

1. БІОЛОГІЧНІ АСПЕКТИ РОСЛИННИХ УГРУПОВАНЬ



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New insights into the role of phytopathogenic fungi vectored by pine bark beetles in pine decline

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*The study presented was carried out in response to the alarmingly increasing number of outbreaks of bark beetles and associated fungi in European forests including Ukraine. Our study was aimed to generate new knowledge on bark beetles and understand the possible role of vectored fungi. The obtained data can contribute to the increased knowledge and will allow adequately responding to an expected intensification of bark beetle disturbances, as well as may allow better understanding tree diseases and how to cope with them. In total, eleven ophiostomatoid fungi (*Graphium* sp., *Leptographium sosnaicola*, *Grosmannia olivacea*, *Grosmannia penicillata*, *Ophiostoma bicolor*, *O. ips*, *O. canum*, *O. piceae*, *O. minus*, *O. nigrocarpum* *Graphilbum rectangulosporium*) were found here in association with five species of bark beetles (*Hylurgus ligniperda*, *Hylastes ater*, *Hylastes angustatus*, *Ips acuminatus* and *Ips sexdentatus*) on Scots pine trees infested by bark beetles. Scots pine seedlings were inoculated with ophiostomatoid fungi and sterile medium (control) to evaluate their pathogenicity. The inoculated seedlings were examined finally 6 months later after inoculation. Inoculation with *Leptographium sosnaicola*, *Graphium* sp and *O. minus* produced significantly large lesions and mortality of pine seedlings. In total, all the eight fungal-inoculated species caused resin exudation and staining on the bark around inoculations in Scots pine seedlings and different rate of the seedlings decline.*

It can be concluded that the studied bark beetles are vectors for different functional groups of fungi including aggressive pathogens, and that ophiostomatoid fungi are the most closely associated symbionts. Moreover, high virulence fungi can help bark beetles to overcome the tree resistance and may significantly contribute to tree mortality.

Key words: ophiostomatoid fungi; Scots pine; bark beetles; pathogenicity.

Introduction. Bark beetles (Coleoptera: Curculionidae, Scolytinae) are insects known all over the world that are among the most economically and ecologically important pests that are very dangerous for forests, especially for stressed and weakened trees. One of the

most distinguished characteristics of bark beetles is the ubiquitous association with different fungi that were recognized more than one hundred years ago (Six, 2003; Six & Wingfield, 2011). Bark beetles are well-known for promoting the spread of these fungi into the

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living trees as well as to untreated timber and wood products (Linnakoski, 2011). There are a few dramatic examples of the invasion of bark beetle species and vectored fungi into new areas where they became aggressive and caused threats to forests after they had been accidentally introduced (Linnakoski 2011; Bezos, Martinez-Alvarez, Diez, & Fernandez, 2015; Hulcr et al., 2020).

Bark beetles comprise a highly diverse group of insects with a worldwide distribution. The overwhelming majority of bark beetle species are capable to attack weakened or dying trees when their populations are low in abundance, but a mass-attack on large numbers of healthy trees can take place once bark beetle populations are numerous (Hlásny et al., 2019).

Among aggressive bark beetles that have developed adaptations to colonize trees are mostly from the *Pinaceae* family. Despite the strong mechanism of tree resistance, these insects are economically and ecologically dangerous as during their outbreaks, they can devastate both managed and natural forests (Raffa, Bonello, & Orrock, 2020). The abundance of windthrown or coniferous trees weakened by drought, as e.g. Norway spruce (*Picea abies* L.) and Scots pine (*Pinus sylvestris* L.), can also trigger the growth of the bark beetle population. In case of the lack of fallen or stressed trees with low level of resistance, bark beetles can attack healthy trees (Linnakoski, 2011, 2012; Bezos et al., 2015; Hulcr et al., 2020).

