ЗАХИСТ ЛІСУ

K. V. DAVYDENKO[1], D. O. BATURKIN[1,2]
PINE ENGRAVER BEETLE *IPS ACUMINATUS* AS A POTENTIAL VECTOR OF *SPHAEROPSIS SAPINEA*
[1]*Ukrainian Research Institute of Forestry and Forest Melioration named after G. M. Vysotsky*
[2]*State Forest Protection Service "Kharkivlisozahyst"*

The pine engraver beetle *Ips acuminatus* Gyll. is a potential vector of the Sphaeropsis tip blight pathogen according to Leach's postulates. The specimens of *I. acuminatus* were associated with numerous fungi species, namely *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton and ophiostomatoid species. The association between opportunistic pathogen *S. sapinea* and *I. acuminatus* has been confirmed for 62.9 % of all branches (44 % of needle samples and 82 % of wood samples). The presence of *S. sapinea* in the galleries and on the surface of the beetle indicates that *I. acuminatus* may transport the pathogen and later introduce it into healthy trees. The bark beetle can transfer pathogenic fungus during maturation feeding on the shoots of healthy pine crowns and into the branches during making galleries.
K e y   w o r d s : Sphaeropsis tip blight, insect-fungus interaction, Scots pine.

Introduction. Scots pine (*Pinus sylvestris* L) is the main forest-forming tree species in Ukraine. Pine forests occupy about 2.5 million ha of land in Ukraine or 34 percent of all forest-covered area in all climate zones: Forest, Forest-Steppe, and Steppe. Having the widest in Eurasia range of all conifers, the southern boundary of the Scots pine range passes through the territory of Ukraine. Scots pine is among the most important tree species in Ukrainian forests, and occupy vast areas of poor sandy soil and degraded habitats. This species is tolerant of poor soil, drought, wind, and frost (Houston Durant et al. 2016).

For many years, a gradual decline of Scots pine forests has been observed in the Ukrainian regions (Meshkova & Borysenko 2017). Recent studies have shown that drought-induced initial pine decline resulted in significant tree damage by pine bark beetles (Meshkova et al. 2018, Davydenko 2019). Among the bark beetles, a considerable economic damage is caused by the pine engraver beetle *Ips acuminatus* (Gyllenhal 1827) (Coleoptera: Scolytidae) and six-toothed bark beetle *Ips sexdentatus* (Börner 1767) (Coleoptera: Scolytidae) (Meshkova & Borysenko 2017, Davydenko 2019). The majority of bark beetles are well-known for developing long-term ecological and evolutionary relations of symbiosis with special fungi, so-called ophiostomatoid fungi (Six 2003, 2012, Linnakoski et al. 2012). However, bark beetles were revealed to be a vector of the vast number of other tree pathogens and therefore then can play a key role in the spread of different forest diseases, for instance, Pine pitch canker (Bezos et al. 2015), Dutch elm disease (Linnakoski et al. 2012), vascular wilt and vascular stain diseases (Linnakoski et al. 2012), increasing the aggressiveness and success of bark beetle attacks (Krokene & Solheim 1998). Many fungal species could be disseminated by insects, and entry into plant tissues is aided by insect damage (Agrios 1997). Most of the fungi involved are Ascomycetes. In some cases, the association involves the creation of infection through wound lesions, but more frequently, the insects are directly implicated in vectoring as the primary facilitator of spore transmission (Agrios 1997).

Sphaeropsis sapinea (Fr.) Dyko & B. Sutton (=*Diplodia pinea*) causes shoot blight, canker, collar rot, root disease, and blue stain of many pine species and conifers of various ages in ornamental plantations, and natural stands in different continents (Oblinger et al. 2013, Davydenko 2018). The pathogen was found in Ukraine for the first time in the 1990s but no records of an outbreak or massive decline caused by *S. sapinea* have been observed until 2010 (Meshkova et al. 2012). *S. sapinea* has been registered in many areas in Europe, but the data on the first records in Ukraine are controversial. Probably, most results of *S. sapinea* findings have not been reported in the available literature, or identification was made only to the genus level based on symptoms (Sphaeropsis sapinea 2020). Generally, a southern fungus moves north during drought periods: it is known that *S. sapinea* can be released from its latent stage by host water stress (Stanosz et al. 2007,

