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PHOMA BLIGHT OF PLANTING STOCK OF *PINUS SYLVESTRIS* L. AND *PICEA ABIES* L. IN FOREST NURSERIES OF BELARUS

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Abstract: *In the Republic of Belarus reforestation is carried out on considerable surface areas. Therefore, much attention is paid to the health of the planting material in forest nurseries. Modern DNA analysis methods have shown for the first time the prevalence and harmfulness of Phoma blight of Scots pine (Pinus sylvestris L.) and Norway spruce (Picea abies L.) in most forest nurseries of Belarus. The main symptoms of Phoma blight depend on the type and age of plants, and the elements of the cultivation technology. The species composition of causative agents of disease in planting material has been updated by DNA analysis methods and the pathogenicity of Phoma spp.was proven on plant tissues.*

Key words: *Phoma blight, forest nurseries, planting material, Phoma spp., harmfulness, forest pathology survey.*

1. Introduction

In the Republic of Belarus the main forest forming species are *Pinus sylvestris* L. and *Picea abies* L. For the purposes of reforestation planting stock of pine and spruce is cultivated in more than 120 forest nurseries with a total area of over 1.4 thousand hectares located throughout the country. The total volume of planting stock production reaches more than 300 million plants per year. Diseases significantly affect the yield of certified planting material in forest nurseries, since thin external protective tissues of young plants are easily exposed to the introduction of phytopathogenic organisms. This entails the infestation of planting material with obligate and facultative parasites and, as a result, leads to mass mortality of plants.

In classical scientific literature on forest phytopathology of the studied region mainly fungi of *Fusarium* Link.; *Alternaria* Nees.; *Rhizoctonia* DC.; *Pythium* Nees.; *Botrytis* P. Micheli; *Cladosporium* Link.; *Lophodermium* Chevall.; *Phacidium* Fr.; *Gremmeniella*

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M. Morelet and others are identified as the main disease excitants of planting material of coniferous woody plants in forest nurseries [11, 12]. At the same time, global climate change, plant introduction, the active movement of seed and planting material, the introduction of new technologies of cultivation of seedlings and saplings as well as the flexibility of the microorganisms and many other factors cause the emergence of cases of mass infection of plants in nurseries with new pathogens. At the present time, a proportion of plant affection by facultative parasites, living on dead organic matter, but able to colonize plants with poor disturbed cover and weakened immune system, has increased [9]. In this regard, the list of common pathogens of planting material requires a significant adjustment.

The widespread adoption of molecular genetic analysis methods of phytopathogens into forestry allows not only to diagnose disease excitant of planting material in forest nurseries with a high degree of accuracy but also to revise the existing complex of pathogenic organisms that can infect the tissues of seedlings and saplings. The first results of the molecular genetic diagnosis of phytopathogens in nurseries started in Belarus in 2011 – 2012, showed that in every second forest nursery in the tissues infected woody plants contain structures of pathogenic fungi of the *Phoma* Sacc., causing a disease called Phoma blight [14]. Previously, it was believed that *Phoma*spp. are harmful only for agricultural plants, and on woody plants they can cause only various kinds of spotting (most often on fruits and seeds). In this regard, it was necessary to clarify the role of the fungi of the genus *Phoma* as pathogens of woody plants, in particular *P. sylvestris* and *P. abies*.

2. Materials and Methods

2.1. Field Studies

Identification of affected areas with the symptoms of Phoma blight was carried out during 2013 – 2017 in forest nurseries of 37 forest enterprises located in all 6 administrative regions of Belarus (Figure 1). The total survey area was 823.5 hectares, including 147 hectares occupied by *P. sylvestris* and *P. abies*. In total, 457 registration areas were created; the number of recorded seedlings was more than 52 thousand.

On the registration areas the percentage of infected plants (disease prevalence) was calculated. Also the nature of the damage was described and samples for subsequent analysis under laboratory conditions were collected.

2.2. Laboratory Work

In the laboratory, the fungi were isolated from the tissues of affected plants into pure cultures. Identification of pure cultures was performed using the manual for the identification by G. Boerema [4], as well as methods of molecular genetic diagnosis [8].

Pathogenicity test of the fungus *Didymella pomorum* (Thüm.) Qian Chen & L. Cai 2015 [5] (syn. *Phomapomorum* Thüm.) towards germinants was carried out according to the method of J. Chelkowski and M. Manka [11]. For artificial inoculation of seedlings, coniferous seeds were sowed in a container soil taken from a forest nursery.

