

ETIOLOGY OF COMMON ASH DISEASES IN PODOLIA, UKRAINE

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*The article summarizes study results of pathological changes of vegetative and generative organs of common ash (*Fraxinus excelsior* L). It is shown that this important forest tree is rather susceptible not only to different systematic and functional groups of mico- and microorganisms, but also to numerous types of harmful entomofauna. Our results demonstrate that the most common and dangerous ash disease is tuberculosis. Its causative agent - *Pseudomonas syringae* pv. *savastanoi* - affects trunks, branches and shoots as well as inflorescences of common ash. There are a variety of other pathogens and pests that considerably slow ash growth and development and reduce its qualitative characteristics.*

Pathogenic microflora, contagious and non-contagious pathology, harmful entomofauna, disease symptoms, ash generative organs, ash tuberculosis, pathogenesis, disease occurrence and hazard levels.

Study objective was scientific analysis of specialized literature on symptomatology and etiology of diseases of vegetative and generative organs of common ash that develop under the influence of pathogenic mico- and microorganisms, such as pathogens of infectious diseases and pests, and their analytic generalization. Another goal was to investigate the primary factors of common ash pathology in Ukrainian Podolia.

The study approach included the application of a variety of research methods, including system analysis, information management, bibliometrics, specific forest pathology and phytopathology methods and surveys. Species diversity of mico- and microorganisms, and pests were classified according to corresponding indicators.

The object of investigation - *Fraxinus excelsior* L and forest plantations that include this component in Podolia, Ukraine. **The subject of investigation** – etiology and pathogenesis of contagious and non-contagious diseases of common ash.

Results. Almost all known groups of microorganisms that are pathogens of diseases (fungi, bacteria, viruses, etc.), as well as algae and lichens, can be found on common ash. There is also a large variety of pests that are especially dangerous for weakened *Fraxinus excelsior* L. plantations.

Disruption of photosynthesis and transpiration processes is often associated with certain leaf diseases (such as spots, deformations, etc.). This is especially true of young common ash plants and seedlings. Premature shrinking and falling of leaves may result from large scale spread of the disease.

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Our common ash survey took place in state forest enterprise «Chortkivske». A variety of microflora species were identified, including those of *Deuteromycota* taxonomic morphological group (*Alternaria tenuis* Nees, *Cladosporium herbarum* (Pers.) Lk., *Phomopsis scobinella* Sacc. et d. Sacc., *Phyllosticta fraxini* Ell. et Mart, *Septoria fraxini* Desm., *Cylindrosporium fraxini* (Ell. et Kell) Ell. et Ev.), *Ascomycota* (*Microsphaera alphitoides* Gr. et Nb, *Uncinula fraxini* Miyake, *Phyllactinia suffulta* Sacc., *Mycosphaerella fraxini* (Niessl) Zindau), as well as *Puccinia obtusata* Otth (*Urediniomycota*). On wet rich soils *Alternaria tenuis* Nees, *Cladosporium herbarum* (Pers.) Lk. and *Phomopsis scobinella* Sacc. et D. Sacc were commonly found.

Alternaria tenuis Nees colony on potato agar (PA) is usually colored black or olive-black. Conidiophores are isolated or gathered in small groups, simple or branched, with partitions, straight or sinuous, sometimes crank, smooth; their dimensions vary within 5-125 4 3-6 μm . Conidia is formed in long, branching chains of 10 or more, egg-shaped, spherical, 7-130 4 6-22.5 μm , with longitudinal and transverse SEPTA, smooth, dark olive or olive-brown. Conidia is without cervical, elliptical, egg-shaped, rounded at the ends, 7-72 4 6-22.5 μm , its cervix 1-58.5 4 1.5-7.5 μm , with 1-4 transverse bulkheads (Fig. 1). Normal saprophyte, cosmopolitan.

Cladosporium herbarum (Pers.) Lk. colonies are well spread, olive green to olive-brown. Conidiophores are direct or sinuous, sometimes crank, often knotted up, light or dark olive-brown, smooth, 250 4 3-6 μm . Conidia is long or elliptical with rounded edges, with a thick shell, with small warts, one-celled, light or dark olive-brown, dimensions vary within 5-23 4 3-8 μm (Fig. 2).Cosmopolitan.

