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BACTERIAL DISEASES OF ENERGY PLANTS

BAKTERYJNE CHOROBY ROŚLIN ENERGETYCZNYCH

Abstract. The results of the study of the bacterial pathology of bioenergetic woody plants (poplar, birch). which lead to a significant reduction in harvest or death are described. Various bacterial diseases of these cultures and their pathogens, symptoms of diseases and characteristics of injuries, distribution and harmfulness of pathogens are described. The characteristics of the main bacterial diseases (bacterial cancer and bacterial necrosis of the bark) and less dangerous ones (wetwood, bacterial blight, root disease and tuberculosis) are presented. The causative agent of bacterial cancer is the Xanthomonas populi bacterium (syn. Aplanobacterium populi). As the accompanying microbiota of the causal agents of canker prevail bacteria of the genus Pseudomonas, Erwinia, Bacillus, Corynebacterium, Chromobacterium bergonzini and fungi. The causative agents of bacterial necrosis of the bark poplar are bacteria from genera Pseudomonas, Xanthomonas, Erwinia. It is showed that bacterial wetwood (dropsy) is caused by Erwinia nimipressuralis (according to the later classification the causal agent is renamed as Enterobacter nimipressuralis (according to the modern classification. - Lelliottia nimipressuralis. In addition to this pathogen from the birch trees from the wetwood symptoms was isolated bacterium Erwinia multivora Scz.-Parf. As related bacteria are involved in the pathological process identified as Pseudomonas syringae, Enterobacter nancerogenus and bacteria of genera Xanthomonas and Bacillus. Methods of protection and prevention bacterial disease with used biopreparation "bio-gel" are given. It was proved the high effectivity of new preparation in process of biologization of agro-industrial.

Keywords: bioenergetic cultures, phytopathogenic bacteria, poplar, birch, methods of protection

Introduction

According to the expert estimates of official statistical data [1], the theoretical potential of biofuel in Ukraine is equal to 50 million Mg (tons) of oil equivalent (toe),

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technical -36 and economically reasonable -27 million toe. That is, on the basis of the present level of the total consumption of primary energy sources in Ukraine (210 million toe in 2007), the economic potential of biomass can satisfy about 13% of the total energy requirement of Ukraine.

Many energy sources of biomass exist in Ukraine. It is mainly the agricultural wastes (straw of the winter and spring crops, trunks and maize ears, husks and trunks of sunflower) and bioenergy wood or the wastes of forest industry.

Materials and methods

The objects of the study were natural plantings of poplar and birch. In the work used methods of forest-pathological surveys and common phytopathological and microbiological methods of research of pathogenic and cultural properties of bacteria – causative agents of diseases of wood cultures [2]. We investigated developed by us "Bio-gel" – a bio preparation based on useful microorganisms of the genera *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Bacilus* for protection of wood species from pathogens. Antibacterial and antifungal activity of the preparation "Bio-gel" was determined by the described method [3].

Bacterial diseases of poplar

Bacterial diseases of agricultural and woody crops cause significant damage to agriculture and forestry. Identification of causal agents is an important step in understanding the epidemiology of the disease. Definition of diseases and their pathogens will allow developing methods of disease limitation, which will lower the extent of injury and reduce the economic losses.

The poplar belongs to fast-growing species. By the productivity of wood per unit area it has one of the first places among the fast-growing species in the Polesye, Forest-steppe and Steppe zones. In favourable conditions at the age of 25 the volume of poplar wood reaches 700 m³ per 10 000 m² of crop. Poplar wood is widely used in the industry. In addition, the poplar is used in creation of field windbreaks and in all types of urban plantations.

The poplar as fast-growing bioenergy wood draws attention of forestry specialists, energy workers on alternative energy sources of many countries across the world in hope to cover at its expense the ever-increasing deficit of industrial wood and as the energy source of biomass. However, their cultivation is connected with considerable difficulties, as the poplar is affected by diseases more often than other species.

Bacterial wetwood of poplar

The disease received the name "wetwood" due to severe flooding of tissues [4–6]. In literature this disease is also known under other names, in particular "bacterial wet canker", "bacterial brown slime flux" or "brown slime flux" [5], "dropsy" etc. Bacterial wetwood (dropsy) of poplar in Ukraine is caused by *Erwinia nimipressuralis* (causal

agent is renamed as *Enterobacter nimipressuralis*; according to Brady C. et al. – *Lelliottia nimipressuralis* [7]). Scott [4] noted wetwood taken from euramerican hybrid poplars (*Populus×euramericana*) contained large and diverse populations of bacteria. Most of the common bacterial strains were capable of altering wood components. The bacteria most consistently associated with wetwood were identified as *Enterobacter* and *Clostridium* species. These bacteria may play an important role in the development of wetwood in poplar. Schink et al. [5] revealed that isolated from wetwood *Populus deltoides* Bartr. bacteria included *Clostridium*, *Bacteroides*, *Erwinia*, *Edwardsiella*, *Klebsiella* and *Lactobacillus* species. Only two strains of *Erwinia* and *Clostridium* species were prevalent in wetwoods of all the trees examined [5].

Symptoms of disease. According to many authors, who studied the wetwood of poplar disease is characterized by the following symptoms. Poplars commonly develop wetwood, a water-soaked condition accompanied by a darkening of the wood. Premature death of poplars, especially of Lombardy poplar, has been associated with wetwood. Lumber made from wetwood is weaker than sound wood and is subject to checking and collapse during drying [8].

Longitudinal cracks emerge in the early spring on the bark of branches and trunks of trees, and from them appear the colorless exudate or the one of amber color, which darkens on the air. The thick exudate can appear during the whole vegetative period at high humidity (Photo 1) [9]. The bark dies, cracks and hangs down in patches, which is the specific feature of disease. Wood becomes brown-red. The infected bark and wood are wet, liquid flows out from them when pressing on them, sometimes lots of it. The maceration of tissues of phloem and wood happens under the influence of bacterial activity, and gas is accumulated in the wood, and that produces substantial pressure in its middle (0,05–0,5 H/m²). It mainly consists of the methane (53.6%), nitrogen (28.4%) and carbon dioxide (17.6%).