Hanso & Drenkhan 2009). Unfortunately, no data about the spread of *S. sapinea* in Ukraine are known, excluding some regions in the Left-Bank Ukraine (Davydenko 2018, Meshkova et al. 2012). Moreover, this pathogen was found to be in close relation with *Ips acuminatus* (Davydenko et al. 2017). Previously, associations between *S. sapinea* and other bark beetles, namely *Tomicus piniperda*, *Hylastes attenuates*, *Hylurgops palliates,* have been reported from northern Spain (Bezos et al. 2015). Furthermore, an association between the exotic insect *Leptoglossus occidentalis* and *S. sapinea* has been revealed in Italy on pine cones (Luchi et al. 2012).

The etiologies of bark beetle and associated tree diseases are usually identified as they become apparent. To a great extent, that is due to the fact that causal agents are often easy to isolate and the completion of Koch's postulates is straightforward in practice (Ploetz et al. 2013). However, the regular incidence of secondary and other additional beetle species in damaged trees, which play no role in the development of tree diseases, makes it unclear about the roles of the respective beetle in tree disease transmission (Ploetz et al. 2013). Leach (1940) reviewed insects which vector plant pathogens and developed four rules to confirm that an insect is the vector of a respective pathogen. He pointed out that it is necessary to demonstrate four postulates: (1) a close association of the insect with diseased plants; (2) regular visits of healthy plants by the insect; (3) an association of the pathogen with the insect; and (4) the development of the disease in healthy plants after interaction with pathogen-infected insects. Therefore, our study also provides evidence that *S. sapinea* is commonly associated with *I. acuminatus* in Ukraine, contributing to the decline and dieback of *P. sylvestris*.

The aim of the study was to determine whether the pine engraver beetle, *Ips acuminatus*, is a vector for the pathogen *S. sapinea*. To confirm this, Leach's postulates (Leach 1940, Bezos et al. 2015) were tested: (1) a close, although not constant, the association between *I. acuminatus* and trees affected by Diplodia tip blight; (2) regular visit by *I. acuminatus* to healthy Scots pine forest; (3) the presence of the pathogen on the insect in nature; and (4) whether *I. acuminatus* can successfully vector the pathogen to disease-free host material under controlled conditions.

Materials and Methods. The field study was carried out in 2016. Association between bark beetles and healthy/infested crowns of pine trees has been studied by different authors (Ploetz et al. 2013, Bezos et al. 2015, Lieutier et al. 2015) and different methods were used for testing Leach's postulates (Bezos et al. 2015). In the present study, we tried to demonstrate the capability of *I. acuminatus* to infest symptomless green crowns of *P. sylvestris* in plots affected by *S. sapinea*. To confirm an association between *I. acuminatus* and diseased trees (postulate 1), an inspection of cut Scots pine trees attacked by *I. acuminatus* was carried out to find trees infested by bark beetles and *S. sapinea*. Field study sites were pure pine forest stands located in Sumy Region in Ukraine (Ohtyrske Forest Enterprise). To make sure that *S. sapinea* is present in sites, visual symptomatic branches have been collected (Figure 1).

**Fig. 1 – Pycnidia of *S. sapinea* on cones (left) and needles (right) and typical damage (in the middle)
of the host *(Pinus sylvestris)***

The stands at all sites were ca. 60–80-year-old plantations of Scots pine (*Pinus sylvestris*) where *S. sapinea* was found according to morphological symptoms using wet chamber and light microscopy (Figure 2).
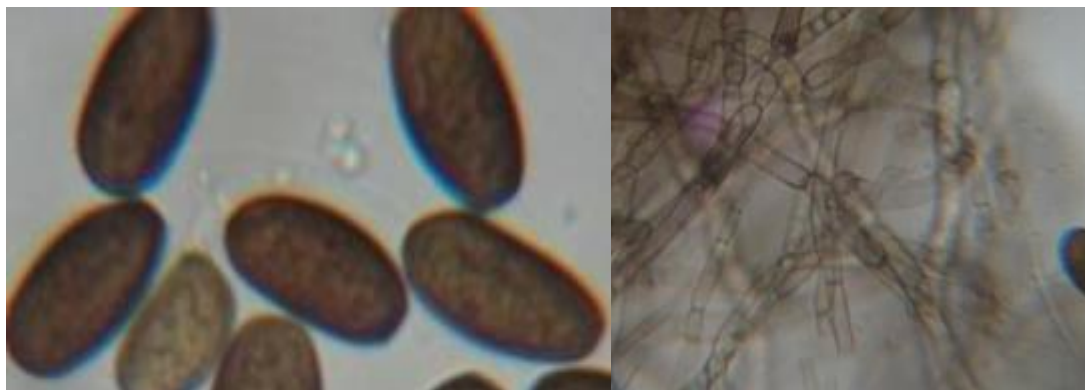
Fig. 2 – Mature conidia (left) and branched mycelium (right) of *S. sapinea* (400 ×)

