5.ЗАХИСТ ЛІСІВ І МИСЛИВСЬКЕ ГОСПОДАРСТВО

Forestry Academy of Sciences of Ukraine

Наукові праці Лісівничої академії наук України
Proceedings of the Forestry Academy of Sciences of Ukraine

http://fasu.nltu.edu.ua
https://doi.org/10.15421/411912
Article received 2018.10.05
Article accepted 2019.03.28

ISSN 1991-606X print
ISSN 2616-5015 online
@ ✉ Correspondence author
Kateryna Davydenko
kateryna.davydenko74@gmail.com
Pushkinska str., 86, Kharkiv, 61024, Ukraine

UDK 630.4

A comparative characteristic of fungal communities associated with *Ips acuminatus* in different regions of Ukraine

K. Davydenko[1]

Pine bark beetles are typically associated with complexes of fungi that could reveal different functional interaction. Thus, previously nonaggressive bark beetle Ips acuminatus is considering now to be among the most serious pests of pine forest in Ukraine and other European countries and vectored fungal community is very important to assess total harm of this bark beetle. The aim of this study was to reveal the vectored fungal community associated with the pine engraver beetle, I. acuminatus with special emphasis on pathogenic fungi for further evaluation of harm bark-beetle - fungi association for Ukrainian forest.

In total, 288 adult beetles were collected from Scots pine trees at six different sites through Ukraine. DNA sequencing as fungal culturing from all beetles resulted in 1681 isolates and amplicons representing 42 fungal taxa. NCBI BLAST search revealed that the overall fungal community was composed of 94 species, of which 80.85% were Ascomycota, followed by Basidiomycota and unidentified fungal group, which accounted for 10.6% and 8.5 % of the total sequences, respectively. Among these, the most commonly detected fungi for pooling dataset were Sphaeropsis sapinea (23.6%), Cladosporium pini-ponderosae (19.44%), Ophiostoma ips (19.1%), Ophiostoma canum (19.1%) and Cladobotryum mycophilum (18.06%). In the pooled dataset of isolates and amplicons for each site, Shannon diversity indices ranged between 1.9 and 2.9 while Simpson diversity index varied between 0.69 and 0.89 indicating rich species diversity.

In total twelve ophiostomatoid species were detected. All ophiostomatoid fungi were showing varying degrees of virulence and O. minus was the most aggressive fungus in previous studies. It is concluded that I. acuminatus vectors a species-rich fungal community including pathogens such as ophiostomatoid fungi, Sphaeropsis sapinea, different needle pathogens and wood decay fungi that seems to be very important for the assessment of threat of I. acuminatus to the pine forest in Ukraine.

Key words: *Scots pine; bark beetles; Ips acuminatus; Ophiostoma; Sphaeropsis sapinea; pathogens; forest health.*

Introduction. Bark beetle species (Coleoptera: Scolytinae) that colonize trees from the Pinaceae family are economically and ecologically important forest insects, and high-density outbreaks can devastate both managed and natural forests. Bark beetles are often divided into notional clusters: aggressive and nonaggressive beetles (Krokene & Solheim, 1998), however both climate changes and humans have been

[1] *Kateryna Davydenko* – Member of Forestry Academy of Sciences of Ukraine, PhD, (agricultural sciences), senior researcher, G. M. Vysotsky Ukrainian Research Institute of Forestry & Forest Melioration, Pushkinska str., 86, Kharkiv, 61024, Ukraine; Visiting Researcherat Department of Forest Mycology and Plant Pathology, Uppsala BioCenter, Swedish University of Agricultural Sciences, P.O. Box 7026, SE-75007, Uppsala, Sweden. Tel.: +38-098 66 755 26. E-mail: kateryna.davydenko74@gmail.com ORCID http://orcid.org/0000-0001-6077-8533

bringing about unprecedented changes to environments worldwide result in the shift in classical aggressive interactions among certain pests and tree species to less or nonaggressive and vice versa. Generally, nonaggressive beetles occupy weakened, dying-up, or dead trees, but under certain conditions, populations of these nonaggressive beetles can cause massive outbreaks, killing healthy trees (Alamouti et al., 2007). Aggressive beetles are well-known to attack, and even kill healthy trees, however, the majority of bark beetles occupy only dying or very weakened trees (Krokene & Solheim, 1998).

Such climate change as increasing temperature and drought in Ukraine are common now and can play an important role as a selective pressure shaping species traits and assemblages in the forest ecosystem. Thus, previously nonaggressive bark beetle *Ips acuminatus* (Gyllenhal, 1827) is considering recently to be among the most serious pests of pine forest in Ukraine and other European countries (Colombari et al., 2013, Siitonen, 2014, Davydenko et al., 2017; Meshkova et al., 2017, 2018).

Majority of bark beetles are well-known for their symbiosis with various microorganismes, for instance, fungi, resulting in various types of symbiotic interactions between fungi and bark beetles from obligate mutualism to commensalism or even parasitism and antagonism (Six, 2012). Bark beetle-fungus symbiosis is established in long-term ecological and evolutionary processes (Six. 2012).

Most studies demonstrated that fungi can provide the nutrient source (Six, 2012; Villari, 2012; Davydenko et al., 2017). Last decades, classic hypothesis postulate that both fungi and aggressive bark beetles exist in a relationship in which each individual benefits from the activity of the other. Regarding this classic paradigm (Six, 2012), some fungi, mostly aggressive, may assist beetles in overcoming tree defences. The actual role of fungi in helping bark beetles to overcome the defenses of the host trees is under debate but at least some species (e.g., *Ophiostoma novo-ulmi* or *Endonophora polonica*) can weaken or even kill healthy trees.

The relationship between fungi and bark beetles has been studied for more than one century, but there are many fundamental and practical aspects poorly known around the topic (Linnakoski et al., 2012; Villari 2012). The pathogenic fungi can play a key role in overcoming the host defense increasing the aggressiveness and success of bark beetle attacks (Krokene & Solheim, 1998), but at the same time this interaction can also play an important part for fungal life cycle to use beetles as transports for the fungi (long-distance movement) to facilitate inoculation the fungi into suitable host-trees (Six, 2003, 2012). Moreover, fungi are also reported to be an important nutrition for some bark-beetle species (Linnakoski et al., 2012, Villari et al., 2012). The bark beetles-fungus interaction appears to vary between different species, regions and countries (Six, 2012), however, a very little is known about ophiostomatoid fungi associated bark beetle species colonizing Scots pine in Ukraine (Davydenko et al., 2017).

The aim of this study was to reveal the vectored fungal community associated with the pine engraver beetle, *Ips acuminatus* (Gyllenhal) in different regions of Ukraine with special emphasis on pathogenic fungi for further evaluation of harm bark-beetle – fungi association for Ukrainian forest.