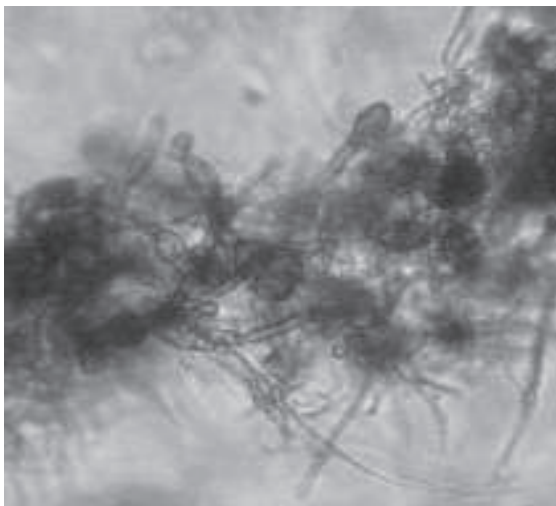


Figure 1. *Alternaria tenuis* Nees (x 400 optical magnification)

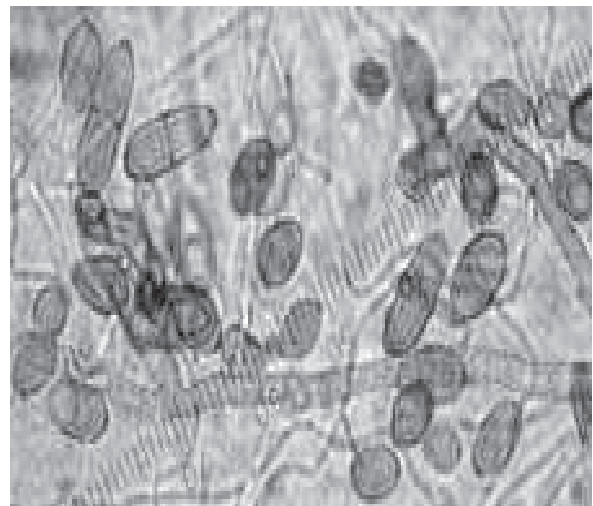


Figure 2. *Cladosporium herbarum* (Pers.) (x 400 optical magnification)

As for *Phomopsis scobinella* Sacc. et d. Sacc, its anatomical-morphological features are similar to those described in the literature [2].

Ash seeds, or keys, make a good growing media for a variety of fungi species. Usually, the seeds are infested by *Deuteromycota* fungi, such as *Ascochyta* spp., *Cercospora* spp., *Cylindrosporium* spp., *Diplodia* spp., *Gleoeosporium* spp., *Heterosporium* spp., *Phoma* spp., *Septoria* spp. The

disease is characterized by the presence of larger or smaller black pectidia on necrotic (lighter) areas of ash seeds, in groups or separately.

By affecting vital organs, causative agents of necrotic diseases of shoots, branches and trunks can not only cause their significant weakening, but also dieback of entire trees. Also, through forming necrotic areas these agents create favorable conditions for timber damaging fungi colonies, particularly on trunks [7,9].

In Podolia, the most common pathogens of necrotic diseases on branches and trunks of common ash are *Cytospora spp.*, *Phoma spp.*, and *Hysteroglyphium fraxini* de Not. *Cytophoma pulchella* (Sacc.) Gutn. and *Endoxylina stellulata* Rom. are causative agents of ash cancer, which lead to the excessive uneven proliferation of isolated parts of plants with the formation of growths and tumors. It was reported that ash cancer may spread into shoots if root tops are infected [7].

Fungi pathogens on common ash were previously studied by Pidoplichko [8, 9, 10], who notes a number of pathogenic fungi, in particular *Basidiomycetes* (*Tyromyces fissilis* (Berk. Et Curt.) Donk, *Spongipellis spumeus* Pat., *Fomitopsis cytisina* Bond. et Sing., *Inonotus hispidus* Karst., *Phellinus conchatus* Quel., *Phellinus torulosus* Bourd. Et Galz., *Polyporus varius* Pers. Ex. Fr, *Funalia gallica* Bond. Et Sing., *Oxyporus populinus* Donk., *Puccinia obtusata* Otth., as well as *Uncinula fraxini* Miyake, *Nectria galligena* Bresadola, *Nectria cinnabarina* Fb and *Phytophthora cactorum*.

As part of our forest pathology survey in state forest enterprise "Chortkivske", we discovered a disease with symptoms similar to "ash dieback infection". Its main symptoms are characterized by massive drying and blackening of ash leaves, by deformation of shoots, and local discoloration of necrotic areas on branches and trunks (fig. 3 a, b, c). Despite the fact that the current etiology of "ash dieback infection" is explained by mycosis (in particular, *Chalara fraxinea*/*Hymenoscyphus pseudoalbidus*), this viewpoint remains controversial. Similar disease was also found on beech trees and is known as the "black bacteriosis" [1], and also on poplar trees – known as "scab."

