sticky bacterial mass.

Over time, the affected areas of the trunk become flattered, crack, the cambium and bast in the middle part die off and collapse. In the center of tuberculous formations a crack forms which heals over time. However, complete overgrowth does not occur. From year to year, new tumors form, which spread both along the length and the perimeter of the trunk (branch). As a result, specific long-term lesions are formed, which outwardly resemble a 'scab' (Figure 1).

a b c

Figure 1. Symptoms of tuberculous pathology
Source: I. Kulbanska.

Note:
a – general view of the affected *Pseudomonas syringae* pv. *savastanoi* tree;
b – tuberculous formations on the trunk;
c – the consequences of joint colonization of the ash trunk with the causative agent of tuberculosis, nectriosis and wood-coloring fungi.

The number of wounds on a tree depends on the degree of injury and the age of the tree, but generally, on one affected tree, dozens and even hundreds of foci of tuberculosis can be found. The presence of lesions deep in the trunk, which is overgrown with wood, usually spread along with the annual rings. Rotten areas on the trunk of an ash tree are formed exclusively during mixed infection with the formation of open ulcers with the participation of wood-destroying and wood-coloring fungi, in particular from the *Ascomycota* and *Basidiomycota* divisions (Goychuk et al. 2020b). In the formation of open wounds (ulcers), usually the causative agents of common or stepped cancer, mainly *Nectria galligena* Bres. (syn. *Neonectria galligena*), are involved or *Endoxylina stellulata* Rom. And then the disease proceeds with the symptoms characteristic of these pathogens (stepped open ulcers are formed) (Table 1).

Table 1. Location of inspected forest stands and the percentage of ash trees affected

Forestry	Latitude, N	Longitude, E	Number of plots	Number of ash trees	Spread of the disease, % ±SE
Ulashkivske	48°53′58″	25°49′07″	4	358	40.8 ±24.6
Hermakivske	48°40′45″	26°10′45″	6	470	33.6 ±10.8
Monastyryske	49°05′18″	25°10′00″	3	201	26.9 ±13.4
Borshchivske	48°48′05″	26°02′32″	4	186	29.8 ±9.6
Terebovlyanske	49°18′02″	25°41′58″	1	34	36.1
Budanivske	49°09′53″	25°42′12″	1	60	30.0
Buchatske	49°03′45″	25°23′16″	2	87	32.7 ±7.3
Dorogychivske	48°51′05″	25°31′03″	2	78	26.4 ±6.4
Skala-Podilske	48°51′06″	26°12′04″	1	59	25.5

Source: Own research.

As a result of determining the species composition of the pathogenic microflora of tuberculous pathology of common ash from 7 selected samples (from the affected vegetative and generative organs) (Figure 2), 7 species of bacteria were detected. They belong to the following species and genus: *Pseudomonas syringae* pv. *savastanoi*, *P. fluorescens*, *P. syringae*, *Pseudomonas* sp., *Erwinia herbicola*, *Xanthomonas* sp., *Bacillus subtilis* Eh. and *Bacillus pumilus* Eh. According to the results of artificial inoculation of the ash trees, the isolates showed pathogenicity both on ash-key, young ash trunks (branches), and on indicator plants. At the same time, they were non-pathogenic to the leaves of the common ash (only minor damage to the leaf blade remained at the sites of the introduction of the bacterial inoculum) (Table 2).

Table 2. Number of colony-forming units (CFU) bacteria isolated from vegetative and generative organs

Strains	Location	Strains	Colony forming units (CFU) ±SE	Mean CFU, %
Pseudomonas syringae pv. *savastanoi*	tuberculous ulcer, bark, bast, single-winged, leaf	K3, Kr5, Kr4, N1, K7, Kr2	74 ±(24–168)	41.57
Pseudomonas fluorescens	stem and petioles	1K, 8K2, 11-3	1 ±(0–4)	0.56
Pseudomonas syringae	tuberculous ulcer, bark	9K1, 9K2	77 ±(31–137)	43.26
Erwinia herbicola	bark	K8, 10-1	2 ±(0–6)	1.12
Xanthomonas sp.	bark, single-winged	K5	9 ±(0–20)	5.06
Others	bark, bast	10-7, N3-1	15 ±(0–31)	8.43

Source: Own research.