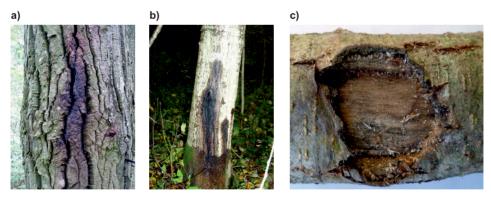


Photo 1. Bacterial wetwood of poplar: a), b) the foveate spots due to dieback of phloem parts of the bark and cambium of red-brown color, c) maceration of tissues of phloem and wood [9]

As a result of dieback of the bark on the trunks are formed the foveate ulcers, sometimes covered with the died bark. Cracks on the trunk and branches can be healed by smooth bark. Disease can get the chronic form in old trees: from year to year on such trees is observed the excessive appearance of exudate on the trunks and dieback of some

branches. Similar symptoms of disease under artificial inoculation of lignified shoots by *E. nimipressuralis* bacterium observed in the autumn and in the early spring. In such a case in the second half of summer appeared necrotic foveate spots of gray-brown color, which increase in size. The wrinkled bark cracked and exfoliated in patches till the end of summer, branches died. In the spring of next year near the basis of dieback branches appeared exudate, and bacterium caused the local quickly healing necroses on young branches. Infection of buds led to the browning of internal scales, and the buds became empty by itself. If the buds continued to develop, then leaves became deformed, and the tops blackened.

Specialization. The causal agent of bacterial wetwood of poplar is polyphage, infects almost all woody and shrub species, though not to the same extent. Among them are the pine, spruce, fir, larch, juniper, European yew, oaks of all species, beech, hornbeam, Tatarian maple, birch, alder, aspen, elm family, Amur cork tree, horse chestnut, linden, Persian walnut, pear, European spindle tree, grape, ginseng. *E. nimipressuralis* bacteria, isolated from the poplar, infect the leaves of oak, beech and hornbeam cause necroses on the leaves of bean (*Phaseolus*) and tomatoes. Tobacco leaves, pods of Phaseolus beans, cotyledons of sunflower, leaves of fruit trees, birch, linden and other plants are not affected by *E. nimipressuralis*.

The affected species of poplar. Bacterial wetwood is most often met in nature on the poplar species: Chinese, berry-bearing, Californian, Bolle's, *Dopulus laurifolia*; white poplar (*Populus alba*) is resistant. The intermediate position is taken by *Populus berolinensis* and black poplar.

By means of artificial inoculation in the field conditions was established that white poplar (*Populus alba*), gray poplar (*Populus canescens*), *Populus bolleana*, berry-bearing (*Populus deltoides*), large-leaved poplar (*Populus candicans*) are resistant to the causal agent of bacterial wetwood *E. nimipressuralis*. Black poplar (*Populus nigra*), Bolle's poplar (*Populus pyramidalis*), Chinese poplar (*Populus simonii*), black Chinese poplar (*Populus simonii nigra*), balsam poplar (*Populus balsamifera*), fragrant (*Populus suaveolens*), Californian poplar (*Populus trichocarpa*) are infected by the causal agent of bacterial wetwood.

Spread and harmfulness. Bacterial wetwood in Ukraine is widespread everywhere, in particular in nursery gardens, forest plantations and plantations of green belt. The disease is of special danger to young poplar at the age of 4–8 years. The harm done by the specified phytopathogenic bacterium in the Western areas of Ukraine, considerably increased. Disease is widespread in Krasnodar Krai, where certain cultivars and species of poplar died completely. In Belarus it is the most dangerous disease of poplar and infection rate of plantations is equal to 50, and sometimes 82.9%. Poplar disease like wetwood is described in Czechoslovakia, Bulgaria, USA and other countries.

The birch is also affected by bacterial wetwood.

Bacterial wetwood of birch

Birch is one of the main species of field protection forestation. The birch is very valuable for forestry, because it is the pioneer species during the planting of open forest spaces. As well as the poplar it is the fast-growing bioenergy wood and draws attention

as the raw material for alternative energy sources. However, similar to the poplar it has the bacterial disease – bacterial wetwood (dropsy) [10].

Symptoms, affection, specificity, spread and harmfulness are described for the poplar. Strains of bacteria were allocated from infected wood, and the part of them was classified as *Erwinia multivora*, (the causal agent is outdated and is not mentioned in the Determinant of bacteria). From the other part were isolated phytopathogenic bacteria, identificated as *Pseudomonas syringae*, very close by properties to *E. hancerogena* (the modern name of causal agent is *Enterobacter hancerogenus*). Both species of bacteria were pathogenic for the birch, though at the action of *P. syringae* were developed necroses of the smaller size. More accurate necroses developed when using the mixture of mentioned above bacteria.

Territories of birch forests in Ukraine are intensely affected by bacterial diseases; therefore there is the need to speak about the danger for this crop.

External symptoms of disease are the thinness of the crown and the presence of dry branches in it. Leaves in the crown are smaller, than in healthy trees, the leaf has



Photo 2. Bacterial wetwood of birch [11]

yellowish tinge. Water shoot, sometimes numerous, appear in the lower part of the crown. On the bark are noticeable the red spots from appearances of exudate from the wet bact. The bast and wood in the places of infection are wet, have dark-brown color with characteristic musty smell (Photo 2) [11].

Swellings are formed above those places, where the bast and cambium dies due to development of bacterial disease. Phytopathogenic bacteria, which develop, form the gases during bacterial activity, which, at accumulating under the dense and impenetrable for the gases bark of the birch, form the swellings, which are filled with exudate. The trees, on which such swellings were formed, die if the spots of the died bast and cambium ring-bark the trunk in its lower part. If the swelling did not ring-bark the trunk, then the tree continues to live. Water shoots are formed on the trunk, and they can live 1–2 years. Ripe low- and tall trees, and also the medium-grown overmature forests are the most affected by bacterial disease.

Bacteria (*Erwinia multivora* Scz.-Parf.), by penetrating into the wood through the damages, which are made by woodpecker, frost cracks, mechanical damages, destroy pectic partitions of cells, cell sap fills by itself the whole volume of infected wood. Wood becomes wet and very heavy (Photo 3) [11].





Photo 3. Damages of woody by phytopathogenic bacterium Erwinia multivora Scz.-Parf [11]

Carriers of bacterial disease are woodpeckers, who make openings in the trunk of birch and drink juice in the spring, and also such insects pests – birch bark beetle, birch ship-timber beetle.

