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ASPECTS REGARDING THE IDENTIFICATION OF THE MICROFLORA PRESENT ON FOREST SEEDS INTENDED FOR SOWING IN SPRING 2022

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Abstract
Forest crops (nurseries and plantations) impose difficulties on foresters, the main cause being disturbing or harmful factors that compete and interact in different directions, but whose result is the weakening of seedlings, culminating in their death. Given that the number of seedlings in nurseries and plantations is high compared to the unit area, the risk of disease and pest attacks is very high. For this reason, it is necessary to monitor the annual forest crops, both in terms of biological material and soil, in order to control or eliminate possible sources of infestation.
In order to achieve the proposed objectives, ash, maple and lime seeds were harvested from Mihai Viteazu Nursery, spruce seeds from Voivodeni Nursery, Forest district Someșu Rece, Forest district Beliș and larch from Forest district Beliș. Specific phytopathological analyzes of the seed samples were performed on the seeds in order to accurately determine the possible pathogens. In the analyzes, the pathogens Fusarium oxysporum, Alternaria alternata and Botrytis cinerea were identified on both deciduous and coniferous seeds.

Keywords: Nursery, pathogens, seeds, infestation

1. INTRODUCTION

Forest crops (nurseries and plantations), given that the number of seedlings is high compared to the area, present an increased risk in terms of attacks of diseases and pests (Tăut, 2018).
These impose difficulties on the administrators, the main cause being the disturbing or harmful factors, which cause diseases or injuries, which result in losses, sometimes considerable. Thus, there are diseases of biotic nature (some of them being infectious) and diseases of abiotic nature (non-infectious) that compete and interact in different directions, but whose result is the weakening of seedlings, culminating in their death (Olenici, 2021).
Pathogens that infest the seeds and implicitly attack the seedlings, during the emergence period, enter the plants through the stomata, directly through the cuticle, etc., in certain circumstances using, for this purpose, also wounds. These can be called optional traumatic parasites or parasites with optional traumatic penetration (infection). Thus, the fungus *Botrytis cinerea*, which has the ability to directly pierce the cuticle, can enter the plant and through wounds; *Fusarium* species can also be mentioned. In contrast, other species of the genus *Fusarium* attack the roots of some plants and penetrate tissues only if the roots are injured. The number of traumatic parasites is quite large, they have representatives in all major groups of agents: viruses, bacteria and fungi (Albrectsen, 2012).

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Active, direct penetration by mechanical or chemical perforation of intact external protective structures of seeds and plants (cuticle, epidermis, periderm, etc.) is characteristic of a relatively large number of parasitic fungi (La Porta, 2008). This ability to actively penetrate through the mechanical perforation of the cuticle and the outer walls of epidermal cells is related to the formation when the hyphae come in contact with the wall of the host cell at the end of the germ tube of a swelling (thickening) globular or widened like a disc. - suction cup - called apressor, which adheres tightly to the surface of the plant (Yarmalovich, 2019). The role of the oppressor is to provide the mechanical force necessary to perforate the cuticle or wall of the epidermis and the penetration of the fungal filament inside the cell (Cooke, 1993, Desprez-Loustau, 2016).
For this reason, it is necessary to monitor annual forest crops, both in terms of biological material and soil, to control or eliminate possible sources of infestation (Tăut, 2018).

2. MATERIALS AND METHODS

In order to achieve the proposed objectives, ash, maple and lime seeds were harvested from County Forest Administration Cluj, Mihai Viteazu Nursery, spruce from Forest District Someșu Rece, Voivodeni Nursery, Forest District Beliș, respectively larch from Forest District Beliș.

Figure 1. Mihai Viteazu nursery

Figure 2. Voivodeni nursery

Figure 3. Forest district Beliș (left) and Someșu Rece (right)

Specific phytopathological analyzes were performed on the seeds in order to accurately determine the possible pathogens. These analyzes consisted of disinfecting the seeds, rinsing them under running water for half an hour and placing them on CGA (potato-glucose-agar) culture medium.

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The period in which the seeds were left on the culture medium was 10 days, during which the pathogens were monitored (figures 4-10).