In the experimental version, fungal inoculum was put to a depth of covering. A 10-day culture *D. pomorum* grown in a liquid wort medium was used as an inoculum. The experimental and control variant was kept for 3 weeks after germination of first seedlings under day light and at room temperature (22 – 24 °C). Throughout the experiment the soil in the containers was uniformly moistened with the same volume of sterile water from a pulverizer. Statistical processing of research results was carried out using generally accepted methods on a personal computer in the Microsoft Excel 2010 program.

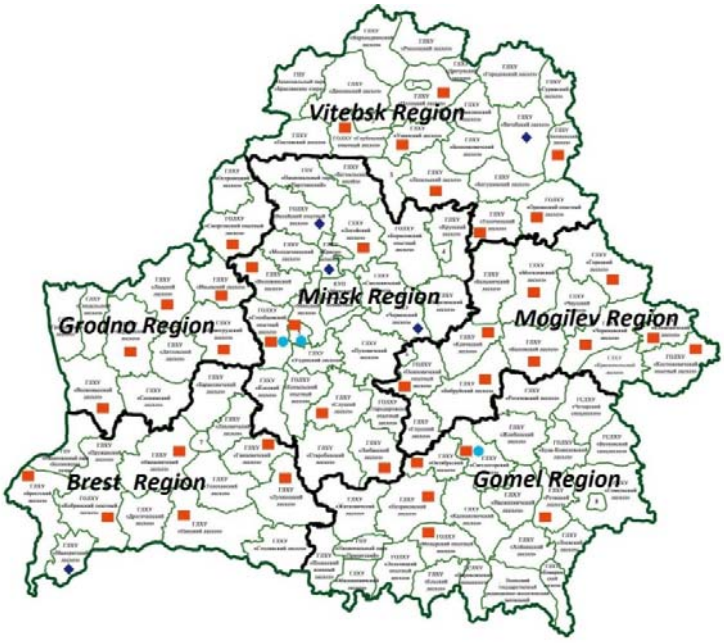

Fig. 1. *Map of field research objects location:*
places of registration areas and collection of samples of infected plants;
places of additional collection of samples of infected plants;
experimental protective treatment sites

3. Results

3.1. Occurrence of *Phoma* Blight in Forest Nurseries

The results of the work showed that in forest nurseries of Belarus Phoma blight is found on 11.4% of the growing area of pine and spruce planting material. Mean while, *P. sylvestris* has some damage features in average on 10.5% of the area, *P. abies* on 11.7%. The disease was equally widespread in nurseries of the Mogilev, Grodno and Brest regions (15.0 – 16.0%), least often in nurseries in the northern part of the country of the Vitebsk region (3.5%) - Table 1.

In seedling sections of nursery in infected area of pine plants the prevalence of Phoma blight is on average 13.4% that is about 2 times higher in comparison with cold greenhouse (7.6%). Spruce is more intensively infected in greenhouses (15.6%) in comparison with

the 1–2 years old seedling section of open ground (Table 2).

Table 1

Occurrence of Phoma blight on growing areas of P. sylvestris and P. abies in forest nurseries

Region	Surveyed [ha]	Damaged [ha/%]	Including growing areas of tree species [ha/%]	
			Pinus sylvestris L.	*Picea abies* L.
1. Brest	14.66	2.2 15.0	1.5 19.2	0.7 10.2
2. Vitebsk	37.01	1.3 3.5	0.4 10.0	0.9 2.7
3. Gomel	6.38	0.4 6.3	0.2 4.0	0.2 14.1
4. Grodno	26.65	4.0 15.0	1.3 25.2	2.7 12.6
5. Minsk	11.48	0.8 7.0	0.3 7.0	0.5 7.0
6. Mogilev	50.77	8.1 16.0	0.3 2.6	7.8 19.9
Total:	146.95	16.8 11.4	4.0 10.5	12.8 11.7

Table 2

The occurrence of Phoma blight of P. sylvestris and P. abies in sections of forest nurseries

Region	The incidence of the disease by tree species and sections [%]				
	Pinus sylvestris L.		*Picea abies* L.		
	Open ground	Protected ground	Open ground		Protected ground
			seeding	the area of sapling cultivation	
1. Brest	11.7	1.2	9.7	15.1	11.6
2. Vitebsk	6.4	6.2	4.1	6.3	–
3. Gomel	12.0	–	–	–	8.8
4. Grodno	14.5	–	5.4	19.5	15.1
5. Minsk	17.0	12.5	5.8	13.4	–
6. Mogilev	18.5	10.4	8.2	22.4	26.7
Average value	13.7	7.6	6.6	15.3	15.6