From the 2010s and on, two species of bark beetles have been found to be the most destructive pests in *Pinus sylvestris* forests, becoming aggressive, attacking and killing healthy trees in Finland, Italy, Poland, Spain, Germany, Slovakia and also in Ukraine (Linnakoski, 2011; Davydenko, Vasaitis, Meshkova, & Menkis, 2014; Bezos et al., 2015; Davydenko, Vasaitis, & Menkis, 2017; Meshkova, Borysenko, & Pryhornytskyi, 2018; Hulcr et al., 2020; Davydenko, 2021). In Ukraine, massive *Pinus sylvestris* dieback and considerable economic damage were caused by the pine engraver beetle (*Ips acuminatus*) (Gyllenhal, 1827) (Coleoptera: Scolytidae) and the six-toothed bark beetle (*Ips sexdentatus*) (Börner, 1767) (Coleoptera: Scolytidae) (Davydenko et al., 2014, 2017; Meshkova et al., 2018). Therefore, *I. sexdentatus* and *I. acuminatus* can kill a considerable number of healthy trees given that their population density is high. The drought-induced weakening of *Pinus sylvestris* trees and their further infestation by bark beetles led to a rapid tree dieback in Ukraine, resulting in vast areas of lost plantation.

Moreover, bark beetles from the genera *Hylurgus* (*Hylurgus ligniperda*) and *Hylastes* (*H. angustatus* and *H. ater*) are the serious pests causing extensive damages to young plantations, stands and timber of *Pinus sylvestris*. *Hylurgus ligniperda* is among the most common bark beetles in Ukraine (Meshkova & Davydenko 2012; Davydenko et al., 2014), Davydenko, 2021). *Hylurgus ligniperda* breeds in logging residues including stumps, roots and logs of *Pinus sylvestris* trees while larvae of *H. ligniperda* may also feed

on roots and butts of healthy-looking and diseased seedlings and saplings (Davydenko et al., 2014).

Material and methods. *Field study and sample collection.* During the 2012-2018 period, to examine the presence of ophiostomatoid fungi, samples of beetles and blue-stained wood were taken from pine stands located in the different regions of the forest-steppe zone of Ukraine. The sample plots are given below (Tab. 1).

Stands at all sites were ca. 50-70 year-old mostly monoculture plantations of Scots pine. Sampling of bark beetles has been carried out randomly from standing trees of *Pinus sylvestris*. Trees were living but slightly or severely weakened by bark beetle and drought. The time of sampling coincided with the period when the dispersal flight period of young generation has completed and their galleries have already been built. Samples of bark with bark beetle galleries, phloem and sapwood were taken from the relevant part of standing trees using sterilized forceps, individually placed in sterile Ependorff tubes and transported to the laboratory. Half of the beetles from each site were stored at 4°C for fungal culturing and the other half – at -20°C for direct DNA sequencing of fungi.

Study of fungal communities of bark beetles. Cultural method. The half of bark beetles of each species and each site were used for fungal isolation. The isolation from bark beetle and wood from their galleries target to gain as much as possible ophiostomatoid fungi for further pathogenicity tests as well as other fungi (Davydenko et al., 2014, 2017). Fungal cultures were divided into groups based on their morphology and, for species identification, representative cultures from each group were subjected to sequencing of the internal transcribed spacer regions of the fungal ribosomal RNA (ITS rRNA). The isolation of DNA, amplification and sequencing were performed according to methods described by Davydenko (Davydenko, 2021). The amplification by PCR was done using primers ITS1F and ITS4. In addition, β -tubulin gene and the partial elongation factor 1-alpha (EF1-a) gene were amplified and sequenced for fungi from the genera *Ophiostoma*, *Graphilbum* and *Grosmania*. The β -tubulin gene was amplified using the primers Bt2a and Bt2b (and EF1-a gene was amplified using the primers EF1F and EF2R). Databases at GenBank were used to determine the identity of ITS rRNA sequences. The criteria used for the identification were as follows: sequence coverage > 80%; similarity to species level 98-100%, similarity to genus level 94-97%.

Study of fungal communities of bark beetles. Direct sequencing. While the conditions vary significantly in phloem compared with nutrient media, the cultural method is selective for fast-growing fungi and is biased towards those fungal species that are able to utilize successfully artificial media. The use of molecular methods circumvents this problem and allows filling a gap by extracting DNA directly from bark beetles and then amplifying it using nested PCR reactions (Persson et al., 2009). The fungal specific primers

NLC2 (GAGCTGATTCCCAACAACACTC) and NSA3 (AAACTCTGTCGTGCTGGGGATA) were applied first (Davydenko, 2021), then, in a second (nested) PCR ribosomal internal transcribed spacer (ITS) region has been targeted as the main primer for the identification

of a fungal community (Davydenko et al., 2014, 2017; Davydenko, 2021). After sequencing, taxonomic identification of derived sequences was carried out by comparing with known sequences in databases such as GenBank.