Objects and methods. Study sites were pine forest stands located in the different regions of Ukraine (Fig. 1). Stands at all sites were ca. 50–60-year-old plantations of Scots pine (*Pinus sylvestris*). *I. acuminatus* adults were collected from infested logs of *P. sylvestris* sampled in Kharkiv (Kh), Luhansk (Lh), Poltava (P), Sumy (S), Kyiv (K) and Zhytomir (Zh), and stored singly until analyses.

Fig. 1. Locality of sampling sites Kharkiv (Kh), Luhansk (Lh), Poltava (P), Sumy (S), Kyiv (K) and Zhytomir (Zh)

Sampling and study of bark beetles *I. acuminatus* was carried out in 2014-2016. Forty-eight individuals per site were collected randomly and analysed. Half of the beetles from each site were stored at 4°C for fungal culturing and the other half at -20°C for DNA analysis.

Fungal culturing and molecular identification. Samples for fungal isolation were plated on 2 % malt extract agar (MEA, Difco, BD, Franklin Lakes, NJ, USA) containing 200 ppm of cycloheximide and 300 ppm of streptomycin (Sigma-Aldrich), in order to be selective for Ophiostoma species and avoid growth of bacterial isolates and fast growing fungi as *Trichoderma* spp, *Penicillium* spp. etc. Obtained cultures were used to get pure isolates by transferring mycelium from the edges of single colonies to fresh 2% MEA. Cultures were incubated at 22°C and grouped according to morphological characteristics of colonies and conidiophores, and single spore cultures were prepared from germinating conidia of isolates representing morphological groups of different sites (Villari et al., 2012).

Morphological identification was based on macro- and microscopic characteristics of the isolates. Specimens were observed both under a stereomicroscope and a light microscope, after anamorph fruiting structures were mounted on glass slides in cotton blue.

DNA was extracted from the single spore cultures of the isolates representing morphological groups of different sites. Approximate DNA concentrations were determined at 260 nm using the Nano-drop 2000 spectrophotometer (Nano-drop Technologies, Wilmington, DE, USA), and extracts were diluted to 10 ng µl−1 in double-distilled water (Sigma-Aldrich, St. Louis, MO, USA). Internal transcribed spacer (ITS) regions 1 and 2, including the ribosomal 5.8S gene, were amplified using the ITS primers pairs (Davydenko et al., 2017). The reaction mixture contained, in a total volume of 15 µl, 200 µM deoxyribonucleotide triphosphates, 0.2 µM of each primer, 0.03 U/µl Thermo Green Taq polymerase with reaction buffer Green, and 2.75 mM final concentration of MgCl2. The thermal cycling was carried out using an Applied Biosystems GeneAmp PCR System 2700 thermal cycler (Foster City, CA, USA). An initial denaturation step at 95°C for 5 min was followed by 35 amplification cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. The thermal cycling was ended by a final extension step at 72°C for 7 min. The protein coding gene β-tubulin (partial gene) was also amplified using the primers Bt2a and Bt2b (Glass and Donaldson 1995). Amplification was performed in 15 µl of reaction mix (1x PCR Go Taq Flexi buffer, 2 mM MgCl2, 0.09 mM dNTPS, 0.66 µM each primer, 4% DMSO, 0.5 U of Taq polymerase and 1µl of template DNA). Thermal cycling condition were 2 min at 94°C followed by 15 cycles of 94°C for 30 s, 58°C for 45 s, and 72°C for 45 s, and further 20 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 45 s, with a final extension of 72°C for 5 min.

PCR products were size separated on 1% agarose gels and visualized under UV light. The PCR products were purified with Qiagen DNA extraction PCR M kit (Qiagen, Hilden, Germany). Sequencing was carried out by Macrogen Inc., Korea. Raw sequence data were analyzed using the SeqMan Pro version 10.0 software from DNASTAR package (DNASTAR, Madison, WI, USA). Databases at GenBank and at the Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, were used to determine the identity of ITS rRNA sequences. The criteria used for identification were: sequence coverage > 80%; similarity to taxon level 98-100%, similarity to genus level 92-97%.

Sequencing of fungi from the beetles. Isolation of DNA, amplification and sequencing of fungal ITS rRNA obtained directly from the beetles was carried out as described by Davydenko et al. (2014, 2017). PCR products were size separated on 1% agarose gels and visualized under UV light. Resulting single-band products were sequenced in both directions using the same primers as for PCR amplification. Sequencing and analyse of sequencing data were performed as described in section above.

Statistical analyses. A NCBI BLAST (National Centre for Biotechnology Information, www.ncbi.nlm.nih.gov) search was run with the edited sequences for preliminary species identification. Sequences were then aligned and visually inspected for the identification of polymorphic loci. A preliminary neighbor-joining tree was obtained with a Kimura 2-parameters substitution model, using only the β–tubulin partial gene sequences since they are known to better distinguish among Ophiostoma species than ITS region. Assessment of average evolutionary divergences over sequence pairs within and between groups defined by the tree were assessed for both ITS region and β-tubulin partial gene. All analyses were performed with MEGA 5.05 software.

Maximum likelihood (ML) analysis was conducted using the RAxML, and the 198 RAxML-HPC BlackBox was selected with default parameters, especially, in order to distinguish further *O. canum, O. canum*-like and *O. cf. canum,* and *O. piceae* complex with MEGA 5.05 software.

Richness of fungal taxa detected in beetles from sites was compared using chi-squared tests (Mead 2017). The relative abundance of fungal taxa was calculated from actual numbers of observations (presence/absence data) as the percentage of observations (isolates/ sequences) for the total fungal community. Shannon diversity indices and Chao indices were used to characterise the diversity and composition of fungal communities (Mead 2017). The Simpson diversity index was used to indicate dominance in fungal diversity to take into accounts both richness and evenness. Fungal dominance and most common species were determined by Camargo's index (Mead 2017). Statistical analyses were carried out using the software JMP®, Version 11.0.0. SAS Institute Inc., Cary, NC, 1989-2007.

Results and discussion. In total, 1681 isolates and amplicons representing 42 fungal taxa were detected when pooling the results from culturing and direct sequencing. The distribution of the fungal species according to site is given in Table 1. Of the all beetles of *I. acuminatus* used for fungal culturing, 272 individuals (94.4%) yielded fungal isolates and amplicons. So, most of all of the investigated beetles were associated with one, or more, species of fungi and 5.8 on average different fungal cultures and amplicons were obtained from each beetle.

The spectrum of fungi mainly consisted of ascomycetes (Tab. 1), but a few fungi from phylum Mucoromycotina and Basidiomycota were also identified.

The most abundant fungal phylum was Ascomycota for all sites accounting for an average of 80.85% of the total sequences, followed by Basidiomycota and unidentified fungal group, which accounted for 10.6 % and 8.5% of the total sequences, respectively (Fig. 2).