In the spring, during the period of feeding of nestlings, the woodpecker, or greater-spotted woodpecker (*Dendrocopos major*) allures insects – by drilling the ranks of accurate rectangular openings on the trunk of birch, then woodpecker drinks sugary sap (carbohydrate component of its diet) and hides for a while, waiting for insects to get into this sugar trap, and they provide the menu of woodpecker with proteins and fats.

Thus, woodpeckers are disease carriers (*Erwinia multivora* Scz.-Parf.) on long distances, infecting with bacterial wetwood the ever more new forests, the part of which is the birch.

In Kazakhstan, the symptoms of the disease are described as the appearance on the trunk of small swellings, which in time increase in size and tearing forming a lacerating epidural wounds. Change in color, size of leaves and degree of sparseness of the crown occurs at the last stage of the disease, immediately before the death of the tree [12]. Among the identified species dominated pathogenic bacterium *Pseudomonas* sp. In 30% of the samples was detected bacterium from family Enterobacteriaceae with the greatest similarity in genetic structure to phytopathogenic bacteria *Pectobacterium carotovorum* and *Dickeya dadantii*. In rare cases the bacteria isolated from the genera *Listeria*, *Acinetobacter* and *Xanthomonas* [12].

Shvets [13, 14] established that causative agent of bacterial dropsy birch in the Zhytomyr Polissia of Ukraine is *Enterobacter nimipressuralis*. From the pathology of bacterial dropsy the associated bacteria of the genera *Xanthomonas*, *Bacillus* and micromycetes (*Penicillium*, *Mucor*, *Rhizopus*) are isolated.

Bacterial canker of poplar

Among various diseases bacterial canker is one of the most damaging diseases of poplars throughout much of Europe, resulting in branch and stem cankers and dieback [15–17].

Etiology of canker. As well as at the other bacterial diseases, as the reason of affection were considered insects, abiotic factors, fungi, the mixed infection caused by bacteria and fungi, and in some cases it could not be defined. Hypothesis about the abiotic nature of canker of the poplar should be rejected, as all researchers, who artificially inoculated the plants with natural mucus, noted appearance of typical symptoms of disease. It is also revealed that infectivity of mucus depends on the year and seasonality and quickly decreases after appearance of exudate [18].

At the present time from the canker of poplar are allocated various phytopathogenic bacteria, however even the acceptance of bacterial nature of disease by itself does not provide final clarity in this question, because it is unknown, which of the described species of bacteria is the real causal agent of disease. For the first time the bacterial nature of canker was highlighted by G. Delacroix. The author allocated from canker tissues the bacterium, which he called as Micrococcus populi and considered that it causes thickening and cracking of the bark on young random-shaped branches, and on old branches - tumors. Bacterium Pseudomonas rimaefaciens is isolated from ulcers on the poplar in the Netherlands. The artificial inoculation of poplar with bacteria caused the fast dieback of branches without typical symptoms of canker. In England failed to infect the poplar plant with the bacterium allocated, which by the properties is close to P. rimaefaciens. At the same time the typical symptoms of canker developed in the case of infection with natural mucus, from where later was allocated the bacterium, which by the properties reminded P. rimaefaciens. In the USSR the typical picture of disease of Populus balsamifera after 24–40 days from infection with P. rimaefaciens was revealed. This bacterium was allocated from infected poplar in Poland, Turkey and Belarus. Pseudomonas syringae f. populi and P. cerasi bacteria were isolated from infected popular trees in Ukraine. The strains of both species were highly-virulent,

infected leaves, branches and trunks. At artificial inoculation *P. syringae* f. *populi* caused development of necroses on the branches, there took place the increase in the sizes to the full ring-bark of branch, and at inoculation with *P. cerasi* there developed the localized necroses-ulcers, fringed of calluses with the cracks in the bark, similarly to the natural manifestation of disease. The causal agent remains in the ulcers and restores its activity in the spring. In the modern taxonomy of phytopathogenic bacteria both species of causal agents (*P. syringae* f. *populi* and *P. ńerasi*) have one name – *P. syringae*. Perhaps the authors worked with one causal agent, which had strain differences.

P. cerasi, unlike *P. syringae* f. *populi*, moves along the vessels of leaf and causes necrosis of adjacent tissue. If we consider the existence of these two species as independent, it becomes clear why Sabet and Dowson [19], having allocated *P. syringae* f. *populi* from the canker infection of poplar, could not receive the typical picture of the symptoms of disease in experiment.

While describing the disease of poplar, which causal agents are *P. syringae* f. *populi* and *P. cerasi*, gave it new name as "the canker-ulcer disease", so that to distinguish it from the canker of poplar, as the causal agent of which at that time has already been identified the *Aplanobacterium populi* by Ride (now *Xanthomonas populi* [16]). The main causal agent in association at canker-ulcer disease is *P. cerasi*. Bacterial canker and die-back of poplars in Britain is caused by *Aplanobacterium populi* Ride [18]. *Pseudomonas syringae* van Hall, which has been suggested as the causal agent, plays only a minor role in canker lesions. It may cause a shoot blight in spring, a distinct pathological condition frequently associated with bacterial canker. Marked variation in infectivity of bacterial slime between seasons and at different dates of collection within one year appears to be the main cause for situations in which varieties, resistant in trials to inoculation with slime, have proved susceptible in the field. Leaf scars afford the main avenues for infection but their infectibility declines rapidly during October [18, 20].

Bacterial canker and bark necrosis combined with freezing stress are big problem. Several bacterial species, for example *Pseudomonas syringae*, *Erwinia* sp. and *Xanthomonas* sp., can be involved and are usually ubiquitously present within plantings. *Xanthomonas populi* (= *Aplanobacterium populi*) bacterium has been a priority pathogen for resistance breeding in poplars for many years in Europe. The poplar canker disease caused by this bacterium is common in Europe but is not known to be present in North America [16].

The *A. populi* bacterium in case of artificial inoculation is capable to cause the typical picture of bacterial canker on the poplar Regenerata, and that was confirmed by other researchers on different species of poplar in the Netherlands, France, Germany, England, Poland, on the territories of Soviet Union.