Table 1

General information on bark beetle sampling in Ukraine

| Sample plot | Latitude, Longitude | Beetle species | Host species | Number of beetle samples |
|------------------|---------------------------|----------------------------|-------------------------|--------------------------|
| Eastern Ukraine | | | | |
| Luhansk | N 48°43', E 039°05' | <i>Hylurgus ligniperda</i> | <i>Pinus sylvestris</i> | 48 |
| | | <i>Ips acuminatus</i> | | 192 |
| | | <i>Hylastes ater</i> | | 138 |
| | | <i>Hylastes angustatus</i> | | 144 |
| Kharkiv | N 49°10' E 037°14' | <i>Hylurgus ligniperda</i> | <i>Pinus sylvestris</i> | 48 |
| | | <i>Ips acuminatus</i> | | 192 |
| | | <i>Hylastes ater</i> | | 138 |
| | | <i>Hylastes angustatus</i> | | 144 |
| Kharkiv | N 49°48'40 E 36°21'24 | <i>Ips sexdentatus</i> | <i>Pinus sylvestris</i> | 96 |
| Donetsk | N48°57'12 E37°54'58 | <i>Ips sexdentatus</i> | <i>Pinus sylvestris</i> | 96 |
| | | <i>Hylastes ater</i> | | 138 |
| | | <i>Hylastes angustatus</i> | | 144 |
| Northern Ukraine | | | | |
| Sumy 1 | N 51°57'18 E 33°34'27 | <i>Ips sexdentatus</i> | <i>Pinus sylvestris</i> | 96 |
| Sumy 2 | N 51°23'02. E 33°23'33 | <i>Ips sexdentatus</i> | <i>Pinus sylvestris</i> | 96 |
| Sumy 3 | N 50°50'40 E 33°55'41 | <i>Ips sexdentatus</i> | <i>Pinus sylvestris</i> | 96 |
| Central Ukraine | | | | |
| Kyiv | N50°55'40 E30°02'27 | <i>Ips sexdentatus</i> | <i>Pinus sylvestris</i> | 96 |

Pathogenicity test. As mentioned above, some ophiostomatoid species are more or less pathogenic to their host plants. Therefore, to confirm pathogenicity and define pathogenic extent, the reappearance of the original symptoms after artificial inoculation by tested species is defined. For this, pathogenic ophiostomatoid fungi are inoculated into phloem by making wounds to similar bark beetles damage as described in our previous studies (Davydenko et al., 2017; Davydenko & Baturkin, 2020; Davydenko, 2021). Further, the fungal inoculation results in the formation of necrotic lesions in the phloem and colonization of sapwood, and in some cases, in the tree mortality (Davydenko & Baturkin, 2020). Thus, the virulence of the fungi was evaluated by measuring lesion sizes and monitoring the mortality of host plants after inoculation (Jankowiak, & Bilański, 2013; Repe, Bojovic, & Jurc, 2015).

Statistical analyses. Statistical analysis was carried out using the statistical software package PAST: Paleontological Statistics Software Package for Education and Data Analysis (Hammer et al., 2001). All the obtained data were tested for adherence to the normal distribution using the Kolmogorov–Smirnov test.

Sorensen similarity indices were used to characterise the diversity and composition of fungal (Magurran, 1988). The data from the inoculation and vector tests were analysed using analysis of variance (ANOVA). Significant treatment differences were further evaluated by Fisher's exact test followed by Tukey's HSD post hoc test. The significance was evaluated at the 0.05 p-level.

Results and discussion. Ophiostomatoid and other fungi associated with bark beetles infesting *Pinus sylvestris* in Ukraine were investigated in this study. Among countries neighboring Ukraine, the most comprehensive and detailed review of fungi associated with bark beetles infesting many conifers and hardwoods, including coniferous living trees, cut down, fallen trees and logs, has been done in Poland by Jankowiak (Jankowiak & Hilszczanski, 2005; Jankowiak & Kolarik 2010; Jankowiak, 2013; Jankowiak & Bilanski, 2013). These studies indicate numerous ophiostomatoid fungi associated with a bark beetle in Poland, the country nearest to Ukraine. Another Ukraine neighboring country is Slovakia, where only one research work has been recently

published by researchers, focused on ophiostomatoid fungi and pine bark beetle (Pastircakova et al., 2018).

