OPHIOSTOMATOID FUNGI COLONIZING SCOTS PINE (PINUS SYLVESTRIS) TREES INFESTED BY *IPS ACUMINATUS* IN SUMY REGION (UKRAINE)

Denys Baturkin[1,2], Kateryna Davydenko[2,3].
[1]Forest Protection Service „Kharkivlisozahyst", baturkin.denis@ukr.net
[2]G. M. Vysotsky Ukrainian Research Institute of Forestry & Forest Melioration
[3]Swedish University of Agricultural Sciences, kateryna.davydenko74@gmail,com

ИЗУЧЕНИЕ ОФИОСТОМОВЫХ ГРИБОВ ВЫДЕЛЕННЫХ ИЗ СОСНЫ ОБЫКНОВЕННОЙ (PINUS SYLVESTRIS), ЗАСЕЛЕННОЙ *IPS ACUMINATUS* В СУМСКОЙ ОБЛАСТИ (УКРАИНА)

Батуркин Д.А., Давиденко К.В.

Представлены результаты исследований офиостомовых грибов, связанных с вершинным короедом (*Ips acuminatus* (Gyllenhal, 1827: Curculionidae, Scolytinae) в сосновых насаждениях Сумской области (Ахтырский и Шосткинский лесхозы). В ходе исследования было выяснено, что 93,86% всех деревьев III, IV и V категорий санитарного состояния были заселены вершинным короедом и инфицированы офиостомовыми грибами. Всего восемь видов офиостомовых грибов было изолировано в чистую культуру и идентифицировано, из них *O. minus* был наиболее агрессивным видом и вызывал гибель сосновых культур, а *Graphium* sp. *Grosmannia* sp.1 и *Ophiostoma* sp.1 были менее вирулентны, но также вызывали усыхание саженцев сосны обыкновенной.

Introduction. For many years, a gradual decline of Scots pine forest has been observed in Ukraine (Meshkova and Borysenko, 2018). Recent studies have shown that drought-induced initial pine decline can result in significant tree damage by pine bark beetles (Davydenko, 2019; Meshkova et al., 2018). Therefore, a rapid and excessive dieback of Scots pine trees has been observed in north-eastern Ukraine in the Sumy region due to the drought and bark beetle attacks.

A high abundance of the bark beetle, namely *Ips acuminatus* (Gyllenhal, 1827: Curculionidae, Scolytinae), *Ips sexdentatus* (Boerner, 1766: Curculionidae, Scolytinae), *Tomicus piniperda* (Linnaeus, 1758: Curculionidae, Scolytinae), *Tomicus minor* (Hartig, 1834): Curculionidae, Scolytinae), and

Orthotomicus sp. have been recorded in 2017–2019. Among them, pine engraver beetle *Ips acuminatus*, is likely to play a crucial role in this dieback resulted in considerable damage to the forest (Meshkova et al., 2018). Furthermore, *I. acuminatus,* as other many bark beetles, vectors microbial spores to trees during the colonization or feeding of pine trees (Bueno et al., 2010; Six 2013).

The aim of our study to identify ophiostomatoid fungi associated with weakened and dying Scots pines infested by *Ips acuminatus* in Sumy region in north-eastern Ukraine.

Material and methods. *Field study and sample collection.* Samples of blue-stained wood were taken from galleries into Scots pine trees damaged by *Ips acuminatus* in Sumy region (State Forest Enterprise "Ohtyrske LG" and State Forest Enterprise "Shostkinske LG") from 28 randomly selected pine trees in 2017-2018 to examine the presence of ophiostomatoid fungi. Forest health condition (FHC) of sampled trees was evaluated visually before cutting on a range of visual characteristics according to "Sanitary rules in the forests of Ukraine" (Sanitary rules in the forests of Ukraine 2016). Each tree was ranked according to one out of six categories (1st – healthy; 2nd – weakened; 3rd – severely weakened; 4th – drying; 5th – recently died; 6th – died over a year ago).

Samples were checked in the laboratory using a microscope for the presence of the fungal fruit-bodies in the bark beetle galleries. Wood samples were sterilized by 96% ethanol for 15 second and small wood fragments were used to place on selective media for ophiostomatoid fungi (2% malt extract agar with cycloheximide. Pure cultures were incubated at 22 °C in the dark, grouped morphologically and iden-tified using identification keys and molecular methods (Davydenko et al., 2017).

Inoculation of pine seedlings. To investigate the pathogenicity of the ophiostomatoid fungi to *P. sylvestris*, inoculation experiments were carried out on 3–5-year-old seedlings of *P. sylvestris*. All species from ophiostomatoid group were used in pathogenicity test carried out in May, 2019. The pine seedlings were inoculated by making a wound on the stem and pouring 10 μL of spore solution suspension ($5x10^3$ spores mL−1) onto the wound and covered with a strip of Parafilm afterwards. The morphological condition of all pine seedlings, their vitality, and necrosis length were evaluated for six months. After all, each seedling was cut and final sizes for each lesion (length, depth and width) were measured.

Small pieces of wood were cut from the edge of necrosis lesions and transferred onto 2 % malt extract agar with 0.8% cyclohexamide and 0.2%

streptomycin sulphate (CSMA) to confirm the presence of the inoculated fungus.

Statistical analyses. Raw sequence data were analyzed using the SeqMan Pro version 10.0 software from DNASTAR package (DNASTAR, Madison, WI, USA). The criteria used for identification were: sequence coverage > 90%; similarity to taxon level 99-100%, similarity to genus level 92–98%. A NCBI BLAST (National Centre for Biotechnology Information, www.ncbi.nlm.nih.gov) search was run with the edited sequences for preliminary species identification.