Pooling all data from isolation and sequencing, all high-quality sequences representing 94 distinct fungal taxa among which 56 (59.7%) were identified to taxon level, 28 (29.8%) to genus level and 10 (10.6%) remained unidentified (Table 1).

The most commonly detected fungi for pooling dataset were *Sphaeropsis sapinea* (23.6%), *Cladosporium pini-ponderosae* (19.44%), *Ophiostoma ips* (19.1%), *Ophiostoma canum* (19.1%) and *Cladobotryum mycophilum* (18.06%). The less abundant were *Ophiostoma bicolor* (15.97%),

Ophiostoma piceae (15.63%), *Graphilbum rectangulosporium* (13.89%), *Sydowia polyspora* (13.54%) and *Cyclaneusma niveum* (12.15%). The frequency of other species detected was lower than 11% (Tab. 1).

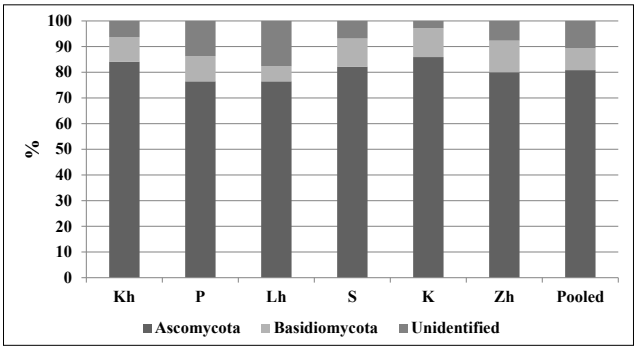

Fig. 2. Fungal phyla relative abundance associated with bark beetles. Sampling sites: Kharkiv (Kh), Luhansk (Lh), Poltava (P), Sumy (S), Kyiv (K) and Zhytomyr (Zh)

Chi-square test showed significant difference ($p > 0.001$) in richness of fungal taxa detected by the different sites (Fig. 3). Differences were observed in the abundance within some of the dominant groups, classified as the phylum and order. For the fungal communities (Fig. 3, Tab. 1), the relative abundances of the taxa Ascomycota and Basidiomycota were significantly lower ($p < 0.001$) in Luhansk and Poltava sites, and significantly increased ($p < 0.05$) in Kharkiv, Zhytomir, Kyiv and Sumy sites respectively reaching maximum value in Sumy region. At the phylum level, for the fungal communities the relative abundances of the dominant taxa *Cladosporium* sp., *Cyclaneusma sp., Grosmannia* sp., *Mucor* sp., *Ophiostoma* sp., *Penicillium* sp., *Sphaeropsis* sp., *Sydowia* sp. and Basidiomycota were significantly different ($p < 0.001$) between all sites, while the relative abundances of the taxa *Cladosporium* sp., *Graphilbum* sp*., Graphium* sp., *Lophodermium* sp., were significantly similar for all sites (data not shown).

Table 1

Pooled relative abundance of fungal taxa obtained from adults of *Ips acuminatus* collected on *Pinus sylvestris* grown in six regions in Ukraine

Fungal taxa	Kh	P	Lh	S	K	Zh	All samples
1	2	3	4	5	6	7	8
Ascomycota and other species							
Alternaria alternata (Fries) Keissler	0	2.08	0	14.58	4.17	6.25	5.21
Alternaria sp.	4.17	0	0	22.92	14.58	0	6.94
Anthostomella pinea Crous	2.08	0	0	12.50	10.42	4.17	4.86
Apiospora montagnei Sacc.	0	8.33	4.17	0	16.67	10.42	6.60
Aspergillus pseudoglaucus Blochwitz	0	10.42	0	16.67	12.50	12.50	8.68
Aspergillus versicolor (Vuill.) Tirab.	6.25	14.58	0	0	4.17	4.17	4.86
Beauveria bassiana (Balsamo-Crivelli) Vuillemin	10.42	16.67	2.08	25.00	12.50	0	11.11
Bionectria ochroleuca (Schwein.) Schroers & Samuels,	6.25	6.25	0	16.67	4.17	6.25	6.60
Bionectriaceae sp. Samuels & Rossman	0	0	0	10.42	6.25	4.17	3.47
Botryotinia fuckeliana (de Bary) Whetzel	6.25	6.25	0	4.17	2.08	8.33	4.51
Cadophora sp	0	0.00	4.17	12.50	10.42	10.42	6.25
Candida sp.	4.17	4.17	0	0	0	0	1.39
Chaetomium globosum Kunze ex Fries	0	0	2.08	25.00	0	0	4.51
Chaetomium sp. Kunze	8.33	0	4.17	0	6.25	0	3.13
Chalara sp.	4.17	4.17	0	12.50	4.17	6.25	5.21
Cladosporium sp. Link	14.58	10.42	0	4.17	10.42	10.42	8.33
Cladobotryum dendroides (Bull.) W. Gams & Hooz	2.08	25.00	0	10.42	4.17	25.00	11.11
Cladosporium herbarum (Pers.) Link	0	0	39.58	0	0	0	6.60
Cladobotryum mycophilum (Oudem.) W. Gams & Hooz	6.25	0	0	25.00	37.50	39.58	18.06
Cladosporium cladosporioides (Fresen.) G.A. de Vries	10.42	0	0	0	0	0	1.74
Cladosporium pini-ponderosae K. Schub	4.17	18.75	0	29.17	41.67	22.92	19.44