The use of both cultural sequencing methods of bark beetle discovered species-rich fungal communities associated with pine bark beetle in Ukraine, encompassing different groups of the fungal kingdom and representing several different ecological roles. The overall

fungal community was composed of 83.5% Ascomycota, 9.5% Basidiomycota and 3% Mucoromycotina, 4% of species remaining unidentified. The most commonly detected groups for all species were saprotrophic fungi (28.1%), ophiostomatoid fungi (18.8%) and pathogens (17.3%), while the frequency of the other ecological group vary from 0.8 to 7.02 % (Fig. 1).

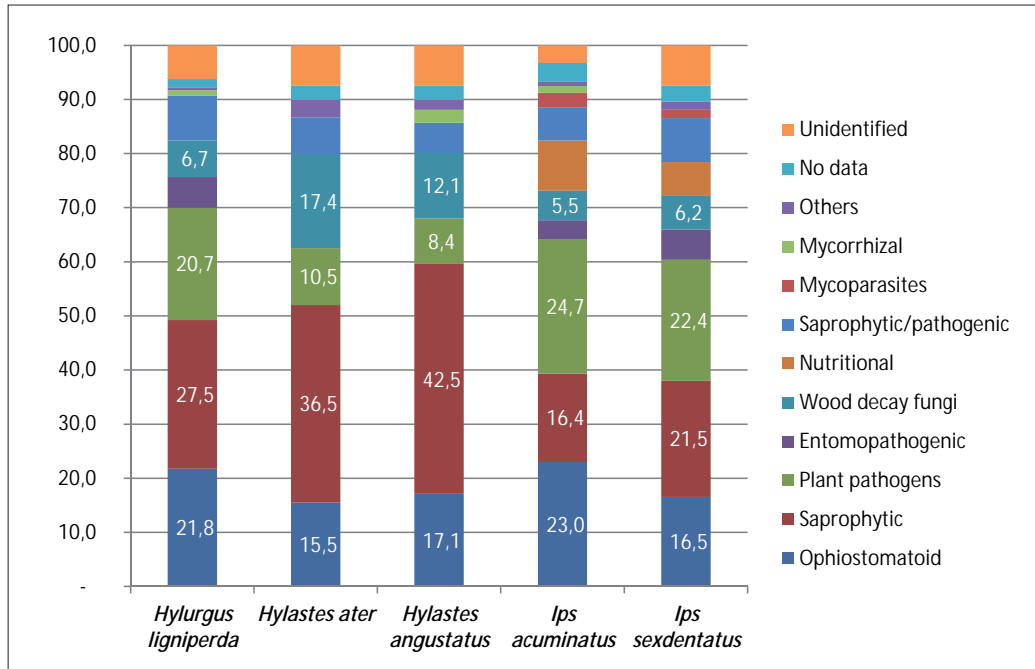


Fig. 1. Ecological composition of fungal communities associated with different pine bark beetles, Oph – ophiostomatoid fungi; Ept – entomopathogenic fungi; Wd = wood decay fungi; Sf = saprophytic fungi; Pp = Plant pathogen, Sp – fungi combined saprophytic and pathogenic strategy; Mp = mycoparasites; My = mycorrhizal fungi; Or = other ecological roles; Une = no data about ecology available, Und – unidentified fungi

In particular, Ascomycota species were common to be associated with all insects at all sites. Among ophiostomatoid fungi, only *Ceratocystis ips* and *Pesotum piceae* (syn. *Ophiostoma piceae*) have been detected for all species and sites while *Grosmannia penicillata* and *Leptographium sosnaicola* were associated exclusively with *Ips sexdentatus* and *Ophiostoma pallidulum* with *Ips acuminatus* (Fig. 2). Moreover, *Grosmannia penicillata*, *G. olivacea*, *Graphium* sp., *Ophiostoma canum* *O. minus*, *Ophiostoma pallidulum*, *Graphilbum rectangulosporium* and *Leptographium sosnaicola* were found first in Ukraine and described to be associated with bark beetles in the present study. Other ophiostomatoid fungi *Ceratocystis ips* and *Pesotum piceae* were mentioned as associated with *Pinus sylvestris* logs in Ukraine. Among the basidiomycetes, species belonging to *Polyporales*, species within *Tremellomycetes*, *Corticiales*, *Agaricales* and *Russulales* were also detected. Within Basidiomycota group, wood-decaying fungi (*Bjerkandera adusta*, *Fomitopsis pinicola*, *Heterobasidion annosum*, *Phlebiopsis gigantea*), mycorrhizal fungi (*Hebeloma* sp.) as well plant pathogen species (*Cryptococcus* sp.) and nutritional fungus *Entomocorticium* sp. were found.