Figure 4. Ash seeds from Mihai Viteazu nursery

Figure 5. Maple seeds from Mihai Viteazu nursery

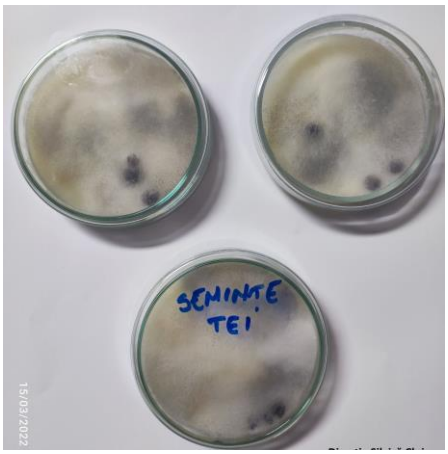

Figure 6. Lime seeds from Mihai Viteazu nursery

Figure 7. Spruce seeds from Forest District Someșu Rece

Figure 8. Spruce seeds from Voivodeni nursery

Figure 9. Spruce seeds from Forest District Beliș

Figure 10. Larch seeds from Forest District Beliș

After ten days, from each colony that presented mycelium specific to the pathogens, fragments of fungi were transplanted on a new medium (Figure 11), in order to obtain the purest colonies, in order to be analyzed under a microscope and to determine as accurately as possible.

Figure 11. Replanting Fusarium

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3. RESULTS AND DISCUSSIONS

Following the analyzes on the deciduous seeds, coming from the Mihai Viteazu nursery, the pathogens *Botrytis cinerea* were identified on 60% of the analyzed samples, *Fusarium oxysporum* on 20% of the samples and *Alternaria alternata* on 10% of the samples.

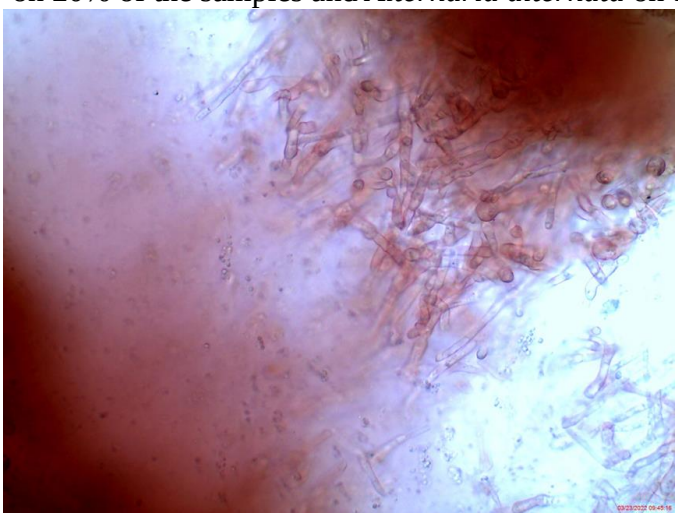

Figure 12. Fusarium oxysporum on microscopic view

Figure 13. Alternaria alternata on microscopic view

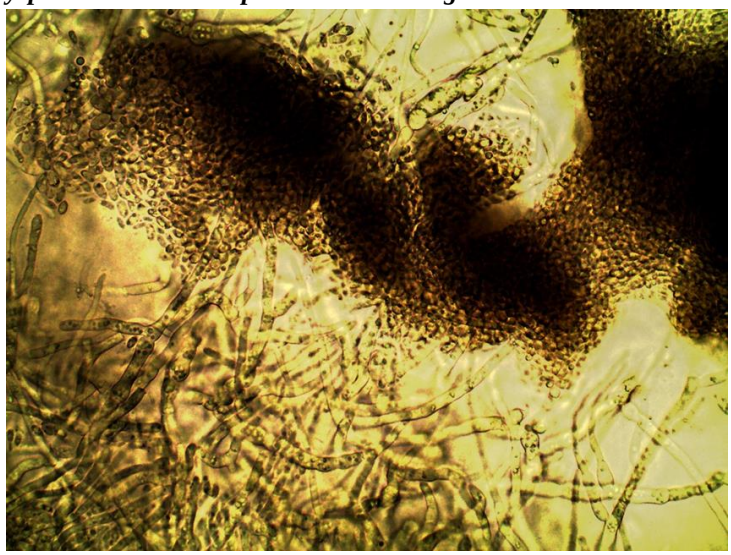

Figure 14. Botrytis cinerea on microscopic view

On the resinous seeds from the Someșu Rece and Beliș Forest District, no pathogens were identified, only saprophytes from the genus *Aspergillius* sp (figure 15).