3.2. Symptoms and Damage

Symptoms of Phoma blight depend largely on the type and age of plants, individual elements of the cultivation technology, in particular, on whether plants are cultivated in the open or in the cold greenhouses and on the way of infection penetration inside the tissues of the plant (the infectious background is created by infected plant residues, the

fungus often preserved in the soil of forest nurseries by chlamydo spores). The diagnosis of the disease is greatly hampered by the absence of emerging fungal sporulation on the infected plant organs, although according to some literature sources numerous pycnidium are formed on the surface or in the thickness of the affected tissue [7, 8]. Plants of pine and spruce are infected up to the age of 3–4 years. In most cases, the first symptoms of Phoma blight begin to appear on plants in early May — the lower needles turn white, become golden brown, then fall off (Table 3).

Fig. 2. *The appearance of infected plants of P. sylvestris (on the left) and P. abies (on the right) by Phoma blight*

The most intensively plants (especially under 1 year) become infected after heavy rains. This is due to the formation of an earth cone at the base of the stem when soil sticks to it because of dirty rain sprays [10]. In such places soil fungi of the *Phoma* genus easily pass into plant tissues. Further, the infection spreads up the stem, therefore in 60.1% of cases the main symptom of Phoma blight is the drying up of the needles in the lower part of the 1–2 years old seedlings (Table 4).

Stem of 3–4 years old seedlings has more developed external protective tissues than young seedlings. That is why the transition of the mycelium from the soil is complicated (the dying off of the needles in the lower part of the plants is 29.3%). Therefore, infection of 3–4 years old seedlings occurs mainly by the aerogenic method (conidium), thus drying of the apical bud together with side shoots occur (25.3%).In the infected area 4–8% of dead plants observed. Besides

damage of Phoma blight appears in a decrease of growth rate of infected seedlings (Figure 4).

Table 3

The main symptoms of Phoma blight of P. sylvestris and P. abies planting material

Tree species	Nursery section		
	Open ground (by years of growing)		Protected ground
	1st year	2nd year and over	
Pinus sylvestris L.	Yellowing of the needles, complete drying that usually starts in the lower part of the seedling. Quite frequently the drying of the whole plant is observed. The plants are most intensely infected when a soil cone is formed around the stem. Affected plants are scattered throughout the area.	Blanching and yellowing of the needles in different parts of the plant, short needles, drying and dying of the central and (or) lateral shoots, a strong weakening of the plant. Affected plants are often grouped.	Yellowing and browning of pine needles, drying in the lower part of the plant and along its height. Infected plants are usually grouped.
Picea abies L.		Browning of apical bud and (or) drying of the stem shoots of the current year; yellowing of the tips of the needles in different parts of the plant, its symptoms are more intense when soil sticks to the stem and in forming of a soil cone. Infected plants are scattered throughout the area.	

Table 4

The main symptoms of Phoma blight infection of P. sylvestris and P. abies

Type of tree	Incidence of the symptoms [%]				
	drying of the needles in the lower part of the plant	dying of an apical bud	infection of an apical bud and shoots	current year's shoots mortality	drying of the whole plant
Seeding section					
Pinus sylvestris L.	66.4	12.1	5.9	8.2	7.4
Picea abies L.	53.8	12.9	20.8	8.9	3.7
Total	60.1	12.5	13.4	8.6	5.6
The area of sapling cultivation					
Picea abies L.	29.3	18.2	25.3	22.1	5.2

Infection of seedlings by Phoma blight leads to inhibition of growth processes, which leads to a decrease in the height of plants compared to healthy species by 23.5–26.0% with a low degree of damage, reaching 65.0–67.0% with a high degree of infection.

In areas affected by Phoma blight, a dicrease in the yield of standard pine planting material by 120–330 thousand units / ha and spruce trees by 100–270 thousand units / ha, or by 5.5–15.0% (Figure 5).