Heterobasidion annosum s.l. has also previously been isolated from *Hylurgus ligniperda* (Davydenko et

al., 2014), *Hylastes ater* and *H. angustatus* (Meshkoba & Davydenko, 2012) but not from *Ips acuminatus* (Davydenko et al., 2017), which can be explained by the localization of bark beetles in the bottom stem and root rot mycelia grown into the wood and therefore the different fungal species can appear on the body surface of insects.

Entomocorticium sp. was the most common fungus associated with *Ips acuminatus* (24,5%) and *Ips sexdentatus* (12,33%), while no nutritional fungi had been found with *Hylurgus ligniperda* (Davydenko et al., 2014). In both cases *Entomocorticium* sp., was detected by direct sequencing first in association to *Ips acuminatus* and *Ips sexdentatus*. This is mycangial fungus, which was the most commonly detected fungus in the present study, has previously only been reported in association with *Dendroctonus ponderosa* Hopkins, (Coleoptera: Curculionidae) and *Dendroctonus frontalis* Zimmermann (Coleoptera: Curculionidae) (Klepzig et al., 2004). *Entomocorticium* sp. may provide nutritional benefits to larvae of bark beetles while *Ophiostoma minus* results in poorly developed larvae, which often fail to reach the adult stage (Klepzig et al., 2004). *Entomocorticium* sp. may provide some protection for larvae of bark beetles against the negative impact of *O. minus*. *Ophiostoma minus* is a high virulent fungus in

our study, apparently, also antagonistic fungal symbiont, that threatens normal development of larvae because it can stop the increase of nutrient concentrations of the phloem as mycangial fungi do. In contrast, *Ambrosiella macrospora* (Franke-Grosch.), which is primarily known as a food source for larvae and thought to be non-pathogenic and which is commonly associated with

Ips acuminatus in Italy (Villary et al., 2012), but was not detected in our study. The latter examples demonstrate that *Ips acuminatus* and *Ips sexdentatus* may vector rather different fungal communities in different parts of Europe including unique nutritional fungus serving an additional source of nutrition for both adults and larvae (Jankowiak, 2013).

