

PHYTOPATHOLOGICAL ASSESSMENT AND COMPOSITION OF *Pinus* sp. PATHOGENS

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Summary. This paper evaluates the state of Moldavian pine forests (nurseries and urban areas) with the use of traditional phytosanitary monitoring in the centers of desiccation. The aim of the research was assessment of the sanitary state of pine plantations, identification of the composition of pathogens and development of recommendations for the improvement of the condition and increase of the biological stability of pine plantations. Results showed that the majority of damages were caused by fungi. In *Pinus* sp. the most common pathogens were fungi: *Alternaria* sp., *Aspergillus* sp., *Rhizopus* sp., *Fusarium* sp. and *Sphaeropsis* sp.

Keywords: *Pinus* sp., phytopathological assessment, reddening of needles, fungal pathogens.

Introduction

Modern globalization facilitates the mass movement of animals, plants, fungi and other organisms outside their natural ranges. The emergence of a previously uncommon (alien) species, its naturalization and introduction into local biogeocenosis (invasion) can cause significant ecological and economic damage, as is the case with the dispersal of phytopathogenic organisms, especially phytopathogenic fungi. Invasions of phytopathogenic fungi have accelerated significantly in Europe over the last 40-50 years [1]. The emergence of dangerous invasive phytopathogens can have the most dangerous consequences for native plant communities, leading to the weakening and even mass mortality of forests, artificial and urban plantations [2]. As a rule, invasions of phytopathogenic fungi are not limited to one country or region, but spread almost all over Europe in certain forest types where host plants of a given phytopathogen grow in masse [3]. An example of destructive invasions of phytopathogenic fungi affecting the whole of Europe in the last 30 years is the spread in forests:

- *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam. which caused severe oak dieback and degradation of oak forests throughout Europe;

- *Fusarium circinatum* Nirenberg & O'Donnell, the causal agent of shoot canker of pines, known in many European countries [4].

Forests are one of the most important vegetation types, fulfilling functions ranging from supporting a healthy watershed to wildlife habitat and produce a wide range of socio-economic goods for the owners of forest lands as well as for the surrounding society [5]. According to archival materials and literature data, the forest area of the right bank of Moldova at the beginning of the nineteenth century was 600 thousand ha (more than 30% of the territory). In 2023, the forest area in the country was 467.9 thousand ha, or 13.8% of the territory [6], which places our Republic among the European countries with the lowest level of afforestation (the average value in Europe is 29%, in the world - 31%). Therefore, forest restoration and expansion of areas covered with forest

vegetation is a national priority. All forest restoration and expansion works are carried out on forest lands managed by the Agency “Moldsilva”. Forest nurseries (25) in Moldova produce annually about two million tree seedlings, of which coniferous trees account for about 20 per cent.

Annual impact of a complex of unfavourable factors of abiotic and biotic character leads to weakening and death of trees, increased (pathological) dieback appears in plantations and sanitary condition deteriorates. Seedlings grown in tree nurseries are also heavily affected by infectious and non-infectious diseases. In order to timely identify and prevent potential threats to forest plantations, monitoring of sanitary and forest pathological condition of the forest fund is carried out. Thus, the monitoring data on the species composition of phytopathogenic fungi affecting woody plants in the south of Moldova indicates the penetration and spread of new species of invasive diseases. New phytopathogens are already causing and may cause serious damage to forestry in the future.

The purpose of the study was to establish the sanitary state of pine plantations, identify the species composition of pathogens and develop recommendations for improving the condition and increasing the biological stability of pine plantations.

Materials and methods

Field study and sample collection.

Three forest areas were selected for observation and sampling as permanent research sites out of 23 forests and reserves managed by Agency “Moldsilva”: the first one in Calarasi district; the second one along the R14 road, near Balti; the third one in Hincesti-Silva, next to a sugar factory; the fourth one was in Chisinau urban forest, Valea Morilor Park (Figure 1). Coniferous species - pine - make up the bulk of the vegetation in these parks.