As the accompanying microbiota of the causal agents of canker diseases of poplar prevail bacteria of the genus *Pseudomonas*, including *Pseudomonas syringae* f. *populi*, *Erwinia*, *Bacillus*, yellow-pigmented bacteria *Corynebacterium*, *Xanthomonas* and *Chromobacterium bergonzini*, fungi from the genera *Fusarium*, *Nectria*, *Cytospora*, *Cladosporium* and *Penicillium* and yeast. From canker bark of *Populus* x *euramericana*

isolated *Acinetobacter qingfengensis* sp. nov. [21] and *Brenneria populi* sp. nov. [22, 23]. But the authors do not report the pathogenic properties of the isolated bacteria. Some of the above mentioned bacteria can cause poplar diseases even with formation of thickenings and die-back of shoots (*Pseudomonas*) or just cause necrosis of shoots (*Erwinia*), die-back of green shoots (*Xanthomonas*, *Pseudomonas*) and necrosis of leaves of bean. In the wounds of wood are formed associations of *P. syringae* and some fungi which are often the cause of disease of poplar [24].

It is possible to draw a conclusion from represented data, that various microorganisms live both in mucus, and in the wounds at canker diseases. Therefore, the studying of relations between them was logical. The first such attempt was made by Urosevic [24, 25]. The author did not reveal any antagonistic relations between the pathogenic and saprotrophic bacteria. Besides, at joint cultivation some of their properties even amplified. The canker of poplar is the mixed bacterial disease, in which the main role is played by one or another causal agent, depending on ecological conditions. In the coastal area of Western Europe the main causal agent is A. populi, in the Central part of Europe – E. cancerogena, in the Eastern – P. cerasi. It is also always allocated P. syringae from affected samples. Considering that the description of new causal agents in different geographical zones lasts till now, it is possible to assume that similar symptoms of infection of poplar can be caused by different species of bacteria. Authors must give to each disease, which is caused by different causal agents, the independent name, so that there was no confusion in literature at definition of disease. The following causal agents of canker diseases of poplar are established nowadays. They are the A. populi (E. cancerogena) – for the canker of popular and P. cerasi × P. syringae f. populi (P. syringae) – for the canker-ulcer disease.

The symptoms of bacterial canker. The first symptoms of disease coincide with the period of spring pruning of trees and are well noticeable on one-two-years shoots [24]. The trunks of trees and branches are infected. In the early spring and at the beginning of summer on the lignified part, usually at the level of internodes, around the infected shoots and near the basis of branches are formed the small damp blisters, which open with the cracks. The last ones can widely open, forming the ulcers. Ulcers can be small (1–3 cm), nodose, "the closed ulcers" and the most typical ones – are large (1–15 cm), extended, "open ulcers" [19, 24]. The wood under infected area is light-red, the bast is dark gray, it is partially painted [26]. The bark and part of cambium at cross-section is half-transparent. The necrosis is formed and branches dieback during the fast disease progression, during the slow – the cork roller is formed, which expands with passing by years [24, 25]. The most intensive development of tumors and ulcers is observed in May, and almost completely declines till August.

Spread of bacteria is happening by the intercellular space of parenchyma and phloem tissue of the bark; bacteria are not found in the heart pith of trees. Bacteria infect the parenchyma of bark, and sometimes the xylem vessels [24, 25]. From the ulcers on the place of leaf scars, cracked blisters, the swollen infected shoots appear the stringy, sticky, nebulous exudate, more liquid in the damp weather, at first it is whitish or gray, which gets the brown coloring as time passes. Exudate is formed in the early spring when the shoots are unfolding, its quantity gradually decreases till autumn and winter.

However, it can also appear in the autumn, which is the symptom of annual infection. Release of mucus depends on climatic conditions and the species of poplar. The outflow of exudate is not spotted in the case of canker of poplar in the North America. Climatic conditions in the Czech Republic and Slovakia also were not suitable for appearance (outflow) of mucous exudate at artificial inoculation [24, 25]. At the same time, the outflow of exudate is considered as the characteristic symptom of bacterial canker of poplar [25].

Appearance of mucus near the basis of small knots of the died branches of trunk is the first external symptom of disease. The leaves prematurely fall, the top of the crown and side branches irregularly dieback at the severely affected trees. As a rule, the leaves are not damaged, only in the case of infection of sprout is sometimes observed the chlorosis of leaves. Only Sabet and Dowson [19] noted the existence of small brown or large black necroses on the leaves and petioles. These differences between the symptoms of diseases, which were noted by authors, perhaps, are connected with the cultivar features of the studied poplars, ecological factors or *P. syringae* f. *populi* infection.

Symptoms of canker-ulcer disease. Disease is characterized by existence of cancerous ulcers on the trunks and branches of different age, and they represent by themselves the nodose, scattered wounds of unequal sizes. At the edges of the ulcers remained residues of the died bark, which have yellowish tinge. Numerous cracks in the bark appear on the trunks and branches of infected trees in the early spring with the beginning of sap flow. The colorless exudate appears from the cracks, over time it is red-brown. The small foveate spots are formed in the places of appearance of exudate till the end of April-May, and they almost do not differ from the healthy tissue by the coloring, and only on the poplars with the light bark they have the low-observable grayish tinge with more dark border. Spots are better noticeable on wet branches. The cambium in infected places is wet, rotten, with musty smell. When the top layers of the bark are removed, the spots are appearing, and they are separated from the healthy tissue by means of red-brown border. The wood is always brown-red, with different tinges. The affected areas on small branches is healed with callus till the end of vegetative period, forming the small wounds-nodules. With disease progression the bark in the places of infection cracks more and more, baring the wood. The formatted wounds can increase in size during several years. They gradually ring-bark the branch or the trunk, and that leads to dieback. The mechanical strength of wood decreases at the severe affection of trunks and the branches and trunks are broken during the strong wind.

The activity of buds on infected trees is weakened, they dieback. Flower buds do not disclosed or do not completely throw out the earring, which, due to its infecting, becomes oily, dark brown, dies and prematurely falls. The cracks and ulcers are formed at the base of such buds – the onset of disease of branches.

On the leaves of some poplars in the middle of July are developing the non-transparent necroses, dark brown or gray-brown, the ones, which are more intensively colored to the edge, with irregular shape, sometimes with chlorotic border. The spots are mainly located along the edge and are closer to the top of the leaf. Necroses affect

the most part of leaf blade till the middle of August, and necrotic tissue cracks and falls. Sometimes the veins with the adjacent tissue of leaf are infected.

The quality of wood sharply decreases in the case of bacterial canker, it is stimulated the development of wood-destroying fungi. The infected wood is unsuitable for industrial use.