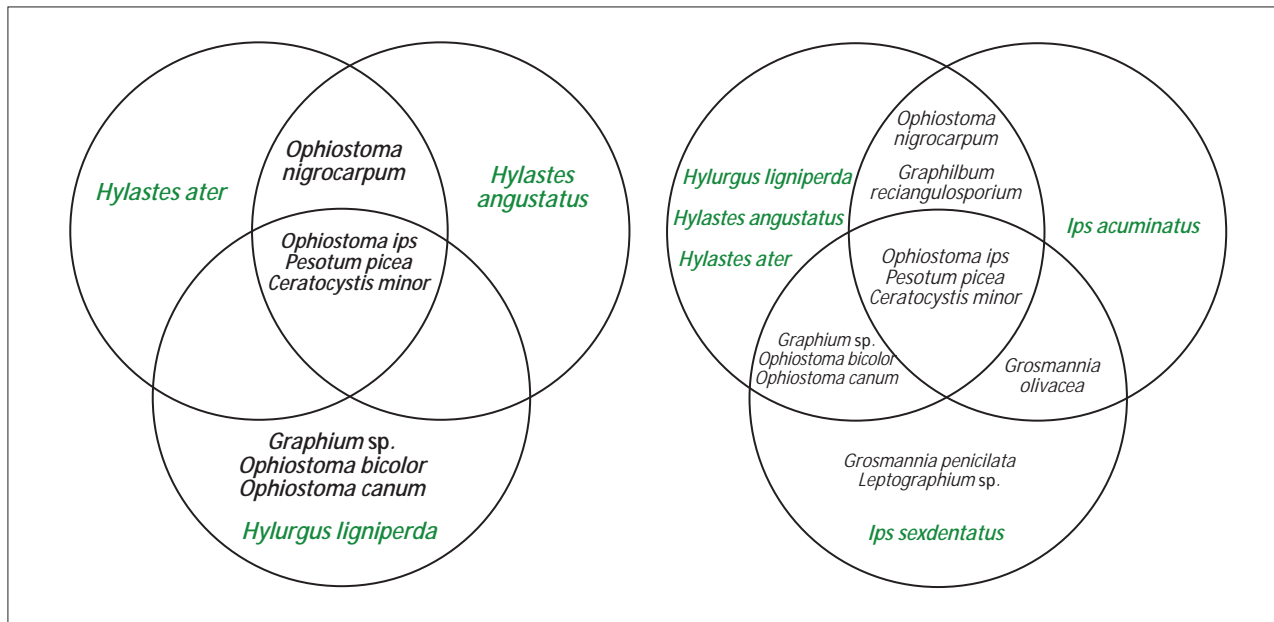


Fig. 2. Venn diagram of the association of five pine bark beetle, *Hylurgus ligniperda*, *Hylastes ater*, *Hylastes angustatus* and *Ips acuminatus* and *Ips sexdentatus* with ophiostomatoid fungi

Sphaeropsis sapinea has been shown to be associated with *Ips acuminatus* in high frequency (24,0%), second after *Entomocorticium sp.*, but quite rare isolated pathogens from the *Ips sexdentatus* and never from *Hylurgus ligniperda* in our study which can be attributed to the absence of *H. ligniperda* and *I. sexdentatus* maturation feeding in the crown.

Sphaeropsis sapinea is a widely distributed pathogen of conifers causing Diplodia tip blight and stem canker disease (Luchi et al., 2014). Previously, associations between *Sphaeropsis pinea* and *Tomicus piniperda* L. (Coleoptera: Curculionidae), *Hylastes attenuatus* Erichson, (Coleoptera: Curculionidae), *Hylurgops palliates* (Gyll.) (Coleoptera: Curculionidae), and *Xyleborus dispar* (F) (Coleoptera: Curculionidae) beetles have been reported from northern Spain (Goldazarena, Romón, & López, 2012). Also, a possible interaction between the exotic insect *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae) and *Sphaeropsis pinea* has been postulated because both these species damage pine cones in Italy (Luchi et al., 2012). Moreover, in contrast to our study, it has been demonstrated that *Sphaeropsis pinea* was effectively vectored by *Hylastes ater*, *Hylastes opacus* Erichson (Coleoptera: Curculionidae) and *Hylurgus ligniperda* in Poland (Jankowiak, 2013) although not for the present study.

Therefore, our study, provides evidence for the first time that *S. pinea* is commonly associated with *Ips*

acuminatus and *Ips sexdentatus* in Ukraine. Generally, complete fungal diversity associated with bark beetle species is better estimated with studies sampling by pooling data for all sites, as can be illustrated by the fact that diversity indices are higher for the *Ips sexdentatus* and *Ips acuminatus* than those for *Hylurgus ligniperda*, *Hylastes ater* and *H. angustatus*.

To determine if the insects actively spread the fungi requires more investigation, but it is clear from this study that fungi can be present on the body surface of insects or in their stomachs and could be vectored by bark beetles. We observed that almost all attacks of pine bark beetles were efficient for some blue-stain fungi which were able to colonize sapwood. Some species (as *Ophiostoma minus*) impair the development of bark beetle larvae whereas other species facilitate bark beetle attack by overcoming tree resistance. This suggests that when established in phloem at the early-stage, ophiostomatoid species competitive abilities are important mechanisms influencing the fungal species community. In term of these abilities of ophiostomatoid fungi, the study of their pathogenicity and degree of virulence seems important for awareness of their influence on forest health as well as for understanding partnerships in bark beetle-fungi symbiosis, which contributes to most of the success and diversity of these insects.

Several methods can be used by researchers to assess and compare the virulence of the ophiostomatoid

fungi associated with bark beetles. The most common method is to measure the size of the lesions forming after the inoculation. Moreover, typical symptoms and damage to the tree would be also useful for assessment. Pathogenicity test revealed different degrees of virulence of different fungal species together with colonization patterns of the plant tissue (Tab. 2).