The average number of bacteria isolated from the vegetative and generative organs of *Fraxinus excelsior* ranged from 1 to 168 CFU (Figure 2). The largest number (116, 168 CFU) of bacteria was obtained by isolating *Pseudomonas syringae* pv. *savastanoi.*

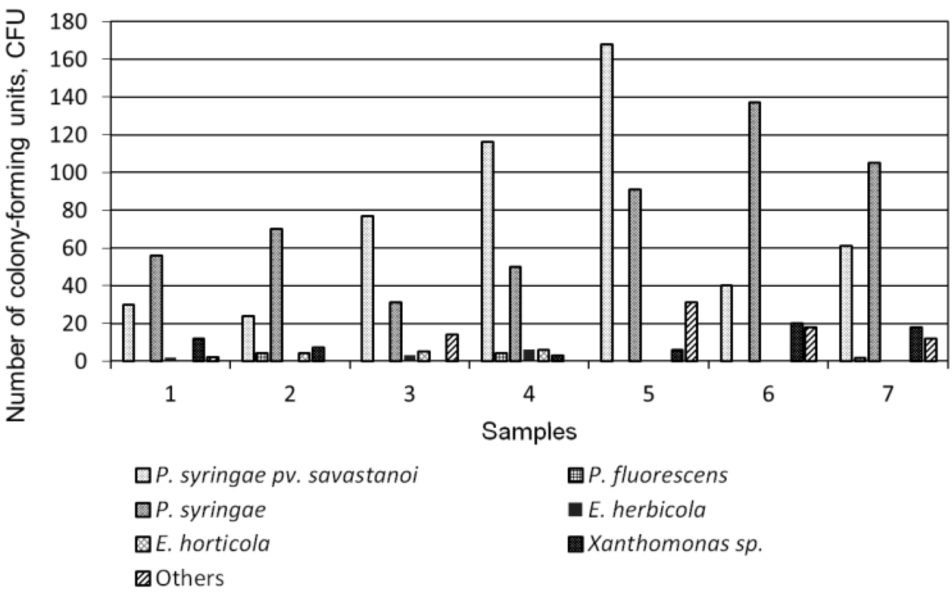

Figure 2. Number of colony-forming units (CFU) bacteria isolated from vegetative and generative organs
Source: Own research.

a b c

Figure 3. The result of artificial infection of the organs of common ash
Source: I. Kulbanska.
Note:
a – infection with a collection strain *P. syringae* 8511;
b – infection with an isolated strain *P. syringae* pv. *savastanoi*;
c – infection with an isolated strain *Pseudomonas* sp.

The isolated species has typical features of the genus *Pseudomonas*. These are non-spore-bearing mobile rods 0.4–0.8×1.3–3.0 μ in size, gram-negative, placed singly, in pairs or in short chains, sometimes in groups, lophotrichs, with 1–6 polar flagella. The ends of the sticks are smoothly rounded. On the potato agar, colonies are grey-white, smooth, round, transparent, with a denser center, with an equal or slightly wavy, sometimes with a blue tint, edge. Colonies on meat-peptone agar are small, 2–3 mm in diameter, grow slowly, grey-white, round, flat, or convex, with a notch in the center, transparent, with an equal or wavy edge. On meat-peptone broth, the growth is moderate, bacteria form uniform cloudiness. No growth was found on the Czapek and Omelyansky medium (Iacobellis et al. 1998, Ramos et al. 2012, Patyka et al. 2017, Goychuk et al. 2021).