Simultaneously, examination and sampling of Scots pine trees attacked by *I. acuminatus* were carried out in disease-free stands (postulate 2). To determine whether *I. acuminatus* regularly visits healthy Scots pines, also fallen branches and shoots of *P. sylvestris* affected by pine engraver beetle were collected and analysed for the presence of the pathogen.

Adults of *I. acuminatus* were collected individually from infested trees of *P. sylvestris* from all sites (disease-free and with the presence of *S. sapinea*) and stored singly until analyses for associated fungal pathogens (postulate 3). For this, 192 individuals from four sites were collected randomly and analysed.

Fungal culturing and molecular identification. To determine whether the pathogen occurs on the insects in nature (postulate 3), sampled adults of *I. acuminatus* were checked for the pathogen presence. Samples for fungal isolation were placed on 2 % malt extract agar (MEA, Difco, BD, Franklin Lakes, NJ, USA) containing 200 ppm of cycloheximide and 300 ppm of streptomycin (Sigma-Aldrich), to be selective for *Diplodia* and *Ophiostoma* species and avoid the growth of bacterial isolates and fast-growing fungi such 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 for 10–12 days and grouped according to the morphological characteristics of colonies and conidiophores, and single spore cultures were prepared from germinating conidia of isolates representing morphological groups of different sites (Linnakoski et al. 2012).

The morphological identification of *S. sapinea* was based on the 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). The presence of *S. sapinea* was verified using the specific primer pairs DpF and BotR described by Stanosz et al. (2007). PCR was performed in a final volume of 50 µl. Each tube contained: 0.8 lM forward primer (Sigma-Aldrich, Schnelldorf, Germany); 0.8 lM reverse primer (Sigma-Aldrich); 12.5 µl TaqManTM AmpliTaq Gold PCR Master Mix (Applied Biosystems, California, USA); 5–10 ng fungal DNA. Each DNA sample was assayed in duplicate. Negative controls (sterile water) and DNA from reference strain were included in all reactions. A Biometra T1 Thermocycler (Whatman Biometra, Gottingen, Germany) was used for the PCR with the following cycler protocol: 95°C for 5 min, 30 cycles of 95°C for 1 min, 53°C for 1 min and 72°C for 1 min with a final extension of 72°C for 5 min. PCR fragments were analysed by agarose gel electrophoresis with 0.7 g in 100 ml 1 9 Tris-boric acid-EDTA buffer (TBE) and visualized by SYBR Safe (Life Technologies, Milan, Italy) staining.

Vector tests. To confirm the vector of the pathogen by *I. acuminatus,* the disease was produced experimentally under controlled conditions (postulate 4) on healthy shoots which were attacked by artificially inoculated specimens of *I. acuminatus*. For this, ten adults of *I. acuminatus* insects were inoculated with a conidial suspension of *S. sapinea*. Each insect was inoculated with 50 µl of the suspension by micropipette. The suspension was obtained from a pure culture of *S. sapinea* growing on MEA and forming typical spores. Scots pine branches (5–7) with diameters 13–18 mm were put in glass receptacles with water to avoid the desiccation. These branches were checked preliminary for disease-free (*S. sapinea*) by molecular methods to be sure that latent infection of *S. sapinea* absents (Davydenko 2018). Branches together with inoculated specimens of *I. acuminatus* were placed onto plastic containers for maturation feeding and colonization for 45 days. Afterward, all branches were visually checked to find symptoms of Spaheropsis shoot blight (SSB). Moreover, 3–5 pieces of wood tissue ca. 1 cm length and 3–5 needles from each shoot with visible entry holes and maturation feeding were removed and plated on MEA containing antibiotics in order to re-isolate *S. sapinea* using classical phytopathological methods (Davydenko 2018).