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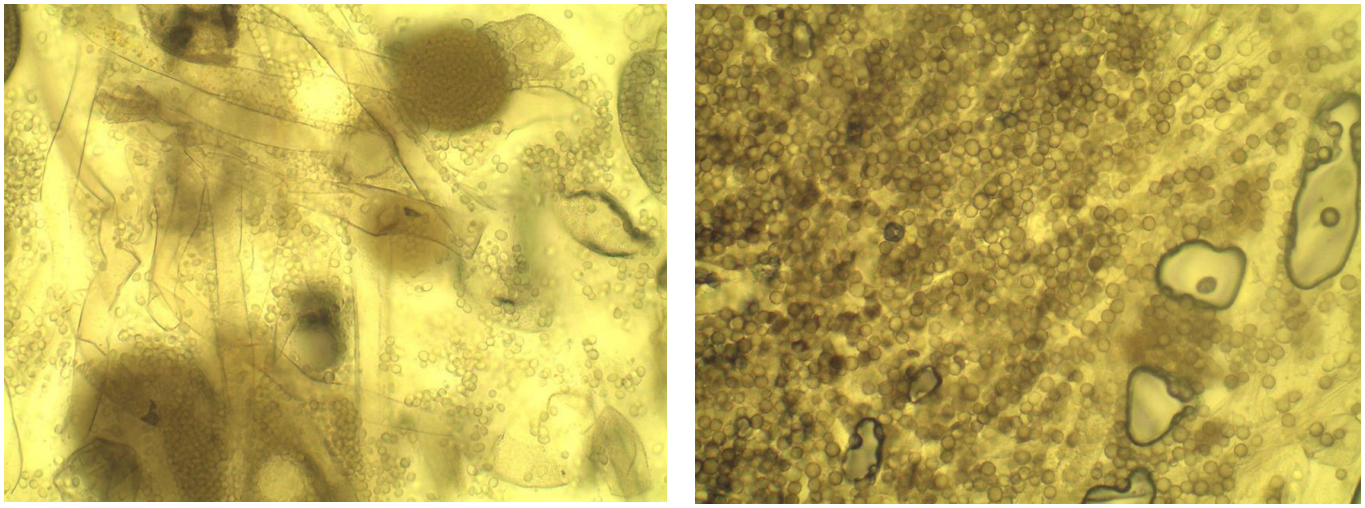

Figure 15. Aspergilius sp on microscopic view on seeds from Forest district Belis (left) and Someșu Rece (Right

On the seeds of conifers from Vojvodeni Nursery, saprophytes of the genus *Rhizophus sp* (figure 16) and *Aspergillius sp* (figure 17) have been identified.

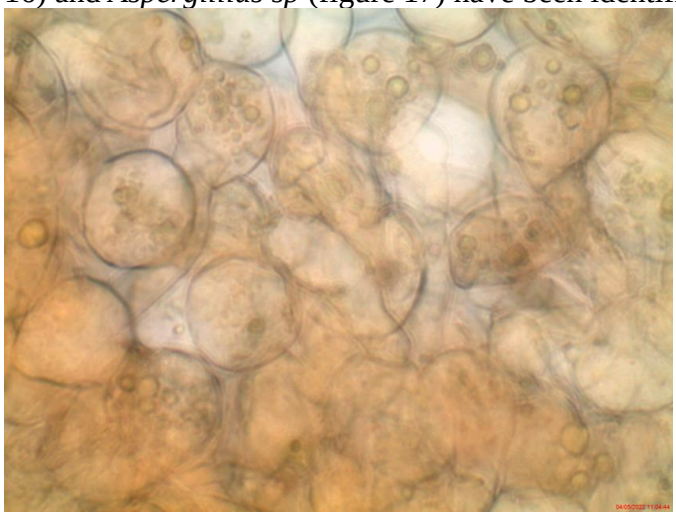

Figure 16. Rhizophs sp. on microscopic view

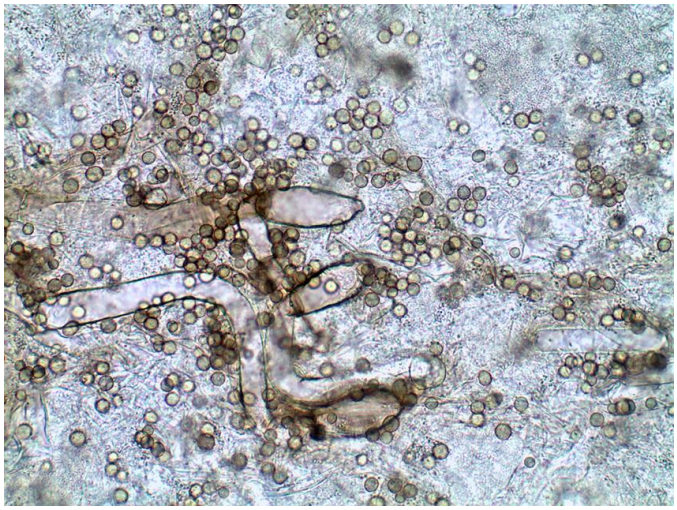

Figure 15. Aspergilius sp on microscopic view

Botrytis cinerea - gray rot.