Table 1 continuation

1	2	3	4	5	6	7	8
Cordyceps sp.	4.17	0	0	12.50	12.50	10.42	6.60
Cyclaneusma niveum (Pers.) DiCosmo, Peredo & Minter	8.33	0	0	25.00	22.92	16.67	12.15
Cylindrocarpon sp	0	0	0	18.75	22.92	8.33	8.33
Dactylonectria macrodidyma (Halleen, Schroers & Crous) L. Lombard, van der Merwe & J.Z. Groenew. & Crous	0	4.17	0	10.42	6.25	0	3.47
Gibberella avenacea R.J. Cook	18.75	0	0	14.58	0	10.42	7.29
Graphilbum rectangulosporium (Ohtaka, Masuya & Yamaoka) Z.W. de Beer & M.J. Wingf.	8.33	10.42	4.17	18.75	22.92	18.75	13.89
Grosmannia olivacea (Math.-Käärik) Zipfel, Z.W. de Beer & M.J. Wingf.	18.75	0	0	10.42	18.75	6.25	9.03
Grosmannia sp.1	12.50	2.08	0	14.58	4.17	6.25	6.60
Eupenicillium sp.	0	10.42	4.17	4.17	10.42	16.67	7.64
Epicoccum nigrum Link	4.17	0	0	2.08	16.67	8.33	5.21
Fusarium oxysporum Schltdl	2.08	0	0	6.25	10.42	0	3.13
Fusarium sp	0	4.17	6.25	0	4.17	0	2.43
Geosmithia sp.1	0	10.42	2.08	16.67	6.25	6.25	6.94
Geosmithia sp.2	2.08	6.25	0	6.25	4.17	0	3.13
Graphium sp.	10.42	0	0	8.33	6.25	10.42	5.90
Ilyonectria radicicola	14.58	2.08	6.25	12.50	8.33	6.25	8.33
Isaria farinosa	4.17	2.08	0	10.42	4.17	0	3.47
Lophodermium seditiosum Minter, Staley & Millar	18.75	4.17	0	12.50	12.50	6.25	9.03
Mariannaea elegans (Corda) Samson	12.50	12.50	14.58	12.50	8.33	0	10.07
Metapochonia bulbilosa (W. Gams & Malla) Kepler & Humbe	8.33	0	4.17	18.75	4.17	0	5.90
Mortierella sp.	0	16.67	6.25	0	6.25	0	4.86
Mucor sp. P. Micheli ex L.	0	4.17	0	12.50	10.42	0	4.51
*Mucor fragil*is Bainier	18.75	2.08	0	4.17	8.33	4.17	6.25
Nakazawaea holstii (Wick.) Y. Yamada, K. Maeda & Mikata	4.17	6.25	6.25	2.08	10.42	0	4.86
Neocatenulostroma germanicum (Crous & U. Braun) Quaedvl. & Crous	14.58	0	0	8.33	12.50	0	5.90
Ogataea henricii (Wick.) Y. Yamada, K. Maeda & Mikata	0	0	4.17	0	4.17	2.08	1.74
Ogataea neopini Nagatsuka, S. Saito & Sugiyama	4.17	2.08	0	0	10.42	0	2.78
Ophiostoma bicolor R.W. Davidson & D.E. Wells	25.00	10.42	0	12.50	22.92	25.00	15.97
Ophiostoma canum (Münch) Syd. & P. Syd	22.92	4.17	10.42	22.92	25.00	29.17	19.10
Ophiostoma ips (Rumbold) Nannfeldt	29.17	12.50	16.67	12.50	20.83	22.92	19.10
Ophiostoma minus (Hedgc.) Syd. & P. Syd [d]	18.75	4.17	0	45.83	45.83	25.00	23.26
Ophiostoma pallidulum Linnak., Z.W. de Beer & M.J. Wingf.	0	18.75	0	0	4.17	29.17	8.68
Ophiostoma piceae (Münch) Sydow & P. Sydow	14.58	2.08	6.25	22.92	25.00	22.92	15.63
Ophiostoma sp.1	0	0	0	18.75	0	29.17	7.99
Ophiostoma sp.2	18.75	4.17	0	0	0	8.33	6.94
Penicillium citreonigrum Dierckx	0	4.17	0	0	25.00	0	4.86

Table 1 continuation

1	2	3	4	5	6	7	8
Penicillium roqueforti Thom	0	0	0	10.42	0	0	1.74
Penicillium sp. HK36 7	6.25	0	0	6.25	0	0	2.08
Penicillium sp. HK80 14	18.75	0	0	4.17	0	4.17	4.51
Penicillium sp. HK83 22	6.25	0	0	4.17	0	0	1.74
Pezicula eucrita (P. Karst.) P. Karst	0	0	4.17	8.33	6.25	12.50	5.21
Phoma macrostoma	0	0	0	22.92	4.17	4.17	5.21
Phomopsis sp. Sacc. & Roum	18.75	0	0	12.50	2.08	6.25	6.60
Pleosporales sp.	2.08	0	0	25.00	4.17	2.08	5.56
Rhizoctonia sp.	0	0	0	14.58	6.25	4.17	4.17
Saccharomycetaceae sp.	6.25	0	0	6.25	4.17	0	2.78
Sordariomycetes sp.	18.75	0	0	10.42	0	4.17	5.56
Sphaeropsis sapinea (Fr.) Dyko & B. Sutton [d]	12.50	22.92	18.75	47.92	0	39.58	23.61
Sydowia polyspora (Brefeld & Tavel) E. Müller	25.00	0	4.17	25.00	4.17	22.92	13.54
Talaromyces minioluteus (Dierckx) Samson, N. Yilmaz, Frisvad & Seifert	0	4.17	0	4.17	2.08	2.08	2.08
Talaromyces purpureogenus Samson, Yilmaz, Houbraken, Spierenb., Seifert, Peterson, Varga & Frisvad	0	2.08	0	8.33	0	2.08	2.08
Trichoderma asperellum Samuels, Lieckfeldt & Nirenberg	16.67	0	2.08	6.25	4.17	4.17	5.56
*Truncatell*a sp. Steyaert	4.17	0	4.17	0	2.08	2.08	2.08
Umbelopsis isabellina (Oudemans) W. Gams	0	0	2.08	0	4.17	4.17	1.74
Umbelopsis ramanniana (Möller) W. Gams 2003	6.25	0	6.25	0	6.25	2.08	3.47
Basidiomycota							
Bjerkandera adusta (Willdenow) P. Karsten	2.08	6.25	0	8.33	4.17	4.17	4.17
Cryptococcus sp. Kütz.	0	0	4.17	6.25	6.25	2.08	3.13
Entomocorticium sp. H.S. Whitney, Bandoni & Oberw [d]	8.33	6.25	4.17	6.25	2.08	12.50	6.60
Fomitopsis pinicola (Swartz) P. Karsten	6.25	0	0	4.17	4.17	4.17	3.13
Hebeloma sp. (Fr.) P. Kumm.	2.08	8.33	0	2.08	4.17	4.17	3.47
Hyphoderma setigerum (Fr.) Donk	4.17	10.42	0	4.17	4.17	2.08	4.17
Heterobasidion annosum (Fr.) Bref.	0	6.25	0	6.25	2.08	4.17	3.13
Phlebiopsis gigantea (Fr.) Jülich,	8.33	0	0	4.17	0	4.17	2.78
Unidentified fungi							
Uncultured Helotiales clone HH79	4.17	4.17	2.08	4.17	2.08	4.17	3.47
Uncultured Pezizales clone HG88	2.08	2.08	2.08	0	2.08	2.08	1.74
Unidentified Basidiomycota FG139	0	0	0	2.08	0	4.17	1.04
Unidentified Basidiomycota FG155	0	2.08	0	2.08	0	4.17	1.39
Fungal sp HH74 18	4.17	0	2.08	2.08	0	2.08	1.74
Fungal sp HH78 19	8.33	0	2.08	2.08	0	0	2.08
Fungal sp HK2 22	2.08	2.08	2.08	0	0	0	1.04
Fungal sp HD6 31	0	2.08	0	0	0	0	0.35
Unidentified culture Mucor-like M19	0	2.08	0	0	0	0	0.35
Unidentified culture Mucor-like M74	4.17	0	2.08	0	0	0	1.04