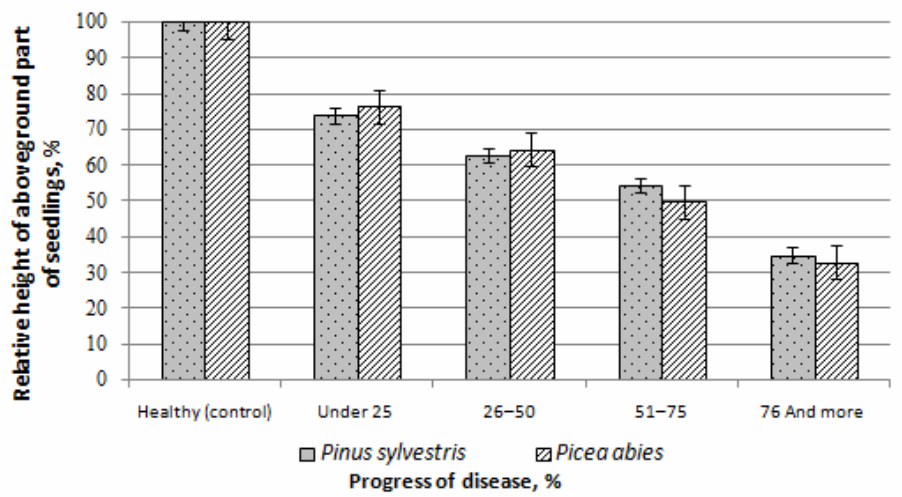

Fig. 4. *Height of the aboveground part of seedlings infected by Phoma blight(in comparison with healthy plants)*

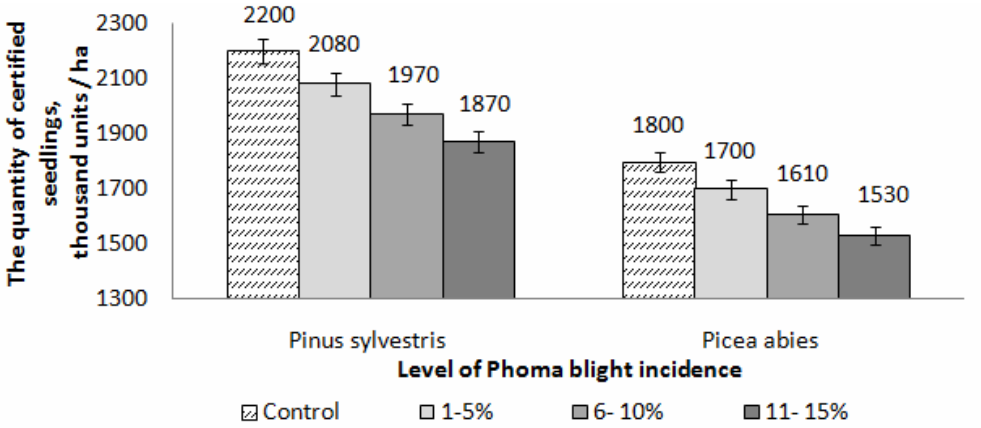

Fig. 5. *Yield of certified seedlings depending on Phoma blightincidence*

3.3. Species Composition of Phoma Blight Pathogens

The application of DNA analysis methodsshowed that the Phoma blightpathogens of *P. sylvestris* and *P. abies* are 3 types of fungi: *D. pomorum*., *D. macrostoma*., *Ph. herbarum* (order *Pleosporales*, class *Dothideomycetes*, section *Ascomycota*). *D. pomorum* dominates (isolated from the tissues of 73.2% of plants), it often affects *Picea abies* in cold greenhouses. *D. macrostoma* is widespread species in the area of 3–4 years old seedlings cultivation of *Picea abies*. *Ph. herbarum*is common only for the greenhouses (Table 5).

3.4. Evaluation of *D. Pomorum* Pathogenicity

Fungi of *Didymella* sectionare relatively rarely parasitize in nature, so the study of their pathogenic characteristics and the

conditions of transition to a parasitic way of life has the big importance in the pathology of plants [1, 2].

Pathogenicity test of the fungus *D. pomorum* in relation to young seedlings, showed that the presence of fungal infection leads to the formation of necrosis. On the 14th day after the start of the experiment, different sizes of spots of dying were observed on the germinants or their complete death was noted (Table 6).