In particular, growth (no growth) is on most nutrient media, significant variability with food sources, relationship in the production of enzymes. The main differences are the absence of fluorescence in liquid nutrient media and the slow absorption of carbohydrates and alcohols. The heterogeneity and plasticity of this species are associated with the possibility of expanding the spectrum of nutritious plants.

The results of mycobiota analysis isolated from tuberculous pathology of ash branches resulted in 7 genera and 10 species of micromycetes. Usually, the affected tissue samples had a mixed infection (Figure 4).

a b

Figure 4. Colonies of micromycetes isolated from vegetative and generative organs of affected trees.
Source: I. Kulbanska.
Note:
a – taxon Mycelia sterilia (orange), M. sterilia (dark), *Cladosporium cladosporiodes*, *Ulocladium botrytis*;
b – *U. botrytis*, *Phoma* sp., *C. cladosporiodes.*

The analysis of the obtained results showed the similarity of the studied samples with the following species: *Acremonium strictum* W. Gams (syn. *Sarocladium strictum* (W. Gams) Summerbell 2011), *Cladosporium cladosporiodes* (Fresen.) G.A. de Vries (syn. *Hormodendrum cladosporioides* (Fresen.) Sacc.), *C. didymium* (Hartig) Wollenw., *Fusarium sporotrichiella* Bilaivar. (Peck) Wol. (syn. *Fusarium sporotrichioides* Sherbakoff, 1915), *Fusarium heterosporum* Nees, *Fusarium* sp., taxon Mycelia sterilia (dark) and M. sterilia (orange), *Phoma* sp., *Ulocladium botrytis* Preuss (syn. *Alternaria botrytis* (Preuss) Woudenb. & Crous, 2013).

Based on the obtained results, it is possible to differentiate the identified species into separate categories. Among all the fungal species *Alternaria botrytis* is a typical dominant species (spatial and seasonal frequency of occurrence exceeds 60 %). The most common species are *Phoma* sp., *Cladosporium cladosporiodes*, taxon Mycelia sterilia (orange). Following the types of rare species – *Acremonium strictum*, *Cladosporium didymum*, *Fusarium sporotrichioides, F. heterosporum*, *Fusarium* sp. and order M. sterilia (dark). There are no random species. *Ulocladium botrytis* is characterized by a high occupancy rate (78,6 %), while *Acremonium strictum*, *C. didymum*, and *Fusarium sporotrichiella* are characterized by a low one (14.3 %). *F. heterosporum* (Figure 5).

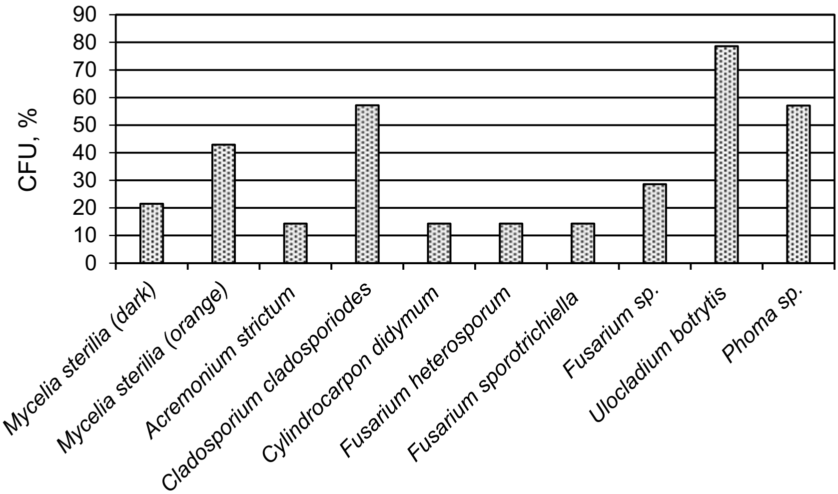

Figure 5. Spatial and seasonal frequency of occurrence of micromycetes of tuberculous pathology
Source: Own research.