Statistical analyses. All data were tested for adherence to the normal distribution using the Kolmogorov – Smirnov test. The differences between the insect and wood samples in relation to the presence/absence of *S. sapinea* were analysed by Fisher's exact test and by the analysis of variance (ANOVA) followed by Tukey's HSD post hoc test. The significance was evaluated at the 0.05 *p*-level. Statistical analysis was carried out using the statistical software package PAST: Paleontological Statistics Software Package for Education and Data Analysis (Hammer et al. 2001).

Results and Discussion. *Postulate 1&2: the association between I. acuminatus and P. sylvestris in both infested by S. sapinea and disease-free sites.*

In general, 120 samples of branch, shoots and needles of Scots pine attacked by *I. acuminatus* were collected from 12 randomly selected trees at four sites. A sampling of needles and wood resulted in 197 morphological groups of fungal cultures. Molecular analyses of fungal morphological groups using *S. sapinea* specific primers demonstrated the absence of *S. sapinea* in free-disease sites attacked by *I. acuminatus* (Figure 3).
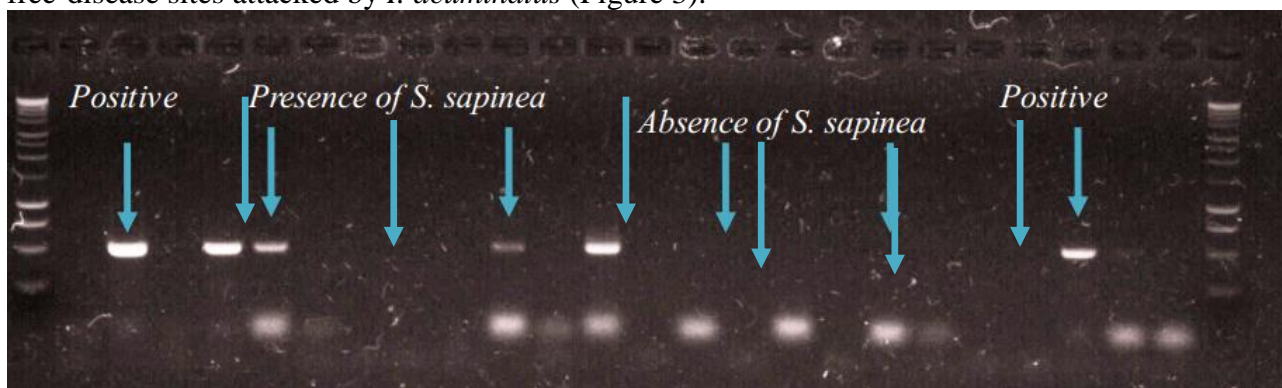

Fig. 3 – UV visualization of PCR on agarose gel electrophoresis (staining 1% TBE buffer)

All shoot samples in disease-free areas where *I. acuminatus* was present demonstrated the absence of *S. sapinea* infection (Table 1).

However, two from 30 needle samples collected from Site 2 disease-free demonstrated the presence of *S. sapinea,* probably in the latent stage, because no symptoms of SSB has been observed. However, Z-test showed that a single case is not significantly different from the sample (Z = -1.6202, *p*-value (two-tailed) = 0.10519), so we can assume that postulate 1 has been proved. All symptomatic samples of shoots and needle collected at sites 3 and 4 showed the presence of SSB pathogen (Table 1). *F*-test showed a significant difference between presence/absence of SSB at diseases-free and disease-presence sites (F = 72.25, *df* = 15, *p*-value =1.744E-09), that is a crucial proof for postulate 2. Furthermore, *F*-test illustrates a significant difference (F = 36, *df* = 7, *p*-value = 0.0009645) between the infection rate into needles and wood (shoots), but probably, this does not prove the general applicability of results to spread the infection. We consider that different

infection rates could be explained by the various qualities of samples for DNA extraction and using species-specific primers. Therefore, our results only reaffirmed the possibility for *I. acuminatus* to visit both infested and not infested by SSB areas (postulate 1 and 2).