Table 5

The structure of the complex of fungi of the genus Phoma and their incidence in forest nurseries

Species of fungi	Frequent detection, %			Section of the nursery	Incidence, %
	General	Including woody species			
D. pomorum	73.2	*P. sylvestris*	27.6	seedlings 1–2 years	10.3
				cold greenhouse	6.7
		P. abies	45.6	seedlings 1–2 years	10.1
				seedling 3–4 years	14.1
				cold greenhouse	20.1
D. macrostoma	11.6	*P. sylvestris*	6.2	seedlings1–2 years	6.1
		P. abies	5.4		6.6
				seedling 3–4 years	16.7
				cold greenhouse	3.6
Ph. herbarum	15.2	*P. sylvestris*	11.4	cold greenhouse	12.5
		P. abies	3.8		8.3

Table 6

The proportion of germinants with varying degrees of fungal infection Ph. pomorum

Damage evaluation	Degree of damage	The percentage of germinants [%]	
		P. sylvestris L.	*P. abies* L.
0	Healthy (without any infection symptoms)	8.9	2.4
1	Spot tissue-necrosis (to 5%)	16.1	16.6
2	Necrosis up to 50% of the surface	34.5	31.7
3	Necrosis of more than 50% or total death of germinants	40.5	49.3

At the end of the experiment, germinants with the highest damage degree (up to 49.3%) prevailed, while the proportion of absolutely healthy germinants did not exceed 8.9%. In the experiment with infecting of plants through the soil the following symptoms of Phoma blight were recorded three weeks after the emergence of sprouts: infected sprouts began to fade, convolve, the tips of the needles turned yellow, and mycelium dispersal was sometimes observed around the stem. Affected plants fell and died, it's looks like damping-off (Figure 3). Fungus did not formed sporulation under the laboratory conditions.

The selection of pure cultures from the affected tissues of seedlings at the end of the experiment allowed to confirm the

belonging of the pathogen to *D. pomorum.*

Thus, the obtained results suggest that *D. pomorum* is a pathogen of young plants of *P. sylvestris* and *P. abies.*

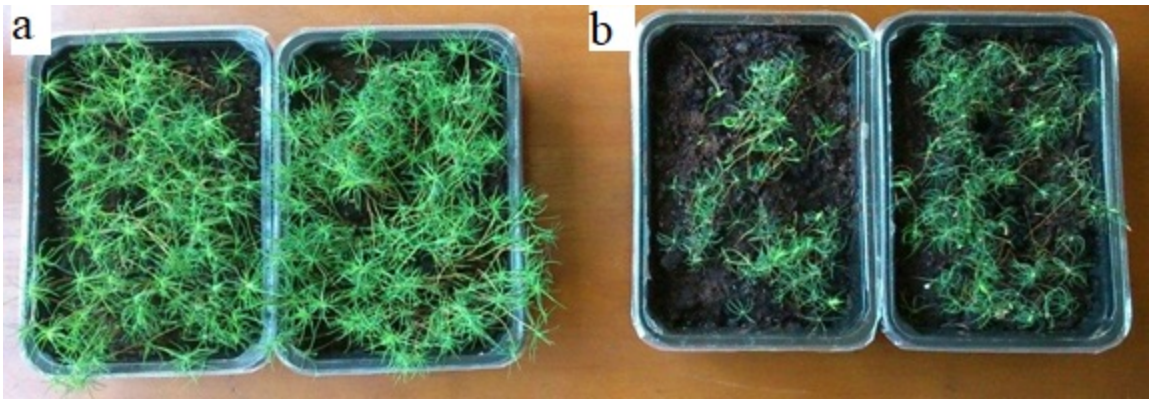

Fig. 3. *Containers with seedlings of Pinus sylvestris (a – control variant; b – variant with soil inoculation by Ph. pomorum), 21 days*

4. Conclusions

In the forest nurseries of Belarus, new disease *Phoma* blight is occurred on 11.4% of the growing area of planting material of *P. sylvestris* and *P. abies*. Pine is more often infected by *Phoma* blight in open ground, while spruce is infected under greenhouse conditions. Visual diagnosis of *Phoma* blight is complicated by various symptoms of the disease, which depend on the species and age of the host, elements of the growing technology, as well as on the ways of infection penetration into the plant tissues. At the same time, conidial sporulation (pycnidia) on affected plants are almost always absent.

The harmfulness of Phoma blight lay in the death of 4–8% of infected plants in the focus of disease inhibition of the growth rate of sick specimens to 65–67% compared with healthy ones, as well as a decrease in the yield of certified planting material of pine and spruce by 5–15%.

Phoma blight of pine and spruce in forest nurseries of Belarus is caused by 3 species of fungi: *D. pomorum* Thüm*., D. macrostoma* Mont*., Ph. herbarum* Westend. *D. pomorum* dominates in 73.2% of cases.

Mycelium of *D. pomorum* causes necrosis of living tissues of *P. sylvestris* and *P. abies.* An infectious background artificially created in the laboratory leads to the emergence of necrotic areas on more than 90% of germinants. The introduction of mycelium into the soil under controlled conditions leads to the death of seedlings like damping-off.

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