Based on the calculation of the Sorensen-Chekanovsky similarity coefficient ($S > 0.5$), there are significant differences between the species composition of the compared stages of the study. In general, the results of the study showed that the mycobiota of infected branches of the common ash is characterized by the presence of a complex of pathogenic species, dispersedly localized over the affected area. The obtained results confirm the assumption of the presence of a complex biological complex, dieback of tree stands. We also found a disease known as ashdieback in the studied stands. It should be noted that the symptoms have fundamental differences in comparison with tuberculosis. In affected plants, there is a rapid gradual (sometimes sudden) crown death 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.

The our study demonstrates that the micromycete *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya is the causative agent of ash dieback (Kowalski 2006, Davydenko et al. 2013, Matsiakh and Kramarets 2014). At the same time, attention was focused on *Hymenoscyphus fraxineus*. We have isolated several species of anamorphic fungi from the pathology of the ash dieback type (*Hymenoscyphus fraxineus*) and bacteria, in particular *Pseudomonas syringae* pv. *savastanoi, Erwinia horticola* and *Xanthomonas* sp. The inoculation of ash by micromycetes isolates did not lead to similar symptoms similar of ash dieback while bacterial infections caused pathological processes similar to ash tuberculosis.

The study of systemic relationships, primarily antagonistic, between different types of myco- and microorganisms, including saprotrophs and pathogens, pathogens of infectious plant diseases, including bacteriosis, in the context of identifying active antagonists to pathogenic components of myco- and microbiota is extremely important and relevant. To study the antagonistic relationships in the system 'bacterium-bacterium' and 'bacterium-micromycete' in the laboratory, we used isolated bacteria and collection strains of bacteria *Pseudomonas syringae* 8511, *Pseudomonas savastanoi* 9174, as well as micromycetes *Ulocladium botrytis*, *Phoma* sp., *Cladosporium cladosporiodes*, *Acremonium strictum*, *Cladosporium didymum*, *Fusarium sporotrichioides*, *F. heterosporum*, *Fusarium* sp. (Brenner et al. 2005).

All the strains were tested in cross-reactions 'basic culture-test culture'. A total of 108 variants of reactions were received. During the research, we did not find antagonistic relationships between test cultures – pathogens of *Fraxinus excelsior* in the system 'bacterium-bacterium'. In contrast to bacteria, micromycetes isolated from the vegetative and generative organs of *Fraxinus excelsior* showed varying degrees antagonistic activity against phytopathogenic bacteria. The most active species were *Alternaria botrytis* and *Cladosporium cladosporiodes* with mean sterile zones of 5.8 and 4.9 mm, respectively. They suppressed to varying degrees all test cultures of phytopathogenic bacteria. The activity of the other three micromycete species, *Acremonium strictum, Fusarium heterosporum*, and *F. sporotrichioides,* was selective. They did not inhibit the growth of *Pseudomonas* sp. (Kr4) and *Pseudomonas syringae* pv. *savastanoi* (Kr4) and weakly inhibited *Pseudomonas syringae* pv. *savastanoi* (H1) and the collection *Pseudomonas savastanoi* 9174. Other species of micromycetes did not show antibacterial activity. Bacteria *Pseudomonas syringae* pv. *savastanoi* (H1), *Pseudomonas syringae* 8511, *Pseudomonas savastanoi* 9174 were the most sensitive to all types of micromycetes (Table 3).

Table 3. Antibacterial properties of micromycetes isolated from tuberculosis of vegetative and generative organs of *Fraxinus excelsior*

Bacterial test culture	A fungal culture test								Average sterile zone, mm
	U. botrytis	***Phoma* sp.**	***C. cladosporiodes***	***A. strictum***	***C. didymum***	***F. sporotrichiella***	***F. heterosporum***	***Fusarium* sp.**	
	Sterile zone, mm								
P. syringae pv. *savastanoi* (Kr2)	5	0	3.5	1	0	2	2	0	1.7
Pseudomonas sp. (Kr4)	2	0	2	0	0	0	0	0	0.5
P. syringae pv. *savastanoi* (H1)	3	0	7.5	6	0	7.5	8.5	0	4.0
P. syringae pv. *savastanoi* (K6)	8	0	3	0	0	0	0	0	1.4
P. syringae 8511	6.5	0	5	5	0	3.5	3	0	2.9
P. savastanoi 9174	10	0	8.5	7	0	7	9	0	5.2
Average sterile zone	5.8	0	4.9	3.2	0	3.3	3.8	0	-

Source: Own research.