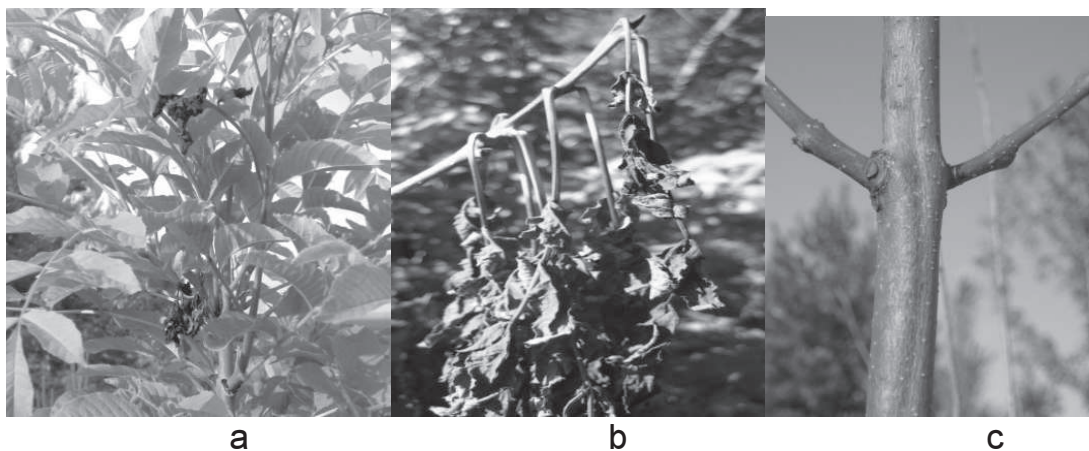


Figure 3. Morphological signs of "terminal illness" Ash

a - blackened leaves as if "burned", b - sprout deformation c – color change of affected branches

Species composition of bacteriosis pathogens for forest woody plants is much less diverse than of mycosis pathogens. However, the most common and dangerous disease of common ash - tuberculosis - is caused by bacteria (*Pseudomonas syringae* pv. *savastanoi* (Smith 1908) Young et. Al. 1978). Bacteria can infect trees of one-two years of age. The disease reveals itself on the branches, trunks, and generative organs of common ash. Instead of smooth greenish bark, there are small elliptical soft tumors filled with grey sticky odorless bacterial mass. Over time, the affected area of the trunk becomes more flat. Cambium dies and disintegrates. In the center of tuberculous formations, a crack is formed that is eventually partially overgrown. From year to year there are new tumors formed that spread both in length and around the perimeter of the trunk (branches). As a result, individual perennial lesions are formed, resembling scab or "parsha" [7].

