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DISEASES OF CONTAINER-GROWN CONIFERS IN THE NURSERIES OF MOGILEV AREA ACCORDING TO MOLECULAR PHYTOPATHOLOGICAL SURVEY

A molecular phytopathological survey of diseased container-grown conifers in the nurseries of Mogilev SFPA were carried out. In plant tissues was detected genetic material of pathogenic fungi, endophytic and saprophytic (non-pathogenic) microflora. The samples studied were characterized by poly-infection and contain a high titer of pathogens. Molecular-genetic diagnostic methods used for identified causative agents of dominant diseases of container-grown pine, fir and larch seedlings. It was established that the predominant disease of one-year seedlings is Cladosporium blight, caused by fungi complex with the dominance of a new species of the genus *Cladosporium*. Cladosporium blight was observed in 60% of the surveyed nurseries, the occurrence of its agents in the affected plant material ranged 50−70%. The disease accompanied by causative agents of Rhizoctonia root rot and Gray mold that registered with the 12% nurseries. Their occurrence in the affected plant material does not exceed 25%. In 37% of the surveyed nurseries two-year seedlings were struck by Phoma blight. Occurrence of its agents in the affected plant material ranged 40−60%. In single forestries along with Phoma blight detected Cytospora stem canker, Epicoccum needle necrosis and Gray mold (50%, 40% and 15%, respectively).

Key words: Container-grown conifers, DNA, PCR, primers, Gray mold, Cladosporium blight, Phoma blight, Rhizoctonia root rot, Epicoccumneedle necrosis, Cytospora stem canker.

Introduction. The use of forest planting material with a clod of soil ("Clod seedlings") in forest restoration has been known since the XVIII century. In 1725, oak wildings were transported for planting in the countries of Central Europe in this way [1]. For almost 300 years the technology of cultivation of container-grown conifers has been improving, special containers and cylinders, providing convenient transportation with minimal damage to the roots, keeping the soil before planting and improving survival have been invented [2]. However, despite a number of advantages over conventional technology and plant isolation from the source of infection – the soil, the problem of diseases of Container-grown conifers remains to the present day [3, 4].

Hence, the diagnosis and identification of agents of infectious diseases of container-grown pine was the purpose of this study.

Main part. The object of the study was Container-grown conifers with signs of infection in forest nurseries in in the nurseries of Mogilev SFPA. As a part of study the phytopathological inspection of forest nurseries in eight forestries was conducted: SEFI "Osipovichi experimental forestry", SFI "Klichev forestry", SFI "Belynichi forestry", SFI "Bykhovs forestry", SFI "Gorki forestry", SFI "Kostyukovichi forestry", SFI "Cherikov forestry", SFI "Krasnopolje forestry". As experimental material there were studied one-year seedlings of Scotch pine, European larch, as well as one- and two-year seedlings of Norway fir. Planting material was characterized by the outward signs of the disease: partial and complete drying of the needles, the withering away of the apical bud.

Diagnosis of infection and species identification of pathogens was performed based on the use of DNA analysis. Fragments of needle tissues, stems and roots were taken from the affected plant for the molecular phytopathological examination. Samples were selected in the triple repetition. 90 samples from each of the surveyed forestry have been studied in total. The sequence of nuclear DNA: 18S-ITS1-5,8-ITS2-28S, which contains the genes coding for the structural components of ribosomes – rRNA was chosen as a marker region. This region is species-specific and constant within a species. Such feature of rDNA marker makes it possible to use this feature as a diagnostic criterion for conducting of species identification. The obtained preparations of total DNA were amplified by the PCR principle using the reagent PCR Green Mix (2X) (Fermentas, Lithuania) and primers ITS1F (F) 5'-CTTGGTCATTTAGAGGAAGTAA-3', ITS4 (R) 5'-TCCTCCGCTTATTGATATGC-3'.

The genetic material of pathogenic fungi, endophytic and saprophytic (non-pathogenic) microflora was revealed in the studies. Electrophoretic fractionation showed that the samples studied were characterized by multifractional PCR-spectra, indicating the polyspecific infection, i. e. infection with two or more kinds of micromycetes. The titer of pathogenic organisms ranged from 10 to 1,000 cells of the pathogen per 5 mg of the plant tissue, which indicated the presence of dominant and related species in the tissues.