Table 2
Effect of inoculation of 3-4-year-old
Pinus sylvestris seedlings with ophiostomatoid
fungi (Davydenko, 2021)*

| Species | Length of the lesion, mm | Dead plants, % | Declining plants, % |
|-------------------------------------|--------------------------|----------------|---------------------|
| <i>Ceratocystis ips</i> | 3.9±0.4 ^a | 0 | 0 |
| <i>Graphium</i> sp. | 14.7±1.22 ^b | 58.33 | 42.7 |
| <i>Grosmannia olivacea</i> | 6.5±0.24 ^c | 16.67 | 16.67 |
| <i>Grosmannia penicillata</i> | 9.6±1.9 ^d | 33.33 | 16.67 |
| <i>Grosmannia rectangulosporium</i> | 11.02±0.2 ^d | 0 | 10 |
| <i>Leptographium sosnaicola</i> | 25.9±1.2 ^c | 75 | 25 |
| <i>Ophiostoma bicolor</i> | 10.2±0.27 ^d | 8.33 | 25 |
| <i>Ophiostoma canum</i> | 3.4±0.06 ^a | 0 | 0 |
| <i>Ophiostoma minus</i> | 19.6±0.9 ^e | 45 | 25 |
| <i>Ophiostoma pallidulum</i> | 2.9±0.2 ^a | 0 | 0 |
| <i>Pesotum piceae</i> | 3.2±0.1 ^a | 0 | 0 |
| Control | 0 ^f | 0 | 0 |

* values for blue-stain depth and lesion length show as mean ± one standard error)

There was a mortality of the inoculated seedlings (75, 58.33 and 45% respectively) after inoculation causing decline of the rest of them (Tab. 2) which was expressed in wilting of the new shoots of the current's years shoots and needle yellowing. *Grosmannia penicillata*, *G. olivacea* and *Ophiostoma bicolor* caused the death of 33.33, 16.67 and 8.33 7% seedlings respectively and also decline of survivors. Inoculation with *Graphilbum rectangulosporium* resulted only declining 10% of seedlings (See Tab. 2). *Ceratocystis ips*, *Ophiostoma canum*, *O. pallidulum* and *Pesotum piceae* did not cause any decline or death of the pine seedlings. No plant dieback or decline was observed in the control.

All testing fungi resulted in lesions of different sizes (See Tab. 2) that were significantly higher than sterile inoculated control plants ($p = 0.000$). *Leptographium sosnaicola* and *O. minus* induced significantly larger necrotic lesions than other ophiostomatoid fungi.

Therefore, all inoculated fungi showed capability to infect plant tissues and cause lesions of different sizes (See Tab. 2). The lesions were generally covered with resin and extended vertically in both directions from the point of inoculation.

Ophiostoma minus, *Leptographium sosnaicola* and *Graphium* sp. showed signs of being the most virulent in this study as well as *G. olivacea*, *G. penicillata* and *O. bicolor* which also caused pine decline and mortality. These results support other studies that have found *Grosmannia* and *Leptographium* species could show high virulence to conifers in Europe and Scandinavia where they are well-known to exist in symbiosis with spruce bark beetles (Linnakoski, 2011; Jankowiak, 2013). *Grosmannia olivacea* appears to be significantly less virulent compared with *Grosmannia penicillata*, while inoculation with *Ceratocystis ips*, *Ophiostoma canum*, *O. pallidulum* and *Pesotum piceae* resulted in relatively small lesions indicating that these species were weak pathogenic although lesion reactions were longer and deeper compared to control seedlings.

Finally, our results and conclusions corroborate a few previous findings, suggesting that pathogenicity is not specific ability and differences in virulence exist between the fungi (Linnakoski, 2011; Jankowiak, 2013; Zhao et al., 2019).