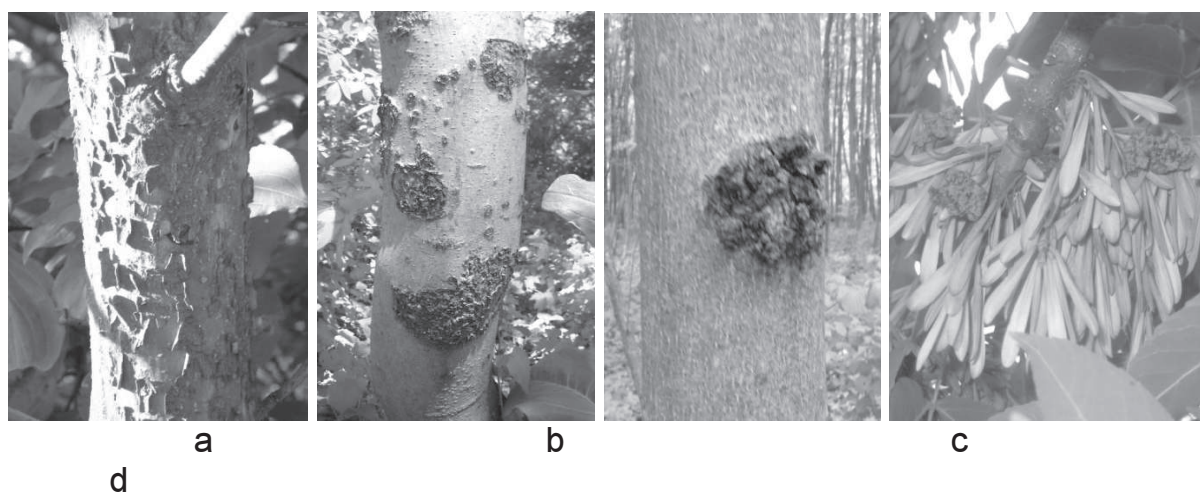


Figure 4. Morphological signs of tuberculosis Ash

a - the initial stage of tuberculosis ("scab"), b - spreading of trunk tumors, c - formation of tubercular tumor, d – metamorphosis of ash generative organs .

We were able to isolate multiple bacteria from affected leaves, inflorescences, keys, branches and trunks of *Fraxinus excelsior* L. In particular, *P. syringae* pv. *savastanoi* and *Xanthomonas* sp. were isolated from infected generative organs [4,5]. The artificial infection with isolates proved them to be pathogenic to ash seeds and branches, and non-pathogenic to ash leaves.

Colonies of *Pseudomonas syringae* pv. *savastanoi* are gray-white or cream, round, smooth, transparent, featuring straight or slightly wavy edge, sometimes with blue tinge; located separately, in pairs or short chains, sometimes in groups. In meat peptone agar bacteria grow slowly, 2-3 mm in diameter; a colony is gray-white, round, transparent, with a smooth or wavy edge. In meat peptone bullion bacteria growth is faster, forming a uniform cloudiness that starts from the top. On potato agar, bacteria form brown pigments. Bacteria is highly pathogenic for both common ash and indicator plants.

Physiological characteristics of isolates are not homogeneous. Bacteria grow well in Eijkman, Kohn , Fermi and Ushynsky environments, slightly slower - Liske environment. No growth was detected in Czapek and Omelyansky mediums.

Bacteria are aerobic, do not ferment glucose under vaseline oil; on 3-5th day absorb arabinose, glucose, galactose, dextrose, mannitol, glycerol; on 7-10th day absorbs raffinose, maltose, starch. All strains absorb lactose and sucrose on 17 - 20-th day. No growth was detected on salicin and eskulin.

On the 4th day of growth bacteria use aspartic, glutamic and aminobutyric acid, alanine, asparagine, tryptophan, and also citric, formic, acetic, succinic, malic, or fumaric acid as a carbon source, in an alkaline environment. The bacteria do not metabolize leucine, cystine, cysteine, oxalic or tartaric acid.

Strains do not dissolve gelatin, do not form hydrogen sulfide or ammonia, do not reduce nitrates, or hydrolyze starch. Thus, tuberculosis pathogen is similar to that described in the literature. As for the yellow-pigmented isolates of bacteria, we are presently studying their anatomical, morphological, physiological and biochemical properties.

The harmful entomofauna that damages ash seed include *Tortrix convayana* F., *Pseudargyrotoza conwayana* F., *Ligniodes enucleator* Panz., and *Dasyneura fraxini* Kieff. From galls of *Dasyneura fraxini* Kieff we were able to isolate bacteria that, in nutritious environments, formed gray, round, translucent colonies, size 0.5 -1 mm in diameter. The colony edge is straight, its surface is smooth and its center is upraised. Isolated bacteria were classified as *Pseudomonas* sp. When artificially introduced, selected isolates caused pathological processes on ash branches. Obviously, *Dasyneura fraxini* Kieff could be one of the carriers of ash tuberculosis, but this hypothesis requires further experimental confirmation.

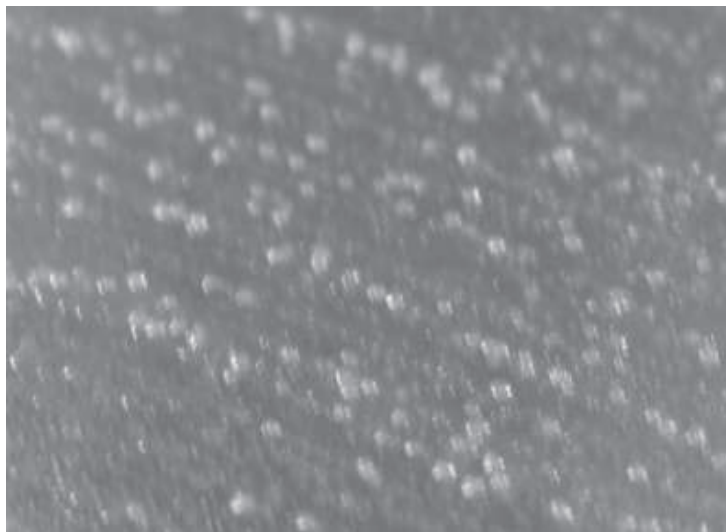


Figure 5. *Pseudomonas* sp.