Data on lesion size of Scots pine seedlings inoculated with fungal species were analyzed using ANOVA in Statistica software (STATISTICA® 7.0 (StatSoft, Inc., Tulsa, USA). For variants with significant effects ($p<0.05$), a post hoc HSD Tukey test was used to compare the means with a significance level of $p< 0.05$.

Results and discussion. In total, 93.86% of all trees III, IV and V categories FHC were infested by *I. acuminatus* together with ophiostomatoid fungi while only 2.5% all trees II categories FHC have larvae galleries of *I. acuminatus* with ophiostomatoid fungi (Fig. 1).

In total, eight species of ophiostomatoid fungi were isolated from sapwood of infested trees by *I. acuminatus* (Table 1).

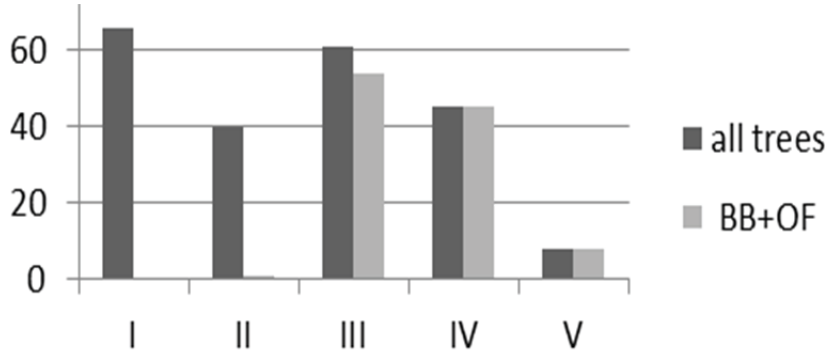

Figure 1. The number of Scots pine trees of different forest health condition damaged by bark beetles (BB) and ophiostomatoid fungi (OF) for both Ohryrske LG and Shostkinske LG.

The most commonly ophiostomatoid fungi for all trees were *Ophiostoma piceae* (82.1%), the slightly less abundant were *Ophiostoma ips* and *O. minus* (67.9%), as well as *Ophiostoma bicolor* (64.3%). The frequency of other detected species was lower than 30%. The six from eight blue-stain fungi (*Ophiostoma bicolor, O. ips, O. canum, O. piceae, O. minus* and *Ophiostoma sp.1)* were isolated from trees III and IV forest health categories. *Graphium* sp and *Grosmannia* sp.1 were found only on severely weakened trees of IV category of FHC (Table 1).

Table 1. Frequency of isolates of ophiostomatoid fungi on weakened *Pinus sylvestris* trees in Sumy

Ophiosomatoid species	Frequency of isolates from galleries	
	III FHC	IV FHC
Grosmannia sp.1	-	57,14
Graphium sp.	-	50
Ophiostoma bicolor R.W. Davidson & D.E. Wells	35,72	92,85
Ophiostoma canum (Münch) Syd. & P. Syd	21,43	42,86
Ophiostoma ips (Rumbold) Nannfeldt	57,14	78,57
Ophiostoma minus (Hedgc.) Syd. & P. Syd [d]	64,28	71,43
Ophiostoma piceae (Münch) Sydow & P. Sydow	50	94,29
Ophiostoma sp.1	14,29	28,57

According to the results of the pathogenicity test, *O. minus* was much more pathogenic than the other fungi, resulting in the highest rate of sapwood desiccation and mortality of 16.7% seedlings in 6 months. *Graphium* sp.also caused the decline of 41.7% pine seedlings as well as *O. bicolor*. *Grosmannia* sp. and. *Ophiostoma* sp.1 caused the decline of 33% pine seedlings while *O. canum* and *O. piceae* caused significant less decline of pine seedlings (16.67 and 8.33% respectively). *O. ips* did not cause seedling decline at all for 6 months. The inoculated fungi were successfully re-isolated from 98% seedlings. No ophiostomatoid species were isolated from the control trees.

Conclusion. Our study demonstrates that 93.86% of all trees III, IV and V categories FHC were infested by *I. acuminatus* together with ophiostomatoid fungi. Among all eight ophiostomatoid species, *O.minus* was the most pathogenic species on Scots pine trees. The ophiostomatoid fungi isolated in our study are commonly found to be associated with bark beetles and detected on conifer timber (Linnakoski et al. 2012; Davydenko et al., 2017).

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A RESEARCH PROJECT AIMING AT REDUCTION OF DEER-INDUCED FOREST DAMAGE

Dziedzic R.M.[1], Kornas R.[1], Gwiazdowicz D.J.[2]

[1] The State School of Higher Education in Chelm, Pocztowa 54, 22-100 Chelm, Poland, e-mail: roman.m.dziedzic@gmail.com, rkornas@pwszchelm.edu.pl

[2] Poznan University of Life Sciences, Faculty of Forestry, Wojska Polskiego 71c, 60-625 Poznanń, Poland, e-mail: dariusz.gwiazdowicz@up.poznan.pl

Analyses conducted on 17 experimental sites showed that deer-induced damage in forests is influenced to the greatest extent by the abundance and quality of their feed base. The research project carried out at one experimental site confirmed high effectiveness of supplemental feeding using a specialist feed mix in limiting the area affected by deer-induced damage. It is planned to conduct studies over a higher number of experimental sites to ensure reliability of the results and facilitate their extensive applicability.