To identify the dominant microflora of polyspecific infection the fungal amplicons were separated by electrophoretic fractionation in a 2% agarose gel and were extracted for further sequencing. Molecular genetic identification of species of fungi was carried out according to database of SSI "Institute of Forest of NAS of Belarus" and international genebank NCBI (National Center for Biotechnology Information, USA) [5].

According to the results of molecular genetic analysis it was revealed that the prevailing disease of one year seedlings of Container-grown conifers is Cladosporium blight, that is caused by complex of micromycetes with the dominance of a new species of the genus Cladospory – *Cladosporium* sp. Cladosporium blight was observed in 60% of the surveyed nurseries, the incidence of its agents in the affected plant material ranked 50–70%. It should be noted that the symptoms of the diagnosed disease were different from olive mold ofseedlings caused by fungi of the same genus (*C. herbarum* and *C. cladosporioides*), and characterized by the shrinkage of the apical bud of a plant.

Comparative genetic analysis of this type in the database of international gene bank NCBI showed close congeniality of *Cladosporium* sp. (99% similarity in the genetic structure) with the uncultivated *Uncultured Cladosporium* clone IBL167f specie, identified by scientists J. A. Nowakowska and members of staff in 2016 on the roots of drying oak trees in the plantations of the west-central part of Poland in Krotoszyn. Lower similarity with the following species (98–99%) has been established: *Cladosporium iridis*, *Cladosporium allii* (identified on wheat by French scientists M. Comby and members of staff in 2015); *Cladosporium* sp. (identified on Thlaspi pierced by German scientists K. Glynou and members of staff in 2015); *Cladosporium subinflatum* (Ukrainian isolate sudstracted by A. Akulov form iris and identified by Dutch scientists K. Benschand members of staff in 2015); *Cladosporium allicinum* (identified by Dutch scientists K. Bensch with staff at Robin in 2015) [5].

Considering the data obtained by Dutch scientists K. Bensch with the staff, in the course of phylogenetic studies of various types of complex of Cladosporium blight pathogens, a new specie identified and closely related fungi above listedare likely to be a complex of species *C. herbarum* [6].

In Kostyukovichi forestry Cladosporium blight was accompanied by the pathogen of Gray mold (*Botrytis cinerea*) of one year pine seedlings. Its incidence in the affected plant material does not exceed 10%.

In Gorki forestry along with Cladosporium blight of one year seedlings of pine, fir and larch root disease *Rhizoctonia solani* J. G. Kuhn, the pathogen of Rhizoctonia root rot has been identified. Its incidence in the affected plant material ranged from 15 to 25%. The highest concentration of this pathogen was observed in the roots and stems of seedlings.

In Cherikov forestry the fungus *Cadophora luteo-olivacea* was identified in 50% of the stems of seedlings of spruce, affected by Cladosporium blight. This specie is a poorly known pathogen associated with the drying of grapes stems ("Grapevine Trunk Disease") [7]. According to the literature in Europe and Asia, the fungus also attacks other fruit crops (apple, pear) and weeds [5]. The cases of its revealing on spruce are found in Canada and Finland [8]. In Slovenia, in 2013, *C. luteo-olivacea* was found in the timber of European ash, afflicted by halarovnecrosis, by scientists T. Hauptman with the staff [9]. The specie is also registered in Antarctica in wood buildings of research stations [10].

The only case of Alternarium blight was observed in Krasnopolye forestry on one year seedlings of fir. The identified pathogen – fungus *Alternaria* sp. – belongs to the specie complex *A. alternata*, popular in nurseries. The incidence of *Alternaria* sp. in the infected plant material was 30%.

Two-year seedlings of container-grown fir were affected by Phoma blightin 37% of the surveyed nurseries. The causative agent of the disease *Phoma* sp.1 is Belarusian strain related to pathogen *P. pomorum* and identified previously in forest nurseries of Belarus on seedlings and saplings of open ground [11]. The incidence of this pathogen in the affected plant material ranged from 50 to 60% in various forestries. Common European specie *Phoma herbarum* was identified along with *Phoma* sp.1 in 40% of cases in Osipovichi experimental forestry.