Regarding the reverse effect, phytopathogenic bacteria on micromycetes, in most cases their antifungal activity was absent. There were also minor areas formed by the action of bacteria on micromycetes. The same a result was obtained in the laboratory on an artificial nutrient medium, which differently affects the growth and activity of bacteria and micromycetes. In particular, bacteria grow intensively on the potato agar, and the growth of micromycetes is inhibited. At the same time, bacteria do not grow on Czapek medium due to the presence in these media of different nutrients for micromycetes and bacteria. Under natural conditions, the interaction in the systems 'bacterium-bacterium', 'bacterium-micromycete', 'micromycete-bacterium' can and obviously should be different, because the mico- and microorganisms are influenced by various factors (woody plant at certain stages growth and development, its physiological state, the presence of a pathology, meteorological (synoptic) factors, including as catalysts of pathology, compliance of forest woody plant with forest conditions, etc.). However, experiments with coniferous forest woody plants have shown that phytopathogenic bacteria (so-called vital obligates) in natural conditions outside the pathological process do not affect the growth of micromycetes (Gvozdyak 2005, Goychuk 2020a). Studies of biological products (Victant and P27ant) based on aerobic spore-forming bacteria *Bacillus* sp. no high antimicrobial activity was revealed for all studied cultures of microorganisms, and their activity depended not only on the type of bacteria but also on which organs of the ash tree it was isolated from (Figure 6).

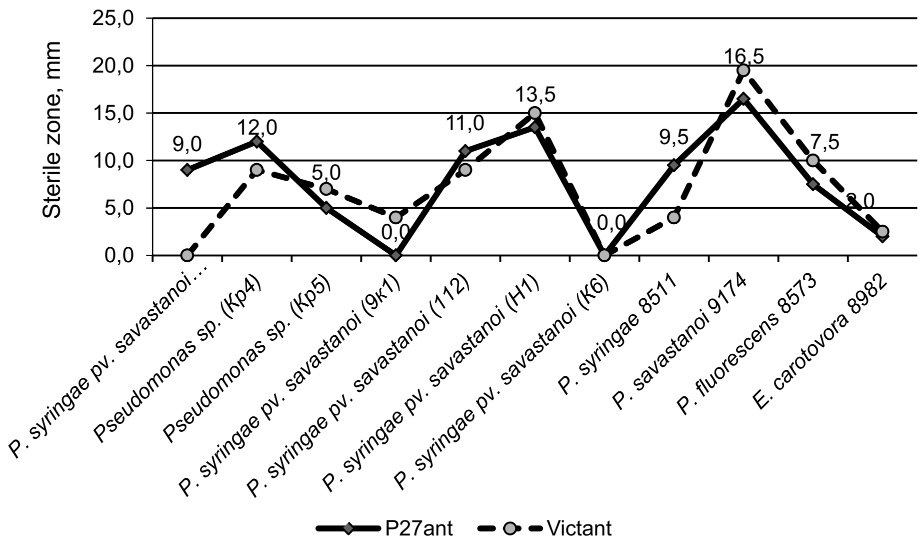

Figure 6. Antimicrobial activity of Victant and the strain P27ant.
Source: Own research