The harmfulness of disease lies not only in the fast and large-scale dieback of trees, thinning of the crown, reduction in leaf numbers, nonresistance of affected trees to wind-fallen trees, but also in decrease of wood increment. The losses are equal to 22.9% of the general wood increment of healthy plants. Economic efficiency from plantings of poplar considerably decreases due to their reordering. The general loss is equal to 49.3% of the cost of wood, whereas the losses from other diseases do not exceed 2.2%.

Bacterial necrosis of the bark

Bacterial necrosis of the bark (brown spotting) of the poplar becomes the second serious obstacle during the cultivation of this valuable crop in comparison with the bacterial canker. The problem of bacterial necrosis of the bark of poplar was discussed at the international conferences in Munich in 1967.

Etiology of disease. Cocco-like bacteria are allocated from affected areas, including *Micrococcus populi*, asporogenous and sporogenous bacilli, *Corynebacterium* [25]. It was also reported about the bacterial nature of this disease of poplar. Besides, the researcers considers fungi as the possible etiologic factor of this disease. *Dothichiza populea* and *Fusarium lateritium* fungi were allocated by Urosevic. However, their role in the etiology of disease, according to the author, is minor.

The works of Urosevic are of considerable interest for determination of etiology of disease [24, 25]. The author drew a conclusion that this disease is caused by the mixture of bacteria P. syringae and bacteria of the genus Erwinia, which by the properties are close to E. cancerogena and E. nimipressuralis. Urosevic pointed out that bacteria of the genus *Pseudomonas* are allocated systematically from necrotic spots both during the dormant period and during the vegetative growth. According to the author, pseudomonades participate in the spread of necroses, and bacteria of the genus Erwinia help in formation of wet necroses. Considering that antagonism between the Erwinia and Pseudomonas species is not established, Urosevic assumes that the main cause for appearance of disease should be searched for in the joint effect of these bacteria. Thus, in conditions of greenhouse bacteria cause necroses on annual poplars. In natural conditions the typical necroses, sometimes with the exudate, develop only on the poplars with the age of more than three years. These species of bacteria are so connected among themselves, so that they are both allocated even from the necroses, artificially caused by one of them. In these cases the symptoms of disease do not differ from those ones, which arise at the natural penetration of infection [24, 25].

A bacterium was isolated from superficial bark necroses on young poplars and its pathogenicity demonstrated by inoculation experiments. The causative agent was identified as *Xanthomonas campestris* pv. *populi* [27].

The frequent joint allocation of bacteria and fungi from infected tissues of the poplar led Urosevic [24, 25] to the thought about possible joint affection of plants by these microorganisms. The artificial inoculation of poplar by the mixture of bacteria and fungi *Fusarium lateritium* gave more clearly expressed symptoms. At the same time, the works of Urosevic [25] also did not bring the full clarity in the etiology of this disease, because, while describing diseases of poplar, the author called them either as the "brown bark spotting", or as "bacterial canker", or as "bacterial bark peeling off poplar" and in all cases he allocated the same bacteria *E. cancerogena* and *P. syringae*. In this regard, it is unclear, whether the author speaks about one disease or about the different diseases. At the same time, Urosevic [25], suggested that the necrosis of the bark is the mixed infection, and *P. syringae* – is one of causal agents, which are causing disease.

Ukrainian scientists isolated from cancer-ulcer disease of poplar along with *P. cerasi* and *P. syringae* f. *populi*, which caused necrosis of the bark in the case of inoculation by pure culture.

Other researchers allocated *P. saliciperda* at the similar disease. This bacterium is identical to *E. salicis*. It should be noted that in the case of artificial inoculation of leaves of poplar with the *P. citriputeale* and *P. syringae* cultures, which are isolated from other plants, necroses are formed, and the fact that *P. citriputeale* is the synonym of *P. syringae*.

With taking into account the literature data that from necroses of the bark of poplar are allocated bacteria, which belong to *P. syringae* species, which pathogenicity is proved by numerous researchers, this species should be considered as the causal agent. Other microorganisms, which are isolated from affected areas, are the accompanying microbiota.

Symptoms of disease. The main sign of necrosis of the bark of poplar is the formation of necrotic spots with the size of 25–30 cm on the trunks, branches and young shoots, and these spots are most often located on the lower branches. The first symptoms of this disease appear in the course of the growing season on the 1-year-old shoots, especially in nursery stock. The attacked bark tissue becomes dark and subsequently necrotic in long streaks. Initially the damage is superficial and does not reach the cambial zone, but when progressing, necrosis reaches the cambium and the bark may be killed. Cracks appear in the bark and the wood becomes exposed. Sap containing numerous bacteria exudes through the cracks and lenticels and dries to a white powder [27].

The first signs in the form of blisters on the bark appear in March–April with the size of 1–3 cm, in some cases reaching 10 cm, at first they are filled with transparent liquid, which gradually darkens later. After the rupture of blister, the liquid outflows and the tissue dries-up [25]. The internal layers of bark are infected at first, then are observed necroses of the top layers. Thus, all elements of the bark are exposed to infection, but the cambium is most often exposed. In this case the bark cracks, the callus is formed, mucus outflows from the wounds, which dries on the bark and turns brown. The cracks in the bark are often covered with white powder mixture of dried-up mucus. The wood under the bark is dark. The bark by itself is brown, at the edge of healthy and rotten bark remains orange or yellow stripe of infected tissue. Along the edge of old damages

at some distance from them appear wet spots, which can become the center of further spread of disease [25]. Some necrotic spots often merge, sometimes they cover branches and trunks of trees, causing their dieback, sometimes branches wilt, even with well-developed leaves.

In addition to these signs there is the excessive slime flux in the summer, openwork of crown, dieback of the top, and Urosevic [25] notes the considerable amount of water in the conduction paths, and that leads to accumulation of intracellular liquid. These symptoms are more characteristic for the wetwood, which causal agent is *E. nimi-pressuralis*. The stated above authors observed the mixed bacterial infection, in which the properties of one of causal agents are more manifested depending on the period of year. Such assumption is also based on the data of Urosevic [24, 25], who showed the participation of bacteria of *Erwinia* genus in disease, in addition to *P. syringae*. And bacteria of the genus *Erwinia* are close by the properties to *E. nimipressuralis* (the causal agent of the wetwood) and, according to the data of Urosevic, they are responsible for the flooding of necroses.

The affected species of poplar. Bacterial necrosis of the bark as if changes bacterial canker of poplar in natural conditions on poplar plantations. It is possible to explain it by the fact that the cultivars of poplar, which are resistant to the causal agent of bacterial canker, are most often affected by the causal agent of necrosis of the bark. Therefore, it is necessary to perform selection of poplar with taking into account the resistance to both causal agents of diseases.