<div align="right">*Table 1*</div>

**Frequency of positive for *S. sapinea* samples collected in Sumy Region
in both disease-free and SSB infested sites**

Site	Forestry, compartment (subcompartment)	Frequency of samples with *S. sapinea*, %			
		Plot 1		Plot 2	
		needles	shoots	needles	shoots
Site 1 disease-free	Ohtyrske, 15 (1)	0.00	0.00	0.00	0.00
Site 2 disease-free	Huhryanske, 96 (2)	6.67	0.00	0.00	0.00
Site 3 infested by *S. sapinea*	Ohtyrske, 30 (16)	60.00	33.33	83.33	26.67
Site 4 infested by *S. sapinea*	Huhryanske, 104 (1)	43.33	23.33	76.67	36.67

Postulate 3 association of the pathogen with the insect: Samples of specimens of *I. acuminatus* and shoots with signs of maturation feeding or breeding galleries were analysed aiming to identify fungal phytopathogens, in particular ophiostomatoid fungi and *S. sapinea*. The most abundant fungal phylum was Ascomycota for all samples accounting for an average of 87.8% of the total species. The most commonly detected fungi from pure culture from the insects were *Sphaeropsis sapinea* (39.58%) and ophiostomatoid fungi (Table 2).

<div align="right">*Table 2*</div>

Relative abundance of main fungal taxa obtained from adults of *Ips acuminatus* and breeding galleries collected on *Pinus sylvestris* in Sumy Region (Ukraine)

Species	SSB-free		Infested by SSB	
	Insects	Galleries	Insects	Galleries
Ophiostomatoid species:				
Ophiostoma piceae (Münch) Sydow & P. Sydow	14.58	16.67	14.58	16.67
Ophiostoma ips (Rumbold) Nannfeldt	10.42	12.5	10.42	12.5
Ophiostoma minus (Hedgc.) Syd. & P. Syd	14.58	18.75	14.58	18.75
Ophiostoma sp.1	8.33	6.25	8.33	6.25
Ophiostoma sp.2	22.92	10.42	22.92	10.42
Ophiostoma sp.3	22.92	0	22.92	0
Other pathogens:				
Sphaeropsis sapinea (Fr.) Dyko & B. Sutton	6.3	0	39.58	16.67
Lophodermium sp.	0	0	4.17	0
Other fungi:				
Mucoromycotina	8.3	6.3	18.75	4.17
Basidiomycota	8.3	4.2	4.17	6.25
Unidentified ascomycetes	14.6	10.4	6.25	8.33
Unidentified species	10.4	0	4.17	2.08

A total of 96 breeding galleries were sampled from symptomatic trees, and 16.67 % of them gave rise to *S. sapinea* colonies when plated on MEAs. The *S. sapinea* pycnidia were also observed on samples placed in wet chambers. Our study demonstrated that *S. sapinea*was found to be associated with *I. acuminatus* (Table 2) that has already been confirmed by our previous study (Davydenko et al. 2017, Davydenko 2019). A few specimens of *I. acuminatus* collected in disease-free sites were identified to be associated with *S. sapinea* (6.3% of all beetles). No *S. sapinea* was

found in the galleries of *I. acuminatus* in disease-free trees. Other pathogens such as *Lophodermium* species were found to be associated with *I. acuminatus,* that confirmed our previous results (Davydenko et al. 2017, Davydenko 2018, 2019).

Postulate 4: Vector test under control condition. To confirm that the bark beetles of *I. acuminatus* can vector pathogen *S. sapinea* during maturation feeding or making breeding galleries into branches with *I. acuminatus,* the disease was produced experimentally under controlled conditions. Individuals of bark beetles were collected from dying Scots pine trees infested by bark beetles (Ohtyrske Forest Enterprise). Our study showed that the most common species was *Ips acuminatus* (82.8% of all samples), while 17.3% were *Tomicus piniperda* and *Ips sexdentatus* that colonize the lower part of the stem with thick bark; only a few specimens of *Tomicus minor* were found which colonize the upper part of the stem with thin bark, that had already been revealed in the previous study (Meshkova & Zinchenko 2013).