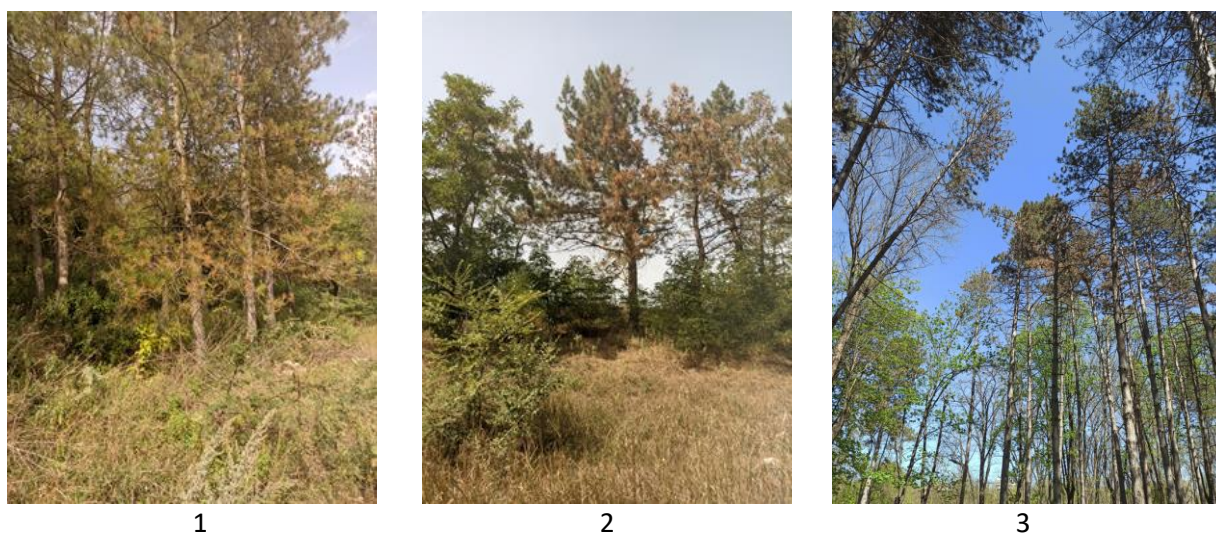


Figure 1. Panoramic trees views: 1- O.S. Calarasi; 2- R14 road, Balti; 3- Chisinau, park Valea Morilor, 2022

The reconnaissance method was used for phytopathological assessment. When assessing the phytosanitary condition, attention was paid to the infestation with a complex of diseases characteristic of coniferous trees. Each pine tree was ranked according to one of six categories of green woody plantations condition according to the results of visual assessment of needles, crown, growth, presence of signs of desiccation, local damage of trunk and root paws, branches as follows: 1st - the tree is healthy; 2nd - the tree is weakened; 3rd - the tree is very weakened; 4th - the tree is in the process of desiccation; 5th - recently died; 6th - died more than a year ago (according to Annex 6K to the Sanitary Rules for Forests of the Republic of Moldova (1998)).

For the assessment of the composition of pathogens the samples of wood, needles and growth, with signs of lesions (reddening) of needles, were taken from *Pinus sp.* for taxonomic characterization of the pathogens. A total of 12 pine samples were collected. Needle samples of

selected trees with a varying degree of drying were taken from three levels (upper, middle and bottom). Fragments of dry and green needles, branch tips, and wood fragments were taken from each sample (Figure 2 and 3).



Figure 2. Sample preparation: fragments of dried and green needles



Figure 3. Sample preparation: growing tips of branches, wood fragments

Identification methods of pathogens included a combination of morphological assessment and fungal culturing methods. Wood and needle samples were sterilized by 96% ethanol for 15 second and small fragments were used to place on PDA nutrient medium agarized in Petri dishes. Then Petri dishes samples were placed in thermostat at temperature of 28°C. Similarly, sections of the branches were performed. When examining the tissue of the branches no symptoms of attack or obvious changes in the woody tissue were observed. Reinoculations of pathogens were performed several times until pure culture was obtained. Pure cultures were incubated at 28°C in the dark, grouped morphologically.

Fungi were identified according to available methods and identifiers [7, 8].

Results and discussions

In the last decade, the pine forests of Moldova have experienced a decrease in biological resistance, accompanied by a massive drying out of forest stands. From the moment of identification of the damaged plantings area, the volume of drying out of pine forests is constantly growing. Counting of dying pine plantations was initiated by the Ministry of Environment of the Republic of Moldova and specialists from Institute of Forestry Research and Management in the fall of 2019, when this phenomenon became widespread. In pine forests on the territory of the Republic, starting from 2019, the development and spread of reddening of needles is observed, which causes its degradation and desiccation. The massive drying up of pine forests, having significantly expanded to the north and west of the republic, affected 6 out of 23 forestry institutions in the country in 2022. The pathological process continued to cover new territories, and in 2020 it was recorded in urban green spaces (Chisinau). The results of the observation of pine plantations managed by Agency “Moldsilva” are presented in Table 1.