Such species are mainly affected: *Populus serotina*, *P. gelrica*, *P. nigra*, *P. nigra italica*, *P. robusta*, *P. berolinensis*; rather high resistance is characteristic to the natural white and gray poplars. *P. saliciperda* (now *Brenneria salicis*) is pathogenic for Chinese poplar, fragrant and Siberian, but laurel-leafed poplar – is resistant. In addition, bacterium affects brittle willow and does not affect mountain ash, apple tree, golden currant, Chinese elm, lilac.

Spread and harmfulness of disease. Necrosis of the bark of poplar – is extremely dangerous and contagious disease. In recent years in Ukraine is revealed the poplar die-back at the age of 15–20 years, and that causes extensive economic damages to households and devalues the operations concerning field-protective forestation. In some forest belts is happening die-back of 50% of trees. Disease can cause die-back of 20–25-year trees within 4–5 years by appearing in the plantings. It is the most serious threat to the cultivation of poplar.

The disease under consideration is well known in Western Europe [25]. From 1384 trees, studied in Slovakia, 90% were infected with the causal agent of necrosis [25]. The disease progresses from year to year and brings more and more notable damage. The trees of different age become affected, starting from 1–2 years. The disease progresses especially quickly on the trees, which were grown on poor soils at the thick planting, at the rapid change of negative and positive temperatures, frosts in the winter and dry summer, at deterioration of microclimate, under the influence of direct sunlight, fall in ground water level.

Bacterial blight

The bacterial disease of poplar like the blight was mentioned for the first time in last century. Later the blight of bark of poplar in the USA is noted, however its causal agent is not allocated. It was only supposed that disease is caused by E. amylovora – etiologic factor of fire blight of fruit trees.

Over time in Czechoslovakia, and later in Ukraine it is described the disease like blight, however, its causal agent turned out to be the *Pseudomonas syringae*, and the permanent companion of phytopathogen – *E. herbicola bacteria* (now *Pantoea agglomerans*). The fungi *Cladosporium*, *Penicillium* and others were isolated at the later stages of infection, but they are not the cause of disease. According to report of Urosevic [24] has also allocated the virulent strain *P. syringae* at this disease.

Given the fact that some researchers consider *P. syringae* and *P. cerasi* as identical organisms and unite them in one species *P. syringae*, it is possible to consider that researchers do not have disagreements concerning the causal agent of blight.

Symptoms of disease. The main symptoms of disease, which are described by Urosevic [24], are such. On the veins of reverse side of the leaf appear edematic spots or streaks of brown color with transition to red color. At the same time it is observed the formation of papillae – small bulges of green color with the smooth surface, in this case the leaves are rolling. Later the centers of necroses merge in the short or long stripes, located along the vein of leafstalk or shoot; the affected tissue cracks, dries, it as if incrustates. The buds, leaves and shoots become infected, leaves fall before their due time, the shoots become rolled, dieback from the top to the base. Disease retards the growth of shoots and leaves, which remain small. The symptoms become apparent in the middle and the last third part of the period of growth. In the next year disease progresses from affected buds.

The symptoms of the blight of poplar in Ukraine are slightly different from described in Czechoslovakia, and that, perhaps, is connected with varietal features of affected trees and they are characterized by the following signs. In natural conditions on the edge of the leaf develop large necrotic zones of yellowish-brown color with the brown, accurately separated brim, behind which sometimes extends the narrow strip of chlorosis. Necroses of the small size of light brown color with dark-brown brim are observed very seldom. In the case of severe affection the leaves are deformed, dieback, hang died for some time, then they fall and the trees die. The disease starts to become apparent in July. At this time the causal agent – *P. cerasi* is allocated.

No damages (cracks, ulcers, necroses) are revealed on the trunks and on the branches. At the same time in the case of artificial inoculation *P. cerasi* infects the stalks, buds, green shoots, spreads by the vessels of the leaf, causing necrosis of adjacent tissue, and it does not affect the lignified shoots. The typical picture of leaf blight in experiment appeared during the spraying of swollen (green cone) buds of annuals seedlings.

The affected species. The species, which are mainly affected are the *Populus angulata* and its hybrids with the black poplar [24, 25]; Canadian poplar, the seldom are affected Bolle's poplar and some varieties of balsam poplar.

In the case of artificial inoculation of leaves by means of the method of wounding *P. cerasi* affects the such poplar species – *Populus alba, Populus canescens, Populus* nigra, *Populus pyramidalis*), *Populus simonii, Populus trichocarpa*, and also it affects the aspen. The laurel-leafed poplar is resistant to the pathogen. The causal agent also affects the leaves of pear, willow, lilac, common oak and red oak, horse chestnut and does not cause any visible pathological changes in the case of artificial inoculation of birch, apricot tree, sweet cherry, hornbeam, maple, linden, cotyledons of sunflower. The leaves and fruits of tomatoes, beans, the leaves of pepper can serve as the indicator crops for preliminary selection of virulent strains.

Spread. The blight of poplar is found in Czechoslovakia, France and, perhaps, in Yugoslavia, in population centers of some areas of Ukraine and it is not revealed in the forest plantings [24, 25]. Though the disease is very harmful – leads to the fast dieback of trees, but the percent of affected plants is low (4–5% or single plants), which, perhaps, is connected with the fact that the died trees are quickly removed from population centers.

Root disease

Root disease research of poplars (the agent *Brenneria salicis* syn. *Erwinia salicis and Pseudomonas saliciperda*) has not yet been a high priority. One exceptionis the water mark disease, caused by the bacterium *B. salicis*, that is especially damaging to *S. alba*. The disease was first described in the early 1900s in England and the Netherlands, and it was recently reported from Belgium and Japan. The bacterium infects the roots and vessels where it resides, and can be asymptomatic in less susceptible hosts or non-hosts such as poplar, alder and other willows species. Disease symptoms include wilting leaves and discoloured wood with a high moisture content. An important agent for spreading the pathogen is infested propagative material. The potential presence of latent infections in roots and vessels of a wide range of species underscores the importance of careful screening of propagation stocks to avoid further spread of this pathogen [16].