Conclusions. To sum up, eleven ophiostomatoid fungi (*Graphium* sp., *Leptographium sosnaicola*, *Grosmannia olivacea*, *Grosmannia penicillata*, *Ophiostoma bicolor*, *O. ips*, *O. canum*, *O. piceae*, *O. minus*, *O. nigrocarpum* *Graphilbum rectangulosporium*) were found in association with five bark beetles (*Hylurgus ligniperda*, *Hylastes ater*, *Hylastes angustatus*, *Ips acuminatus* and *Ips sexdentatus*) in Ukraine on Scots pine trees infested by bark beetles. It can be concluded that the studied bark beetles are vectors for different functional groups of fungi including aggressive pathogens, and that ophiostomatoid fungi are the most closely associated symbionts. Moreover, high virulence fungi can help bark beetles to overcome the tree resistance and may significantly contribute to tree mortality.

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Нове розуміння ролі фітопатогенних грибів, які переносяться сосновими короїдами, у всиханні соснових лісів

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Масове всихання соснових насаджень у багатьох країнах Європи, зокрема і в Україні, вимагає детального вивчення асоціацій ксилофагів і фітопатогенних грибів, насамперед – офіостомових,

що разом з короїдами зумовлюють швидке масове всихання деревостанів. Робота скерована на отримання нових знань про асоціативні зв'язки ксилофагів із фітопатогенними грибами. Отримані дані можуть допомогти своєчасно і адекватно оцінити щкодочинність стовбурових шкідників та агресивність пов'язаних з ними фітопатогенів, шляхи поширення хвороб, способи запобігання і контролю за їхнім поширенням. Під час вивчення соснових стовбурових шкідників було виявлено одинадцять офіостомових грибів (*Graphium* sp., *Leptographium sosnaicola*, *Grosmannia olivacea*, *Grosmannia penicillata*, *Ophiostoma bicolor*, *O. ips*, *O. canum*, *O. piceae*, *O. minus*, *O. nigrocarpum*, *Graphilbum rectangulosporium*), асоційованих з п'ятьма видами короїдів (*Hylurgus ligniperda*, *Hylastes ater*, *Hylastes angustatus*, *Ips acuminatus* та *Ips sexdentatus*). Саджанці сосни звичайної інокулювали офіостомовими грибами та стерильним середовищем (контроль) для оцінки їхньої патогенності. Інокуляція *Leptographium sosnaicola* *Graphium* sp. та *Ophiostoma minus* спричиняла найбільші некрози, ураження та загибель сіянців сосни.

Серед офіостомових грибів лише *Ceratocystis ips* і *Pesotum piceae* (син. *Ophiostoma piceae*) були виявлені у всіх регіонах дослідження, тоді як *Grosmannia penicillata* та *Leptographium sosnaicola* були пов'язані виключно з *Ips sexdentatus*, а *Ophiostoma pallidulum* – з *Ips acuminatus*. Крім того, *Grosmannia penicillata*, *G. olivacea*, *Graphium* sp., *Ophiostoma canum*, *O. minus*, *O. pallidulum*, *Graphilbum rectangulosporium* та *Leptographium sosnaicola* були вперше виявлені в Україні під час виконання наших досліджень. Інші офіостомові гриби – *Ceratocystis ips* та *Pesotum picea* раніше були виявлені в Україні як гриби, асоційовані з *Pinus sylvestris*.

Також в асоціації з ксилофагами було виявлено види, які належать до порядку *Polyporales* (*Tremellomycetes*, *Corticiales*, *Agaricales* та *Russulales*). У групі *Basidiomycota* трапляються гриби, що зумовлюють гнилизну деревини (*Bjerkandera adusta*, *Fomitopsis pinicola*, *Heterobasidion annosum*, *Phlebiopsis gigantea*), мікоризні гриби (*Hebeloma* sp.), а також види – збудники хвороб рослин (*Cryptococcus* sp.) та живильний гриб *Entomocor*.

Heterobasidion annosum s.l. – збудник кореневої губки сосни звичайної, який зумовлює масове всихання і загибель дерев, був виділений з жуків *Hylurgus ligniperda*, *Hylastes ater* та *H. angustatus*, що можна пояснити особливостями екології видів.

Досліджувані види короїдів є переносниками різних функціональних груп грибів, зокрема збудників хвороб сосни, а офіостомові гриби є тісно пов'язаними з ними симбіонтами. Крім того, види грибів з високим ступенем вірулентності можуть допомагати короїдам подолати захисні функції дерев і різною мірою спричиняти їхню загибель.

Ключові слова: офіостомові гриби; сосна звичайна; короїди; патогенність.

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