Sampling sites: Kharkiv (Kh), Luhansk (Lh), Poltava (P), Sumy (S), Kyiv (K) and Zhytomyr (Zh)

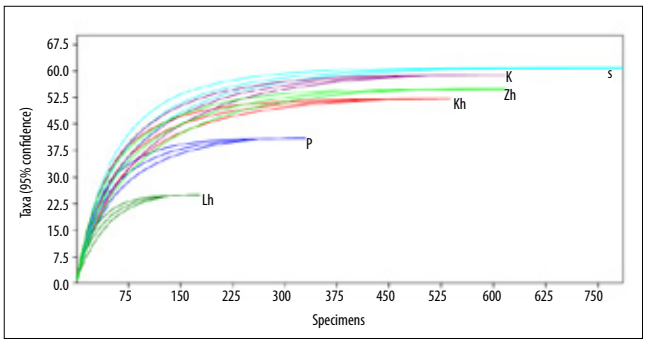

Fig. 3. Richness of observed fungi species for different sampling sites

The PCoA (principal coordinates analysis) based on Bray-Curtis distances revealed that the first principal component explained 99.10% of the variability in the data of the fungal communities (data not shown). Six different clusters were formed for all sites in the respect to the fungal community compositions, indicating the significant difference between fungal community associated with bark beetles.

In the pooled dataset of isolates and amplicons for each site, Shannon diversity indices ranged between 1.9 and 2.9 while Simpson diversity index varied between 0.69 and 0.89 (Fig. 4); Chao index is an estimator based on the abundance of species for each site referring to the abundance of individuals belonging to a certain class in a sample.

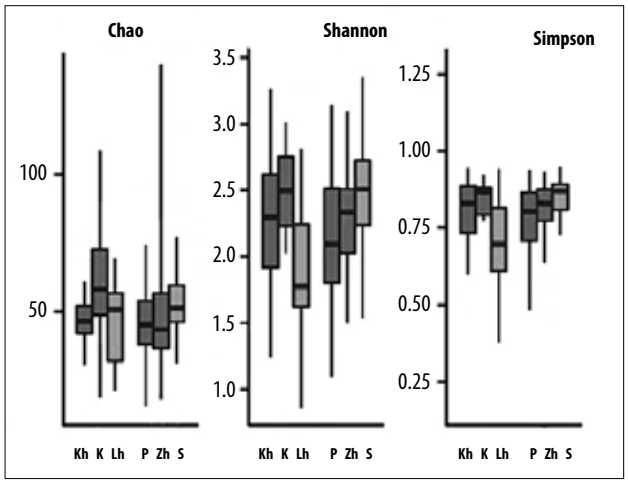

Fig. 4. Richness (Chao1 indices) and Diversity (Shannon and Simpson indices) for different sampling sites – Kharkiv (Kh), Luhansk (Lh), Poltava (P), Sumy (S), Kyiv (K) and Zhytomyr (Zh)

Thus, variation in proportional representation of Shannon and Simpson diversity indices significant taxa changes assigned to species and genus level was observed in this study indicating functional drivers for the difference among sites. It is speculated that the lower bark beetles associated fungal diversity at Luhansk and Poltava sites and the highest diversity at Sumy and Zhytomir ones are probably due to the fact that some microclimate factors as humidity or forest health condition play a more focussed role in fungal diversity between sites.

Opiostomatoid fungi. A total of 438 isolates of morphologically resembling fungi in the *Ophiostomatales* and *Microascales* were obtained from bark beetles from six localities (Table 2). The opiostomatoid group includes genera such as *Ophiostoma* Syd., *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr., *Graphilbum* H.P. Upadhyay & W.B. Kendr., Raffaelea Arx & Hennebert, and *Leptographium* Lagerb. & Melin in the *Ophiostomatales,* and *Ceratocystis* Ellis & Halst., Knoxdaviesia M.J. Wingf., P.S. van Wyk & Marasas and *Graphium* Corda in the *Microascales* (De Beer et al., 2013). The majority of obtained isolated were *Ophiostomatales* (96.1%) and a small number of isolates (3.9%) were from *Microascales* (Tab. 2).

In total twelve ophiostomatoid species were detected (Tab. 2). Within Ophiostoma s. l., the obtained isolates resided in eleven species complexes: *Graphilbum rectangulosporium, Grosmannia olivacea, Grosmannia* sp., *Ophiostoma bicolor, O. canum, O. ips, O. minus, O. pallidulum, Ophiostoma* sp. and one Graphium sp. belong to Microascales (Tab. 2). Analyses of the combined data set that included ITS–LSU, bS, EF-1a, revealed that the majority of isolates obtained in the present study, grouped in a strongly supported clade. Among all obtained isolates, 27 ones were identified as *O. canum* within the *O. piceae* complex, while twenty-four were identified as *O. ips*. The results of the ßT and EF 1-α sequences analysis confirmed that all the obtained isolates in these complexes belonged to *O. canum* and *O. piceae*. Additionally, based on the phylogenetic analysis of SSU ITS2-LSU βT, one isolate was identified as Ophiostoma sp. The comparisons of the SSU ITS2-LSU βT sequences obtained for the Graphium isolates showed identification on genus level.

Beetles collected in Luhansk were associated with four ophiostomatoid species and the frequency was low (rated from 4-16%). Bark beetles from Sumy, Zhytomir and Kyiv regions were associated with the highest number of fungal species from *Ophiostomatales* and *Microascales* group. For all sites, *O. minus* was the most common blue-stain species (23.3% of all bark beetles). *O. ips* and *O. canum* were also isolated at relatively high frequencies as well as *O.bicolor* and *O. picea* (19.1% and 15.97% respectively) from *I. acuminatus* from all sites, whereas rest of *Ophiostoma* and *Graphium* species occurred rarely among all beetles. Interestingly, that O. *ips* was relatively common species for Kharkiv and Luhansk sites (29.2% and 16.7% respectively), frequency of *O. minus* was the highest at Sumy and Kyiv regions (45.8%), while the three Ophiostoma species were the most common (frequency 29.2%) at Zhytomir region (Tab. 2).

Graphium sp. was isolated from specimen of *I. acuminatus* collected in five localities (except Luhansk). *Graphium pseudormiticum* was the closest match (94%) and *G. fimbriasporum* (Morelet) Jacobs, Kirisits & Wingfield (92%) according to the ITS-data. However, the both two different *Graphium* species seem to have different ecological niches associated with

spruce and larch in this study the fungus is presented as *Graphium* sp. because of a low identity score, with other sequenced species, in the GenBank.