It is a polyborne species, parasitizing over 44 genera of plants, found on all aerial organs. It was first reported in 1940 by Ana Hulea.

The disease appears on softwood seeds, in the form of well-defined brown spots. On them, a gray-green mold later develops, under which the tissues soften and rot.

The mycelium is septate, olive-brown in color, which develops on the outside of the attacked organs like a felt or penetrates the tissues to the leading vessels. On the mycelium are differentiated long, brown conidiophores, thicker at the base, thin and hyaline at the apex, septate and branched monopoidally, arborescent at the top. The final branches are short-stemmed, bearing scarigma, on which abundant ovoid, single-celled conidia with a smooth, light brown membrane are formed. The sclerotia are small, 24 mm long and 1-3 mm wide, ovoid or flattened, at first light in color, then

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brown and finally charcoal black. No sexual stage of this fungus has been opened (Williamson 2007).

Fusarium oxysporum - bedding of seedlings.
This pathogen together with other pathogens of the genera *Pythium, Rhizoctonia, Phomopsis, Alternaria, Botrytis, Phytophtora*, etc., leads to the death of seedlings from germination to lignification of the stems.
The young plants, until emergence, are wrapped in a hyaline mycelium, white, very fine, which penetrates into the tissues and eventually either the plant no longer grows or breaks after emergence. The first symptoms of attack from the ground are difficult to follow, but in the second phase of evolution the disease is easily observed because the seedlings turn yellow, then brown spots appear on the package followed by the strangulation of the stems in the attack area. The plants wither and dry out and can be easily removed from the soil (Kamiljanovna, 2020).
During wet periods, the fungus forms a small sleeve of whitish mold at ground level around the stem. The attack is very intense in solariums, where humidity and temperature are high, factors favoring the development of fungi. The critical period is between sowing and up to 45-50 days.
Mycelial hyphae are hyaline or slightly pink. Microconidia occur from simple vials found on short branches of the mycelium. These microconidia are oval or elliptical, straight or slightly curved, 5-12 x 2.2-3.5 μm. They are grouped with the appearance of sporodochia, have 3-5 septa, fusoid shape with a small pedicel. The dimensions vary greatly depending on the number of cells. Macroconidia with 3 septa are 27-46 x 3-5 μm, and those with 5 septa 33-60 x 3-5 μm. Occasionally conidia larger than 50-66 μm in length with 6-7 septa can be found (Farvel, 2003).

Alternaria alternata - staining of seeds and seedlings.
The pathogen is found on softwood seeds and not only in the nutrient bed and causes the "fall" of seedlings.
On the stems appear blackish-brown spots, round or oval, with darker concentric areas.
Gradually the spots grow in size, converge and the affected tissues break, the seedlings fall and rot.
On the seeds the spots are black-brown, deep, and in front of them the tissues rot.
The conidiophores are short, on which are distinguished the typical conidia, peduncled, catenated, septate transversely and longitudinally, in the shape of a bottle, of 90-200 x 12-14 μm.
During vegetation, the pathogen spreads through conidia, and from one year to the next through conidia, clanidiospores and resistant mycelium (Pedroso, 2013).

4. CONCLUSIONS

During the analyzes performed on the resinous seeds from the Voivodeni Nursery and from the Someșu Rece and Beliș Forestry Districts, no pathogens were identified, only saprophytes. The seedlings obtained from these seeds will be monitorized and analyzed in order to obtain a quality planting material.
On all batches of seeds within the Mihai Viteazu Nursery, the pathogens *Fusarium oxysporum, Alternaria alternata and Botrytis cinerea* were identified. Because they cause significant damage, in addition to control measures (application of chemical treatments), cultural activities will have to be strictly observed.

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In order to prevent the spread of diseases caused by the identified pathogens, both in the solarium and in the nursery field, the following should be considered:

- disinfection of seeds with copper products, before sowing;
- sanitation works will be carried out from the first signs of illness.
- appropriate fertilization depending on the deficiency of soil microelements (soil analysis).
- the establishment of the germination bed with a pH below 7;
- bed change every 2-3 years;
- proper aeration after seed germination, so that the maximum temperature does not exceed 25^{o}C, more often in spruce; watering with a temperature not higher or lower than 5^{o}C from the ground temperature.

Regarding the application of chemical treatments, they will be treated using products based on propamocarb hydrochloride, fosetyl aluminum.

5. ACKNOWLEDGEMENTS

This paper is an essential part of the protection of forests, namely the identification of diseases, their control and control in order to obtain a quality planting material. The works were executed by the staff of INCDS "Marin Drăcea" Cluj Section, within PN 19070205 and Cresforlife.

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