Tuberculosis

Tuberculosis of poplar is described for the first time in Italy and France in 1907 under the name "canker of poplar". This disease appears on one-two-year branches. In this case the epidermis blown off, developing into round gall-like tubercles as a pea with the smooth surface. When they are close located, they merge, the surface cracks, folds become deeper, the tumors reach 15 cm in diameter, become wrinkled with the open cracks. The causal agent of disease – sporogenous bacilli – *Bacillus populi* was allocated and described. However, considered *Agrobacterium tumefaciens* as the real causal agent of disease, and *B. populi* only as the accompanying bacteria. *A. tumefaciens* is capable to infect the poplar, and it was confirmed later by the number of researchers.

Urosevic observed disease, very close by the symptoms – tumor-like canker [24]. Unlike other researchers, the author considers bacteria of the genus *Chromobacterium*

as the causal agents of disease, which, in his opinion, are similar by the properties to *A. tumefaciens*. Bacteria cause necroses on the leaves at artificial inoculation in greenhouse. At the same time, Urosevic is of opinion that the question concerning the pathogenicity of these cultures needs to be checked more carefully.

Poplar disease is described in Ukraine, which was called it at first as the canker – tuberculosis disease, and later – as the tuberculosis.

The causal agent of tuberculosis in Ukraine is *B. populi*. By means of artificial inoculation proved the ability of *B. populi* to cause formation of excrescences of tubercular nature, though the natural damages were more extensive. The revealed disease of popular and described phytopathogen *B. populi* are new discovery in fact. Its existence in the nature as the causal agent of disease was not confirmed by other researchers.

Symptoms of disease. On branches appear blown off swellings, most often under the age of 7 years, which develop into ulcers and tumors of various sizes, sometimes they exceed the diameter of the carrying branch by 2-3 times. The form of tumors is spherical, oval, round and nodose, warty. The surface initially is smooth, then – rough. They are located in different parts of the branch, but most often they are located on the place of the dead side shoots, buds, leaf scars, in the internodes. Infection can affect the part or the whole branch. In the latter case at the middle of swelling is always present the slit-like deepening. The thickened knotty and warty rings of wood and cambium are visible if to cut the infected tissue. Internal rings of wood are colored in red-brown of different tinges, external rings of wood have the coloring that has not changed. Inside tumors are formed the cavities, which are filled with the loose dry mass of disturbed wood. Wet spotted damages on cross section are observed only on the cambium. The exudate does not appear. The infection of leaves under natural conditions is not established. At the same time in the case of artificial inoculation of leaves is happening development of dark-brown or gray-brown spots with the narrow strip of chlorosis. The infected tissue sometimes occupies 1/2-3/4 of leaf blade. The petiole, buds and vessels are not damaged.

The affected species. The disease affects *Populus alba*, *P. nigra*, *P. tremula*, *P. tri-chocarpa* (Californian poplar), the poplars from Leuce section [24, 25].

In the case of artificial inoculation *B. populi* causes damages on the leaves of different species, cultivars and hybrids of poplar, and also on the leaves of willow, lilac, red oak. However, the tumors developed only on the Californian poplar. Bacteria do not affect the leaves of birch, common oak, linden, sweet cherry, Sudan grass and tomatoes, cotyledons of sunflower, do not cause the hypersensitivity reaction on the leaves of tobacco, pepper and bean pods. By the type of hypersensitivity reaction to injection of bacteria by means of infiltration react primordial leaves of *Phaseolus*. Brown necroses with the dark bordering and weak chlorosis are formed on leaves of tomatoes. In laboratory experiments under the influence of *B. populi* the tomato fruits become watery and glassy, rotten, with very unpleasant smell. Only pathogenic strains cause the rotting. The specified feature, from our point of view, can be used for selection of virulent isolates.

Spread and harmfulness. The disease was observed in France, Czechoslovakia and Ukraine. Under the influence of causal agent the branches can dieback, but the trees do not die [24].

Protection measures from causal agents of bacterial diseases of forest trees, in particular the wetwood of birch, are not yet developed. The only, so far known method – is the felling of trees.

Protection measures from the causal agent of bacterial canker are mainly of preventive nature and their essence lies in the following. It is necessary to select the stalks only from healthy trees for planting, treating them with 25% solution of methyl bromide in the camera during 6 minutes. Antibiotics are not effective. It is necessary to carefully examine the mother plantations in the nursery gardens of poplar, to thin out the planting in due time, at the same time avoiding mechanical damages of plants, to remove infected branches, burn them, to treat the wounds with disinfecting solutions, to perform the extraction of the trees with the symptoms of infection. If the percent of infected trees exceeds 30–40, it is recommended to perform the extraction of all trees; it is necessary to fight with the insects – disease vectors, it is necessary to breed the varieties and clones of poplar, which are resistant to the causal agent of bacterial canker. The last measures are of exceptional importance in the Netherlands, Belgium and France, where the planting of species and hybrids of poplar, nonresistant to bacterial canker, is limited by the law.

As the means of biological protection measures we recommend the "Bio-gel", which was developed by us. Exactly at the basis of modern microbiological approaches – is the selection of random useful strains of soil microorganisms and creation of optimum conditions for their cultivation in the artificially created nutritional mediums to the high concentration at the level of $10^9 - 10^{10}$ of CFU of microorganisms per 1 cm³ of medium. As the "useful" microorganisms researchers are first of all classifying the nitrogen--fixing microorganism *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, phosphate-mobilizable, mainly from the species Bacilus subtilus, lactic-acid bacteria and other "useful" bacteria, according to researchers. At the same time the scientists-practicians determine that efficiency of artificially created biological products does not exceed 65–70%, especially in the case of extreme environmental conditions, droughts, high and low temperatures, flooding of soils. The bacteria, which are artificially created at the culture mediums, rich in organics, can not quickly adapt to the modern soils, which are exhausted and contaminated by means of chemicalization, and the bacteria quickly die, reducing their initial concentration by thousand times. For example, the bacterium, artificially cultivated on MPA (meat-and-peptone agar) with the level of ammonium nitrogen of 120–130 mg/100 mm^3 , decrease from initial titer of $5 \cdot 10^9$ to $2 \cdot 10^6$ CFU in 1 cm³ just within 1-2 days after getting into the peat mix with the natural level of nitrogen of 30-40 mg.

Exactly for this reason the cultivation of concentrates of these bacteria in artificially created nutritional mediums, which are far from the natural soils by their composition, considerably complicates both the time of adaptation of "strangers" on the new place and creation of necessary biocomplexes "soil-plant-microorganism".