Graphilbum rectangulosporium was isolated from 13.9% all individuals collected in all sampling sited as well as *Ophiostoma canum*, *O. ips* and *O. picea* were also found (Table 2). Most of the collected *I. acuminatus* were associated *Grosmannia* sp.1 and *O. pallidulum*. All detected opiostomatoid species were found only at Zhytomir sampling site while bark beetle collected from Kiev and Sumy region vectored 91.7% (11 out 12) opiostomatoid species. Less diversity in this group were found to be associated with *I. acuminatus* at Kharkiv and Poltava sites whereas only four *Ophiostoma* species were detected for bark beetles at Luhansk site.

Grosmannia olivacea was isolated from 10.3% of all collected bark beetles. ITS-data and β-tubulin-data gave 99% identity match on *O. olivaceum*. Following the new nomenclature, the species is renamed to *G. olivacea*. Reports of *G. olivacea* are very limited (Linnakoski, 2011). However, the fungus is known to be common associate of pine- and spruce infesting bark beetles, such as *Dryocoetes autographus*, *Hylastes cunicularius*, *I. sexdentatus* and *I. typographus* (Linnakoski, 2011). Moreover, *Grosmannia olivacea* along with ophiostomatoid species - *Ophiostoma ips*, *O. minus, O. pallidulum*, *O. piceae* and *G. cf. rectangulosporium* has already been reported to be associated with *I.acuminatus* in Ukraine (Davydenko et al., 2017) whereas *Graphium* sp., *Ophiostoma bicolor*, *O. canum*, *O. ips, O. piceae*, and *G. cf. rectangulosporium* were found to be in close interaction with *Hylurgus ligniperda* (Davydenko et al., 2014).

All ophiostomatoid fungi were tested to be more or less pathogenic to conifers and all detected species showed the ability infect seedlings of *P. sylvestris* with varying degree of virulence in precious studies. Moreover, *O. minus* caused dieback to *P. sylvestris* seedlings in previous studies (Jankowiak, 2013, Davydenko et al., 2017) whereas other ophiostomatoid species were capable to colonise sapwood and caused substantial blue-stain in the inoculation area resulting in decline of *P. sylvestris* seedlings, which was also reported previously (Jankowiak, 2013, Davydenko et al., 2017).

Table 2

Frequency of ophiostomatoid fungi isolated from *Ips acuminatus* collected in different sites

Ophiosomatoid species	Number of beetles (% frequency)					
	Kh	P	Lh	S	K	Zh
Graphilbum rectangulosporium (Ohtaka, Masuya & Yamaoka) Z.W. de Beer & M.J. Wingf.	8.33	10.42	4.17	14.58	22.92	18.75
Grosmannia olivacea (Math.-Käärik) Zipfel, Z.W. de Beer & M.J. Wingf.	18.75	-	-	18.75	18.75	6.25
Grosmannia sp.1	12.50	2.08	-	10.42	4.17	6.25
Graphium sp.	10.42	16.67	-	8.33	6.25	10.42
Ophiostoma bicolor R.W. Davidson & D.E. Wells	25.00	10.42	-	12.50	22.92	25.00
Ophiostoma canum (Münch) Syd. & P. Syd	22.92	4.17	10.42	22.92	25.00	29.17
Ophiostoma ips (Rumbold) Nannfeldt	29.17	12.50	16.67	12.50	20.83	22.92
Ophiostoma minus (Hedgc.) Syd. & P. Syd [d]	18.75	4.17	-	45.83	45.83	25.00
Ophiostoma pallidulum Linnak., Z.W. de Beer & M.J. Wingf.	-	10.75	-	-	4.17	29.17
Ophiostoma piceae (Münch) Sydow & P. Sydow	14.58	2.08	6.25	22.92	25.00	22.92
Ophiostoma sp.1	-	-	-	18.75	-	29.17
Ophiostoma sp.2	18.75	4.17	-	-	10.42	8.33

Sampling sites: Kharkiv (Kh), Luhansk (Lh), Poltava (P), Sumy (S), Kyiv (K) and Zhytomyr (Zh)

Pathogenic fungi. In the present study, different fungal pathogens were detected among which one non-ophiostomatoid species, *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton, was also frequently isolated in this study from all sites except Kyiv (Tab. 1). *S. sapinea* is pathogen of conifers causing Diplodia tip blight and stem canker disease across Europe (Oliva et al., 2013). In total, *S. sapinea* was isolated from 23.6% of the adults of *I. acuminatus* collected from five sampling sites. The presence of *S. sapinea* was not significantly affected by sampling site in which they were collected ($df = 3$, $\chi^2 = 46.389$, $p = 0.059$), although most specimens were found at Sumy site (23 out of 47.9%) followed by Zhytomir site (19 out of 48, 39.6%). In

the six different areas of the 200 trees were analyzed randomly to identify Sphaeropsis tip blight (data not shown), and the presence of *S. sapinea* was not significantly influenced by the forest health condition of sampling trees (df = 1, F = 3.579, p == 0.059) and infection rate. The rate of infection was significantly different among trees with/without bark beetle damage and most of the trees showing symptoms of *Sphaeropsis* tip blight does not damage by *I. acuminatus* (df = 1, X_2 = 5.361, p = 0.021), but nevertheless, the possibility should not be excluded that in addition to the negative effect of *I. acuminatus* and ophiostomatoid fungi, *S. sapinea* has also contributed to the pine decline as disease symptoms were often observed on Scots pine at the time of sampling on study sites (Davydenko et al., 2017).

Among other pathogens, some fungi cause needle disease were found in different extent *(Anthostomella pinea, Cyclaneusma niveum, Lophodermium seditiosum*) as well as fungi from genera *Alternaria, Chametomium, Fusarium, Phoma,* and *Rhizoctonia* are mainly known as generalist saprotrophs and/or facultative parasites (Davydenko et al., 2014, 2017).

A few Basidiomycota were also occasionally detected: wood-decay fungi (*Bjerkandera adusta, Fomitopsis pinicola* and *Heterobasidion annosum*), yeast Cryptococcus sp., mycorrhizal fungus *Hebeloma* sp., common saprophytic fungus that causes white rot of conifer logs and stumps and used as a biological control of annosum root rot (*Phlebiopsis gigantea*) and previously reported mycangial nutritional fungus Entomocorticium sp. (Davydenko et al., 2014, 2017) which of already mentioned in numerous studies as occasional occurrence of basidiomycetous fungi in bark beetles (Jankowiak, 2006, Persson et al., 2011, Davydenko et al., 2014, 2017).

Conclusion. General conclusions that can be taken from our study range different topics which go from the widely discussed role of associated fungi in bark beetles host establishment (Six, 2012, Villari, 2012), to the attempts in understanding ecology and population dynamics of a forest-damaging species as *Ips acuminatus*.