Table 1

Area of pine plantations managed by the Agency “Moldsilva” in which outbreaks of reddening of needles were observed, 2022

No	Forestry enterprises	Area of outbreaks (ha)				
		Total	Including the volume of drying			
			very weak (5-20%)	weak (21-30%)	moderately (31-50%)	strong (> 50%)
1	Calarasi	61,95	34,95	17,6	5,45	3,95
2	Nisporeni-Silva	56,9		5,5	47,4	4
3	Orhei	436,6	163,2	107,2	166,2	
4	Balti	219,84	104,09	71,8	27,16	16,79
5	Hincesti Silva	285	111,2	113,6	30,7	29,5
6	Soroca	501,1		9,7	158,5	332,9
	Total	1561,39	413,44	325,4	435,41	387,14

According to the Agency “Moldsilva” data, the reddening of needles is 1561.39 ha. Foci with intensity of infestation: very weak (5-20%) account for 26.5% (413.4 ha), weak (21-30%) - 20.8% (325.4 ha), medium (31-50%) - 27.9% (435.41 ha) and strong (more than 50%) - 24.9% (35.6 ha).

To assess the pathogen, affected branches were taken from three samples and prepared for pathogen identification using the method described above.

Results showed that the majority of damages were caused by fungi. A majority of fungal diseases were associated with shoots and needles. The symptoms included stunted growth, discoloration and loss of needles, and dieback of the shoots. In many cases, fungal mycelia and fungal fruiting bodies were also observed on damaged tissue.

During the study, a number of pathogenic fungi were isolated and identified (at the genus level). Results of microbiological examination of pine samples (needles, shoots, wood) are presented in the Table 2.

Table 2

List of identified pine phytopathogens

Sample	No.	Pathogenic agent
		Fungi
Needles	1	<i>Rhizopus sp.</i> ; <i>Penicillium sp.</i> ; <i>Alternaria sp.</i> <i>Apergillus sp.</i> . <i>Fusarium sp.</i> ; <i>Sphaeropsis sp.</i> NB! <i>Trichoderma sp.</i> - non-pathogenic
	2	<i>Rhizopus sp.</i> ; <i>Apergillus sp.</i> ; <i>Alternaria sp.</i> ; <i>Penicillium sp.</i> ; <i>Fusarium sp.</i> ; <i>Trichoderma sp.</i>
	3	<i>Rhizopus sp.</i> ; <i>Alternaria sp.</i> ; <i>Penicillium sp.</i> ; <i>Fusarium sp.</i> <i>Trichoderma sp.</i>
	4	<i>Alternaria sp.</i> ; <i>Penicillium sp.</i> <i>Fusarium sp.</i> , <i>Sphaeropsis sp.</i> , <i>Trichoderma sp.</i>
Wood/ shoot	1-4	<i>Rhizopus sp.</i> ; <i>Aspergillus sp.</i> ; <i>Fusarium sp.</i> ; <i>Sphaeropsis sp.</i>

In *Pinus sp.* the most common fungal pathogens were *Alternaria sp.*, *Aspergillus sp.* and *Rhizopus sp.*, *Fusarium sp.* and *Sphaeropsis sp.* (Figure 4).



Rhizopus sp. (needles)



Sphaeropsis sp. (needles)



Rhizopus sp., *Fusarium sp.*,
Sphaeropsis sp., *Aspergillus sp.*
(pine shoots)

Figure 4. Growth of pine phytopathogen colonies on solid media

Results demonstrated that fungal pathogens remain the major threat in *Pinus sp.* in Moldovan forest nurseries.

Thus, the immediate cause of the death of pines in the centers of desiccation is damage by pathogenic organisms that are introduced including by bark beetles when populating living trees.

But, given the geography of this phenomenon, it can be assumed that the massive drying out of pine stands in many European countries, including Moldova, may be associated with climate change. Possible causes of shrinkage can be divided into:

- natural: dry events of recent years, violation of the hydrological regime of soils, massive damage to pine plantations;
- anthropogenic: accumulation in the forest fund of a large number of artificial pine stands of the same age, refusal to utilize pine felling residues by fire in accordance with the requirements of forest certification, low efficiency of forest pathological monitoring, very low funding for forest protection work and scientific research in this area.

No funding for research does not allow speaking about a more complete picture of the reasons for the phenomenon of mass drying of pine forests in Moldova at the moment.

Clearly, there is a great need for research on such invasions, which could help minimize their negative effects in the future. Unfortunately, so far the measures taken to combat these growing threats are not systematic and effective, although in many cases technologies have already been developed to accurately identify invasive phytopathogenic fungi, there is an understanding of their pathways of entry and spread, and control methods have been developed.

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