Analyzing the antagonistic relationship between bacteria in the pathology of the ash and bacteria of the genus *Bacillus* sp. the following results were established: the highest antagonistic activity was shown by the Victant strain to the collection strain *P. savastanoi* 9174 – the zone of growth retardation was 19.5 mm (moderately active). It was also moderately active against the strain P. *syringae* pv. *savastanoi* (H1 – isolated from seeds) and showed a weak antagonistic effect on strains of *Pseudomonas* sp. (Kr4, Kr5 – isolated from the bark), *P. syringae* pv. *savastanoi* (9k1, 112) and P. *syringae* 8511 (growth retardation ranged from 4–9 mm). The culture was inactive to the strain *P. syringae* pv. *savastanoi* (K6). The strain P27ant (as well as Victant) showed the highest antagonistic activity to the collection strain *P. savastanoi* 9174 – the zone of growth retardation was 16.5 mm (moderately active). It was also moderately active against the strains of *Pseudomonas* sp. (Kr4) and *P. syringae* pv. *savastanoi* (112 – isolated from buds, H1) – the zone of growth retardation ranged from 5 to 13.5 mm. A weak antagonistic effect on *P. syringae* pv. *savastanoi* strains was revealed (Kr2), *Pseudomonas* sp. (Kr5) and the collection strain *P. syringae* 8511 (the growth retardation zone ranged from 5 to 9.5 mm). The culture was inactive to the strain *P. syringae* pv. *savastanoi* (K6), (as well as Victant) and *P. syringae* pv. *savastanoi* (9k1). The bacteria isolated by us from the generative organs of the ash did not show antagonistic action against test cultures – the pathogens of the ash (growth retardation zone – 0 mm).

It was found that the bacteria of the genus *Bacillus* sp. can suppress the survival of phytopathogenic *P. syringae* pv. *savastanoi* on the surfaces and tissues of test plants, eliminating the pathogen population or decreasing its density with a possible attenuation of their properties, the mechanism of which requires further research. Thus, our study in combination with the results of other scientists indicate the presence of infected trees *Fraxinus excelsior* complex biocomplex of pathogenic species, which directly involved in the dieback of ash stands. Moreover, we found that the elements of antagonistic relationships between components of different systematic and functional groups of microorganisms indicate the possibility and necessity of using this phenomenon in the context of positive and negative feedback mechanisms to develop methods of biological protection of forest trees, against pathogens including bacteriosis.

CONCLUSIONS

Currently, the deterioration of phytosanitary conditions of stands with the participation of *Fraxinus excelsior* is associated with various biotic and climatic factors including 'ash dieback' and tuberculosis, which indicates the need for a comprehensive study of forest pathology issue, in particular, fungal and bacterial diseases.

The isolated causative agent of tuberculous of the common ash *Pseudomonas syringae* pv. *savastanoi* is close to that described in the literature: grows on most nutrient media with significant variability in relation to nutrient sources.

Erwinia horticola, *Pseudomonas* sp., *P. fluorescens*, *P. syringae*, *P. aglomerans*, *Xanthomonas* sp., spore-bearing bacteria *Bacillius* sp., as well as micromycetes *Acremonium strictum*, *Cladosporium didymum*, *Fusarium* sp., *Fusarium sporotrichiella*, *F. heterosporum*, *Phoma* sp., *Alternaria botrytis* and the like were isolated at different stages of tuberculous pathology of *Fraxinus excelsior*. The affected plants at any age have hidden faults in the wood (blackening, cracks, rotten areas with a significant spread along the trunk), which devalues it.

It was found that both isolated phytopathogenic bacteria did not reveal antagonistic activity. In contrast to the bacteria, the isolated micromycetes are characterized by a different antagonistic activity concerning phytopathogenic bacteria. Thus, we found, like other researchers, that the elements of antagonistic relationships between components of different systematic and functional groups of myco- and microorganisms indicate the possibility and necessity of using this phenomenon in the context of positive and negative feedback mechanisms for the development of means and methods of biological protection forest woody plants, including *Fraxinus excelsior*, from pathogens of infectious diseases, including bacteriosis.