It is known that, for the purpose of increase in yield, especially the yield of bioenergy crops, way back at the beginning of last century agricultural producers planted the seedlings, soaking the roots into the soil of extracted trees. Application of the part of fertile soil for enrichment of new areas with the useful microbiota is used in some cases till now, mainly at garden plots. It is clear that such technology is ineffective, as the average number of microorganisms on fertile soils does not exceed $1 \cdot 10^4 - 1 \cdot 10^5$ CFU/0,001 kg and, furthermore, removal of the layer of soil disturbs the existing environmental balance.

Exactly these reasons led to creation of such LEAN-technology, concerning the enrichment of fertility of soils and the increase in yield of plants, in particular the seedlings, which would have used the whole soil microbiota, reproduced exactly in its habitat, without disturbing the natural balance of lands and without pollution of environment by any artificially-created media.

The product, created by means of such technology, was called "Bio-gel" due to characteristic gel-like physical state. The product received organic certification, as it is made from organic raw materials without use of chemical processes. As raw materials are used fertile soils, bottom sediments of reservoirs, lowland peats and so forth, which contain the initial rather high natural titer of soil microorganisms at the level of $1 \cdot 10^4 - 1 \cdot 10^6$ CFU/cm³.

By using the so-called hydro-thermodynamic technology ("HTD-technology"®, the Eurasian patent No. 006420), it is possible to considerably increase the bioavailability of useful components of fresh raw materials. First of all they are the ammonium nitrogen, available carbon, in the form of humic and fulvic acids, lignins, pectins, microelements, etc.

The low-temperature hydrolysis of raw materials, by means of "HTD-technology" (\mathbb{R}) without use of chemical compounds, allows to keep the initial "natural portrait" of soil microbiota and to reproduce it in available natural medium to the high concentrations of $(1 \cdot 10^9 - 1) \cdot (10^{11})$.

Numerous laboratory and full-scale experiments showed rather high microbiological activity of the product "Bio-gel". Among other things were proved fungicide properties of the product concerning *Fusarium oxysporum*, *Alternaria alternata*, *Botrytis cinerea* and *Septoria glycines*. The data is given in the Table 1. The specified fungi are also accompanying for phytopathogenic bacteria [28].

Table 1 Fungicide properties of the product, concerning phytopathogenic fungi

Name of oution	Zo	Zone of growth inhibition of micromycete [n	n]	
Name of option	Fusarium	Alternaria	Botrytis	Septoria
"Bio-gel"	12	10	23	0
"Bio-gel"-2, 1%	fs	15	fs	0
"Bio-gel"-2, 10%	18	_	25	0
H_2O	0	0	0	0

Note: "fs" – is fungistasis, "—" – it was not possible to take the experiment into account. Fungistasis – is the partial and non-permanent toxic action of the product on fungi, fungicidal action – is the full toxic action of the product on fungi.

"Bio-gel"-2 in the concentration of 10% has average (15–25 mm) and high (from 25 mm) effect on the main causal agents of fungal diseases of plants. No option had effect on micromycete *Septoria*.

Preventive fungicidal action of new product was also proved in conditions of large-scale experiments in various climatic zones on the majority of plants, including maize, willow (Table 2, Photo 4, 5), Crimean pine, etc.

Table 2

The yield of hybrid of maize Orzhitsa 237 MV depending on the spraying of plantings with "Bio-gel"

0.6	Yield	+/– to control	
Options	[Mg/10000 m ²]	[Mg/10000 m ²]	[%]
1. Control (the integrated protection of plants)	9 640	_	_
2. IPP + spraying of plantings of 5–6 leaves with the product "Bio-gel", 1 dm ³ /ha (conc. 0.5%)	10 220	+580	6.0
3. IPP + spraying of plantings of 5–6 leaves with the product "Bio-gel", 2 dm ³ /ha (conc. 1.0%)	10 780	+1140	11.8
HIP ₀₅		0.33	

One-two-phase treatment of plants in the vegetative stage provided the practical absence of bacterial diseases and noticeable increase in harvest.

Thus, the new biological product, which was created, can be used both as the natural adaptogen and as the amplifier of the effect of chemical pesticides.

It is also confirmed by field experiments on different crops, including forest crops with the chemical fungicides of different producers. Experiments showed that the use of half-dose of chemical fungicides together with "Bio-gel" provides practically the same result, as the dose, which is recommended by producers. Thus was proved the rather



Photo 4. Efficiency of "Bio-gel" on perennial tree crops [author's photos]



Photo 5. Influence of the products of humate of the chemical origin and "Bio-gel" on the test crop – rape seed [author's photos]

high efficiency of new product in the process of biological activity of agro-industrial production.

Particular emphasis needs to be placed on efficiency of the product "Bio-gel" in conditions of extremely high temperatures of the South of Ukraine, where air temperature on the sandy grounds reached above 60°C at the relative air humidity of 30–35%. Thus, in the conditions of summer period of 2016 at the level of precipitation of 57 mm and at the norm of precipitation of 132 mm there was proved the "working ability" of microbiota of "Bio-gel", which, in comparison with the control, was greater by 2.5–3 times, than in control (water). Also there was experimentally proved the essential decrease (by 20–25%) of water-use ratio by plants, due to the deeper penetration by root system into the loamy light sands.

Results of field researches in extreme environmental conditions of 2015 and 2016 allowed to recommend new product for large-scale introduction in agro-industrial sector of Ukraine.

Conclusions

The poplar and birch as fast-growing bioenergy wood draws attention of forestry specialists, energy workers on alternative energy sources. However their cultivation is connected with considerable difficulties, as the wood trees are affected by diseases more often than other species.

Among various bacterial diseases of poplar and birch the most damaging diseases that lead to economic losses are bacterial canker and bacterial necrosis of the bark. The harmfulness of diseases lies not only thinning of the crown, reduction in leaf numbers, in the fast and large-scale dieback of trees, nonresistance of affected trees to wind, but also in decrease of wood increment. The less dangerous diseases of woody trees are wetwood (dropsy), bacterial blight, root disease and tuberculosis.

To protect the wood trees from agents of bacterial diseases and accompanying micromycetes was proposed developed by us the biopreparation "Bio-gel", which includes the nitrogen-fixing microorganism *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, phosphate-mobilizable, mainly from the genus *Bacilus* and lactic-acid bacteria.

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