The investigated bark beetles, *I. acuminatus*, were associated with numerous of fungi species. Therefore, *I. acuminatus* is vector for species-rich fungal community, which was generally dominated by tree pathogens. Present study also demonstrated rich community of ophiostomatoid fungi associated with *I. acuminatus* in Ukraine. Such combination of drought, bark beetles and both blue-stain fungi and other pathogens is gaining importance regarding dieback of pines in Ukraine.

It appears that ophiostomatoid fungi are an important factor determining the aggressiveness of bark beetles (Krokene & Solheim 1998), demonstrating a significantly varying degree of virulence. Among ophiostomatoid fungi reported to be associated with bark beetles in Ukraine, *O. minus* has been the most virulent and causes dieback in seedlings of Scots pine. It is speculated that the associated ophiostomatoid fungi may play key roles in overcoming tree defense to facilitate the establishment of bark beetles. Nonetheless, the most important benefits that weal or non-pathogenic fungi may get from the association with bark beetle are transport, spread, and facilitation in entering the host tissues. In this case, bark beetle associated fungi are completely dependent on their vector for dissemination and thus adapted to insect dispersal which has been confirmed by many scientists

The association between non-ophiostomatoid pathogenic fungi as *Sphaeropsis sapinea, Cyclaneusma minus, Lophodermium seditiosum* and *I. acuminatus*, is of considerable importance to forest health and required further attention. Results of this study have shown though that associated fungal community (even weak or non-pathogenic fungi) are able to be as trigger decreasing tree defences comparable to the ones induced by a blue-stain fungus, and may thus participate in exhausting host plant defences. The combined performance of the tree pathogenic fungi and their insect vectors could also be behind the dieback of drought-stressed Scots pines in the study sites.

References

Alamouti, S. M., Kim, J. J., Humble, L. M., Uzunovic, A., & Breuil, C. (2007). Ophiostomatoid fungi associated with the northern spruce engraver, *Ips perturbatus*, in western Canada. *Antonie van Leeuwenhoek*, *91* (1), 19-34. https://doi.org/10.1007/s10482-006-9092-8

Colombari, F., Schroeder, M. L., Battisti, A., & Faccoli, M. (2013). Spatio-temporal dynamics of an *Ips acuminatus* outbreak and implications for management. *Agricultural and Forest Entomology*, *15*, 34-42. https://doi.org/10.1111/j.1461-9563.-2012.00589.x

Davydenko, K., Vasaitis, R., Meshkova, V., & Menkis, A. (2014). Fungi associated with the red-haired bark beetle, *Hylurgus ligniperda* (*Coleoptera: Curculionidae*) in the forest-steppe zone in eastern Ukraine. *European Journal of Entomology*, *111* (4), 561-565. https://doi.org/10.14411/eje.2014.070

Davydenko, K., Vasaitis, R., & Menkis, A. (2017). Fungi associated with *Ips acuminatus* (*Coleoptera: Curculionidae*) in Ukraine with a special emphasis on pathogenicity of ophiostomatoid species. *European Journal of Entomology*, *114*, 77-85. https://doi.org/10.14411/eje.2017.011

Glass, N. L., & Donaldson, G. C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol*., *61* (4), 1323-1330

Krokene, P., & Solheim, H. (1998). Pathogenicity of four blue-stain fungi associated with aggressive and nonaggressive bark beetles. *Phytopathology*, *88* (1), 39-44. https://doi.org/10.1094/PHYTO.1998.88.1.39

Linnakoski, R., De Beer, Z. W., Niemelä, P., & Wingfield, M. J. (2012). Associations of conifer-

infesting bark beetles and fungi in Fennoscandia. *Insects, 3* (1), 200-227. https://doi.org/10.3390/insects3010200

Mead, R. (2017). *Statistical methods in agriculture and experimental biology*. Chapman and Hall/CRC.

Meshkova, V. L., & Borysenko, O. I. (2017) GIS-based prediction of the foliage browsing insects' outbreaks in the pine stands of the SE «Kreminske FHE». *Proceedings of the Forestry Academy of Sciences of Ukraine*, *15*, 12-18. http://fasu.nltu.edu.ua ISSN 1991-606. https://doi.org/ 411714

Meshkova, V., Borysenko, O., & Pryhornytskyi, V. (2018). Forest site conditions and other features of Scots pine stands favorable for bark beetles. *Proceedings of the Forestry Academy of Sciences of Ukraine*, *16*, 106-114. https://doi.org/10.15421/4118

Siitonen, J. (2014). *Ips acuminatu* kills pine in souther Finland. *Silva Fennica 48* (4), article id 1145. 7 p. http://dx.doi.org/10.14214/sf.1145

Six, D. L. (2003). Bark Beetle-Fungus Symbioses. In Bourtzis, K., Miller, T. (Eds) *(*pp. 97-114)/ CRC Press: Boca Raton, FL, USA.

Six, D. L. (2012). Ecological and evolutionary determinants of bark beetle – fungus symbioses. *Insects*, *3* (1), 339-366. doi.org/10.3390/insects3010339

Villari, C. (2012). Fungi associated with the pine engraver beetle *Ips acuminatus* and their interactions with the host tree. (Ph.D. thesis, University pf Padova, Italy: Universita' degli studi di Padova) Retrieved from http://paduaresearch.cab.unipd.it/

Порівняльна характеристика угруповань грибів, пов'язаних із верхівковим короїдом (*Ips acuminatus*) у різних областях України

К. В. Давиденко[1]

В останнє десятиріччя все більше уваги приділяється грибам, які поширюються комахами та спричиняють синяву деревини й інші захворювання дерев (так звана група офіостомових грибів, куди входять види родів *Ceratocystis*, *Ceratocustiopsis*, *Ophiostoma*, *Grosmania*). Ці гриби утворюють ценотичні асоціації з багатьма видами короїдів, виконують різноманітні функції та займають різні екологічні ніші. На території України ця група грибів на хвойних породах практично не вивчена, особливо їхні фітопатогенні властивості, які можуть посилювати негативний вплив короїдів на дерева, викликаючи судинні захворювання, некрози та інші хвороби.

Тому метою дослідження було виявлення угруповань грибів, які переносить верхівковий короїд, а також визначення складу фітопатогенних грибів, асоційованих із *Ips acuminatus*, з метою подальшого складання достовірного прогнозу й оцінювання загрози пошкодження лісових насаджень комплексом «*верхівковий короїд – фітопатогенні гриби*».

Для проведення досліджень на ділянках соснових лісів, де виявлено осередки верхівкового короїда у шести областях України (Харківська, Полтавська, Луганська, Київська, Житомирська, Сумська), було зібрано 288 шт. особин *I. acuminatus* Для визначення всіх видів грибів, які переносить верхівковий короїд, паралельно використовували метод культивування грибів (на агаровому живильному середовищі) і ДНК аналіз послідовностей грибів за допомогою декількох пар праймерів для грибів.