Scots pine branches were cut from disease-free trees and checked preliminary by molecular analyses with Diplodia-specific primers randomly. SSB-free Scots pine branches (5–7) with diameters 13, 15, and 18 mm together with inoculated by *S. sapinea* specimens of *I. acuminatus* were placed into plastic containers. Results of check of branch samples in the presence/absence of *S. sapinea* demonstrate the capacity of *I. acuminatus* to vector *S. sapinea* (Figure 4) as well as other fungal pathogens, e.g. ophiostomatoid fungi (Davydenko et al. 2017, Davydenko 2019).
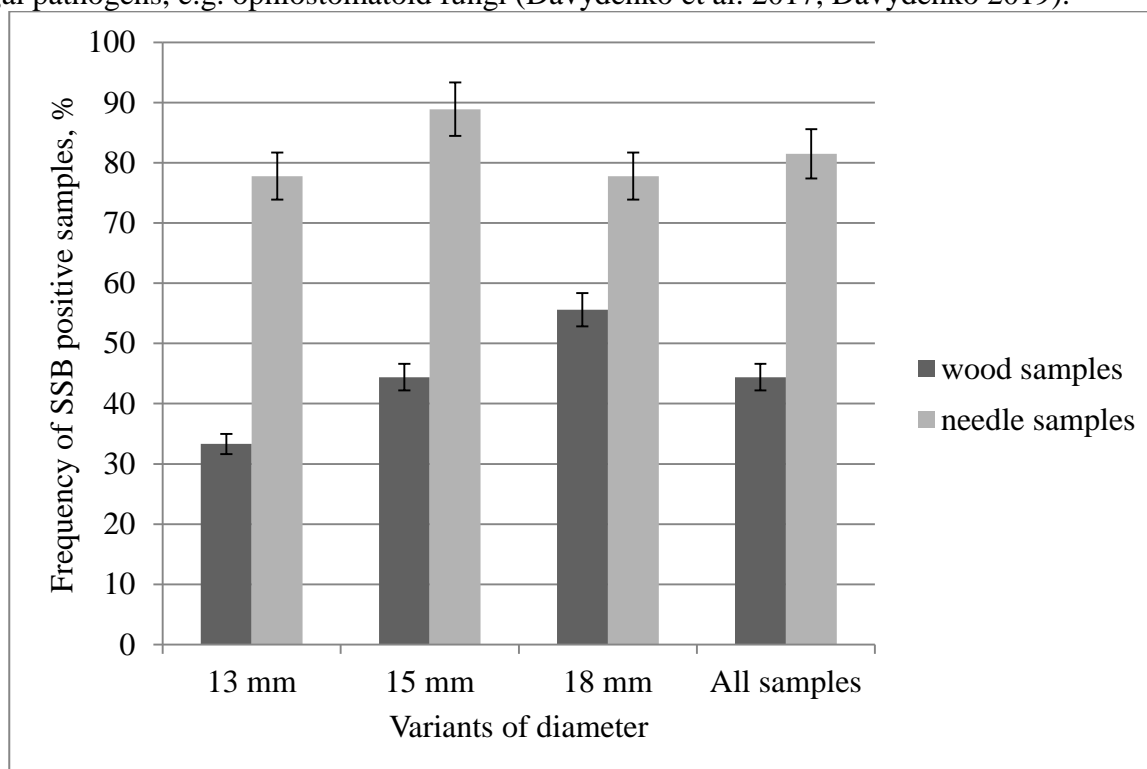

Fig. 4 – Frequency of needle and wood samples indicating presence of Spaheropsis shoot blight (branches diameter 13, 15 and 18 mm)

In general, 62.9% of all branches (44% of needle samples and 82% of wood samples) showed the presence of *S. sapinea*, while any confirmation of the *S. sapinea* presence in control samples was not found and all groups showed a significant difference comparing with control one ($\chi^2_f = 9.49$, *p*-value is 0.0132 Chi-square at Cochran – Mantel – Haenszel test).

According to SSB data for different branch diameter, the chi-squared test statistic is 1.20 with an associated $p < 0.1371$, so the null hypothesis is not rejected, since $p > 0.05$, and a conclusion is made that branch diameter is not associated with SSB infection. As no data from the Chi-square test indicate that the probabilities of the two variables are related, we cannot consider a relationship between variables.

Therefore, our results confirm postulate 4 which indicates the development of the disease in healthy plants after their interaction with pathogen-infested insects. Undoubtedly, not all the samples with *S. sapinea* showed SSB typical symptoms. However, this could be explained by the existence of the latent phase of *S. sapinea* that has been confirmed by various authors worldwide (Davydenko 2019), so the development of typical symptoms could slow down.