Ash leaves are also damaged by *Lytta vesicatoria* L., *Macrophya punctumalbum* L), *Calospilos pantaria* L., *Xanthospilapteryx syringella* L., *Melita camaturna* L., *Sphinx ligustri* L., *Tomostethus nigratus* F., *Dasyneura fraxini* Kieff, *Erannis defoliaria* Cl., *Phytagromyza heringi* Hend., *Prociphilus nidificus* Loew., *Psyllopsis fraxini* L. Branch pests include *Prays curtisellus* Don., *Zeuzera pyrina* L., *Leperisinus fhahini* Ranz, *Fonscolombea fraxini* (Kalt.), and *Chionas pissalidis* L.

Among the most common and dangerous pests affecting ash trunks in the study area are *Leperisinus fraxini* Panz and *Hylesinus crenatus* Fabr. We also located *Zeuzera pyrina* L, *Xyleborus* (= *Anisandrus*) *dispar*) Fabr. and *Platypus cylindrus* F. According to the literature review, other trunk pests include *Phloeotribus caucasicus* Reitt., *Hylesinus cholodkovskyi* Berg., *H.cingulatus* Blandf., *H.eos* Spess., *H. laticollis* Blandf., *H.nobilis* Blandf., *H.pravdini* Stark., *H. striatus* Egg., *H. toranio* (Danth.) (*oleiperda* (F.)), *H. varius* F. etc [9].

Conclusions

The most harmful disease of common ash (*Fraxinus excelsior* L)is tuberculosis (the causative agent - *P. syringae* pv.*savastanoi*), which affects both its vegetative and generative organs. From a variety of ash organs (except leaves) we isolated as a yellow-pigmented and spore bacteria that experimentally demonstrated pathogenic properties. We also studied a variety of fungi, including *Deuteromycota*, *Basidiomycota*, and *Ascomycota*.

Our research demonstrated a wide diversity of harmful entomofauna that damage generative organs, leaves, branches and trunks. The greatest harm to *Fraxinus excelsior* L is caused by some representatives of the *Coleoptera* order.

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Наведено результати досліджень патологічних змін вегетативних і генеративних органів ясена звичайного. Показано, що ця цінна лісова деревна рослина досить чутлива не лише до різних за система-

тичним і функціональним положенням груп міко- та мікроорганізмів, а й до численних видів шкідливої ентомофауни. Акцентується увага, що найбільш поширеним і шкодочинним для ясеня звичайного є туберкульоз, збудник якого – фітопатогенна бактерія *Pseudomonas syringae* pv. *savastanoi*, що уражує як стовбури, гілки та пагони, так і суцвіття ясеня звичайного. Відмічено ряд збудників інфекційних хвороб та види шкодочинної ентомофауни, які суттєво послаблюють ріст, розвиток та знижують якісні характеристики деревини ясеня звичайного.

Патогенна мікрофлора, інфекційна та неінфекційна патологія, шкідлива ентомофауна, симптоми хвороб, генеративні органи ясеня, туберкульоз ясеня, патогенез, поширеність та шкодочинність хвороб.

Приведены результаты исследований патологических изменений вегетативных и генеративных органов ясеня обыкновенного. Показано, что это ценное лесное древесное растение весьма чувствительно не только к разным по систематическим и функциональным положением групп мико- и микроорганизмов, но и к многочисленным видам вредоносной энтомофауны. Акцентируется внимание, что наиболее распространенным и вредоносным заболеванием для ясеня обыкновенного является туберкулез, возбудитель – фитопатогенная бактерия *Pseudomonas syringae* pv. *savastanoi* – поражает как стволы, ветви и побеги, так и соцветия ясеня обыкновенного. Отмечен ряд возбудителей инфекционных болезней и виды вредоносной энтомофауны, которые существенно ослабляют рост, развитие и снижают качественные характеристики древесины ясеня обыкновенного.

Патогенная микрофлора, инфекционная и неинфекционная патология, вредоносная энтомофауна, симптомы болезней, генеративные органы ясеня, туберкулез ясеня, патогенез, распространенность и вредоносность болезней.