У результаті здійсненого аналізу виявлено велику кількість ізолятів і ампліконів (1681 штамів і сіквенсів), які після обробки спеціальною програмою об'єднали у 42 родини грибів. За допомогою програмного забезпечення NCBI BLAST виявлено 94 види грибів, з яких 80,85 % становили *Ascomycota*. Решта видів представлена *Basidiomycota* та невизначеними видами, які становили 10,6 і 8,5 % відповідно. Серед них найчастіше виявляли *Sphaeropsis sapinea* (23,6 %), *Cladosporium pini-ponderosae* (19,44 %), *Ophiostoma Ips* (19,1 %), *Ophiostoma canum* (19,1 %) і *Cladobotryum mycophilum* (18,06 %). Під час аналізу угруповань грибів визначено, що за областями індекс різноманітності Шеннона становив від 1,9 до 2,9, а індекс різноманітності Сімпсона – від 0,69 до 0,89, що вказує на високу різноманітність видів і достовірну різницю видового складу мікобіоти окремих областей.

Серед збудників синяви виявлено дванадцять видів офіостомових грибів. За даними наших попередніх досліджень і європейських дослідників, всі визначені офіостомові гриби демонструють різну ступінь вірулентності стосовно сосни звичайної та інших хвойних. За нашими попередніми дослідженнями, вид *O. minus* виявив найбільшу агресивність, викликаючи всихання й відпад саджанців сосни звичайної. Також встановлено, що *I. acuminatus* переносить багато інших патогенів, окрім офіостомових грибів, а саме – *Sphaeropsis sapinea*, різні патогени хвої і дереворуйнівні гриби, що дуже важливо для подальшого оцінювання фізіологічної й технічної шкідливості *I. acuminatus* у соснових лісах України.

Ключові слова: сосна звичайна; короїди; *Ips acuminatus; Ophiostoma*; *Sphaeropsis sapinea*; фітопатогени.

[1] *Давиденко Катерина Валеріївна* – член-кореспондент Лісівничої академії наук України, кандидат сільськогосподарських наук, старший науковий співробітник, Український науково-дослідний інститут лісового господарства та агролісомеліорації ім. Г. М. Висоцького, вул. Пушкінська, 86, Харків, 61024, Україна; Запрошений науковий співробітник Департаменту Лісової мікології і фітопатології Шведського Аграрного Університету, P.O. Box 7026, SE-75007, Уппсала, Швеція. Тел.: +38-098 66 755 26. E-mail: kateryna.davydenko74@gmail.com ORCID http://orcid.org/0000-0001-6077-8533

Сравнительная характеристика грибных сообществ, связанных с вершинным короедом (*Ips acuminatus*) в различных областях Украины

Е. В. Давиденко[1]

В последнее десятилетие все больше внимания уделяется грибам, которые распространяются насекомыми и вызывают синеву древесины и другие заболевания (так называемая группа офиостомовых грибов, куда входят виды родов *Ceratocystis, Ceratocustiopsis, Ophiostoma, Grosmania)*. Эти грибы образуют ценотические ассоциации со многими видами короедов, выполняют разнообразные функции и занимают разные экологические ниши. На территории Украины данная группа грибов на хвойных породах практически не изучена, особенно их фитопатогенные свойства, которые могут усиливать негативное воздействие короедов на деревья, вызывая сосудистые заболевания, некрозы и другие болезни.

Целью работы было выявление видов грибов, которые переносит вершинный короед, определение состава фитопатогенных грибов, ассоциированных с *Ips acuminatus*, для составления в дальнейшем достоверного прогноза и оценки угрозы повреждения лесных насаждений комплексом «*вершинный короед – фитопатогенные грибы*».

Для проведения исследований на пробных площадях в сосновых лесах в шести областях Украины (Харьковская, Полтавская, Луганская, Киевская, Житомирская, Сумская), где обнаруживались очаги вершинного короеда, было собраны 288 особей *I. acuminatus*. Для определения всех видов грибов, которые переносит вершинный короед, параллельно использовали метод культивирования грибов (на агаровой питательной среде) и ДНК-анализ последовательностей грибов с помощью нескольких пар праймеров для грибов.

В результате проведенного анализа получено 1681 штаммов и сиквенсов, которые после обработки специальной программой объединили в 42 семейства грибов. Направленный поиск в базе данных (NCBI BLAST) выявил 94 вида грибов (на уровне вида и рода), из которых 80,85 % составляли *Ascomycota*; другие грибы были представлены грибами из группы *Basidiomycota* (10,6 %), а также неопределенными видами, которые составляли 8,5 %. Среди установленных видов наиболее часто встречались *Sphaeropsis sapinea* (23,6 %), *Cladosporium pini-ponderosae* (19,44 %), *Ophiostoma ips* (19,1 %), *Ophiostoma canum* (19,1 %) и *Cladobotryum mycophilum* (18,06 %). При анализе было обнаружено, что по областям индекс разнообразия Шеннона составлял от 1,9 до 2,9, индекс разнообразия Симпсона – от 0,69 до 0,89, что указывает на высокое разнообразие видов и достоверные различия видового состава микобиоты отдельных областей.

Среди возбудителей синевы обнаружено двенадцать видов офиостомовых грибов. По данным наших предыдущих исследований и европейских авторов все выявленные офиостомовые грибы демонстрируют разную степень вирулентности для сосны обыкновенной и других хвойных. По результатам наших предыдущих исследований, вид *O. minus* проявил наибольшую агрессивность, вызывая усыхание и гибель саженцев сосны обыкновенной. Также установлено, что *I. acuminatus* переносит многие другие патогены, кроме офиостомовых грибов, а именно – *Sphaeropsis sapinea*, различные патогены хвои и дереворазрушающие грибы, что очень важно для дальнейшей оценки физиологической и технической вредоносности *I. acuminatus* в сосновых лесах Украины.

Ключевые слова: сосна обыкновенная; короеды; *Ips acuminatus; Ophiostoma; Sphaeropsis sapinea*; фитопатогены.

[1] *Давиденко Екатерина Валерьевна* – член-корреспондент Лесной академии наук Украины, кандидат сельскохозяйственных наук, старший научный сотрудник, Украинский научно-исследовательский институт лесного хозяйства и агролесомелиорации им. Г. Н. Высоцкого, ул. Пушкинская, 86, Харьков, 61024, Украина; Приглашенный научный сотрудник Департамента Лесной микологии и фитопатологии Шведского Аграрного Университета, P.O. Box 7026, SE-75007, Уппсала, Швеция. Тел .: + 38-098 66 755 26. E-mail: kateryna.davydenko74@gmail.com ORCID http://orcid.org/0000-0001-6077-8533