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SUMMARY

Over the past few decades, the number and severity of plant diseases caused by pathogens increases in response to higher human mobility, climate change, land use intensification, urbanization and creating new habitat conditions (Anderson et al. 2004, Aukema et al. 2010, Meentemeyer et al. 2012). This study focuses on perspectives and desirability of using antagonistic relations myco- and microorganisms and the manufacture of products based on them for the prevention and protection of trees against pathogens bacterial.

One of the most common and harmful diseases of *Fraxinus excelsior* L. in Ukraine is tuberculosis. The causative agent of the disease is the phytopathogenic bacterium Pseudomonas syringae subsp. savastanoi (ex Smith 1908) Janse 1982. This species affects both trunks, branches and shoots and inflorescences of common ash. *Pseudomonas* bacteria fluorescens Mig., *P. syringae* Van., *Erwinia herbicola* Eh., *Xanthomonas* sp. were isolated from tuberculosis samples as coexisting with micromycetes *Cladosporium cladosporioides* (Fresen.) G.A. de Vries., *Alternaria botrytis* (Preuss) Woudenb. & Crous, *Mycelia sterilia* (dark) and *M. sterilia* (orange), *Fusarium heterosporum* Lin., *Cylindrocarpon didymum* (Harting) Wollenw, etc. The mechanism of systemic association of components myco- and microbiota of tuberculous pathology of the common ash was studied in regulation (self-regulation) of pathogenicity and aggressiveness of life compounds. It has been shown that *Fraxinus excelsior* pathology is a phenomenon multifaceted with the interrelated processes of an infectious and non-infectious nature. It is necessary to distinguish between the etiology and pathogenesis of it negative phenomenon, i.e. not mixing disease catalysing factors with factors causing epiphytotic dieback of common ash.

STRESZCZENIE

W ciągu ostatnich kilku dziesięcioleci liczba i nasilenie chorób roślin powodowanych przez patogeny wzrasta w odpowiedzi na wzrost mobilności ludzi, zmiany klimatu, intensyfikację użytkowania gleby, urbanizację i tworzenie nowych warunków siedliskowych (Anderson et al. 2004, Aukema et al. 2010, Meentemeyer et al. 2012). Niniejsze badanie koncentruje się na perspektywach i celowości wykorzystania antagonistycznych stosunków myko- i mikroorganizmów oraz wyrobienia opartych na nich produktów biologicznych do profilaktyki i ochrony drzew przed patogenami bakteryjnymi.

Jedną z najczęstszych i najbardziej szkodliwych chorób *Fraxinus excelsior* L. na Ukrainie jest gruźlica. Czynnikiem sprawczym choroby jest fitopatogenna bakteria *Pseudomonas syringae* subsp. *savastanoi* (ex Smith 1908) Janse 1982. Gatunek ten atakuje zarówno pnie, gałęzie i pędy, jak i kwiatostany jesionu wyniosłego. Bakterie *Pseudomonas fluorescens* Mig., *P. syringae* Van., *Erwinia herbicola* Eh., *Xanthomonas* sp. zostały wyizolowane z próbek gruźlicy jako współistniejące z mikromycetami *Cladosporium cladosporioides* (Fresen.) G.A. de Vries., *Alternaria botrytis* (Preuss) Woudenb. & Crous, *Mycelia sterilia* (ciemny) i *M. sterilia* (pomarańczowy), *Fusarium heterosporum* Lin., *Cylindrocarpon didymum* (Harting) Wollenw, itp. Mechanizm powiązań układowych składników myko- i mikrobioty patologii gruźliczej jesionu wyniosłego badano w regulacji (samoregulacji) patogenności i agresywności związków życiowych. Wykazano, że patologia *Fraxinus excelsior* jest zjawiskiem wieloaspektowym z powiązanymi ze sobą procesami o charakterze zakaźnym i niezakaźnym. Konieczne jest rozróżnienie etiologii i patogenezy tego negatywnego zjawiska, czyli niemieszanie czynników katalizujących chorobę z czynnikami powodującymi zamieranie epifitotyczne jesionu wyniosłego.