It should be noted that the infectious pressure in this forestry is aggravated by the causative agent of Cytospora stem canker – a new type *Cytospora* sp. fungus detected in 50% of the stems of two years fir. Comparative genetic analysis of this type in the database of the international gene bank NCBI showed almost 100% genetic similarity with the two strains *Cytospora* sp. Olrim 554 and Olrim 784, detected and identified by the Latvian scientists R. Vasiliauskas, V. Lygis and J. Stenlid in the timber of fir with signs of infectious disease [5]. The data indicate that strains compared belong to the same species, mutated in different ecological conditions. The closest related species from the above listed (98% genetic similarity) is the causative agent of cancer *Cytospora cedri* eucalyptus stem, invasion of which has already been registered in USA.

In 15% of cases Phoma blight was accompanied by Gray mold (*B. cinerea*) in Gorki forestry. In Bykhov forestry in combination with causative agents of Phoma blight in 40% of cases *Epicoccum nigrum* was identified – Epicoccum needle necrosis pathogen.

Conclusion. The affection of this types of fungi is related to the primary effect of adverse factors (climate, soil conditions, violation of farming cul-

tivation) that caused the weakening of the plants. Taking into consideration the isolation of planting material from the soil, the defective sterilization of the substrate and the original presence of infectious origincan be concluded. As the factors of weakening of Container-grown conifers the following can be considered – the discrepancy of the substrate in the physical and chemical properties with the grown material (temperature, humidity, acidity, salt content, etc.), the mismatch of container type with the root morphology of grown material, the violation of technology of cultivation because of higher demands to the ongoing efforts compared with traditional planting.

References

1. Toman J., Hocking D. A brief History and Some Perspectives of Ball Planting. *Skogshogskolan*, 1973, vol. 44, pp. 4–6.

2. Barnett J. P., Brissette J. C. Producing southern pine seedlings in containers. *USDA Forest Service*, 1986, pp. 6–14.

3. Lilja A., Poteri M., Petaisto R. L., Rikala R., Kurkela T., Kasanen R. Fungal Diseases in Forest Nurseries in Finland. *Silva Fennica*, 2010, vol. 44, pp. 525–545.

4. Dumroese R. K, James R. L. Root diseases in bareroot and container nurseries of the Pacific Northwest: epidemiology, management, and effects on outplanting performance. *New Forests*, 2005, vol. 30, pp. 185−202.

5. The National Center for Biotechnology Information (NCBI) [Electronic resource]. Available at: http:// www.ncbi.nlm.nih.gov/tools/primer-blast/ (accessed: 10.01.2016).

6. Bensch K., Groenewald J. Z., Braun U., Dijksterhuis J., Yanez-Morales M. J., Crous P.W. Common but different: The expanding realm of *Cladosporium*. *Studies In Mycology*, 2015, vol. 82, pp. 23–74.

7. Gramaje D., Mostert L., Armengol J. Characterization of *Cadophora luteo-olivacea* and *C. melinii* isolates obtained from grapevines and environmental samples from grapevine nurseries in Spain. *Phytopathol. Mediterr*, 2011, vol. 50, pp. 112−126.

8. Berubea J. A., Nicolas G. G. Alien fungal species on asymptomatic live woody plant material imported into Canada. *Canadian Journal of Plant Pathology*, 2015, vol. 37, pp. 67–81.

9. Hauptman T., Piskur B., de Groot M., Ogris N., Ferlan M., Jurc D. Temperature effect on Chalara fraxinea: heat treatment of saplings as a possible disease control method. *Forest Pathology*, 2013, vol. 43, pp. 360–370.

10. Blanchette R. A., Held B. W., Arenz B. E., Jurgens J. A., Baltes N. J., Duncan S. M., Farrell R. L. An Antarctic Hot Spot for Fungi at Shackleton's Historic Hut on Cape Royds. *Microb Ecol.*, 2010, vol. 60, pp. 29–38.

11. Baranov O. Yu., Panteleev S. V., Yarmolovich V. A., Romanenko M. O. Molecular genetic aspects of diagnostics and identification of Phoma Blight agents. *Trudy BGTU* [Proceedings of BSTU], 2014, no. 1: Forestry, pp. 198–201 (In Russian).

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