The association between *I. acuminatus* and *P. sylvestris* trees affected by *S. sapinea* was observed during field sampling in 2015–2016 and during other field studies. Bark beetles and breeding galleries collected from symptomatic trees were positive for *S. sapinea*. This may indicate that the larvaes/beetles were already infected by SSB when making a gallery or that the crown and bark was already infected with *S. sapinea*. The probability of *I. acuminatus* being contaminated with the pathogen would be increased by the insects excavating their breeding galleries in diseased trees (Bezos et al. 2015, Davydenko et al. 2017).

Conclusion. Our study confirmed that *Ips acuminatus* is probably a vector of *Sphaeropsis sapinea*, according to Leach's postulates.

Our study demonstrates that specimens of *I. acuminatus* were associated with numerous fungi species, which were generally dominated by tree pathogens, namely *Sphaeropsis sapinea* and ophiostomatoid species. The association between opportunistic pathogen *S. sapinea* and *I. acuminatus* is of considerable importance to forest health, particular to drought-stressed Scots pines. The presence of *S. sapinea* in the galleries and on the surface of the beetle indicates that *I. acuminatus* may transport the pathogen and later introduce it into healthy trees. Moreover, the shoots are most likely to become infected with the pathogen during maturation feeding. However, further studies are required for a better understanding of the relationship between the life cycles of the *I. acuminatus* and the *S. sapinea*. For this tree disease, improved detection, prediction, and management should be the major goals of future research.

REFERENCES

Agrios, G. N. 1997. Plant pathology. 4th ed. San Diego, Academic Press, 635 p.

Bezos, D., Martínez-Álvarez, P., Diez, J. J. & Fernández, M. M. 2015. The pine shoot beetle *Tomicus piniperda* as a plausible vector of *Fusarium circinatum* in northern Spain. Annals of Forest Science, 72(8): 1079–1088.

Davydenko, K. V. 2018. Sphaeropsis shoot blight in pine plantations under stress conditions. Proceedings of the KhNAU: Phytopathology and entomology, (1-2): 29–36.

Davydenko, K. 2019. A comparative characteristic of fungal communities associated with *Ips acuminatus* in different regions of Ukraine. Proceedings of the Forestry Academy of Sciences of Ukraine, (18): 118–128.

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.

Hammer, O., Harper, D. A. T., Ryan, P. D. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4: 1–9.

Hanso, M. and Drenkhan, R. 2009. *Diplodia pinea* is a new pathogen on Austrian pine (*Pinusnigra*) in Estonia. Plant Pathology, 58 (4): 797–797.

Houston Durant, T., de Rigo, D., Caudullo, G. 2016. *Pinus sylvestris* in Europe: Distribution, habitat, usage and threats. In: San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., & Mauri, A. (Eds.). European atlas of forest tree species. Publication Office of the European Union, Luxemburg, e016b94, p. 132–135.

Krokene, P. and Solheim, H. 1998. Pathogenicity of four blue-stain fungi associated with aggressive and nonaggressive bark beetles. Phytopathology, 88(1): 39–44.

Leach, J. G. 1940. Insect Transmission of Plant Diseases. New York, McGraw-Hill Book Co., Inc., 615 p.

Lieutier, F., Långström, B., Faccoli, M. 2015. The genus *Tomicus*. In: Bark Beetles. Academic Press, p. 371–426.

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.

Luchi, N., Mancini, V., Feducci, M., Santini, A., Capretti, P. 2012. *Leptoglossus occidentalis* and *Diplodia pinea*: a new insect-fungus association in Mediterranean forests. Forest Pathology, 42(3): 246–251.

Meshkova, V. L. and 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.

Meshkova, V. L. and Zinchenko, O. V. 2013. Colonization by stem insects of the pine stands weakened by different causes. The Bulletin of Kharkiv National Agrarian University. Series: Phytopathology and Entomology, (10): 126–131 (in Ukrainian).

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.

Meshkova, V. L., Davydenko, K. V., Tovstukha O. V. 2012. Diplodia blight in pine plantations in Sumy Region. In: Proceedings of Scientific Conference of the Teaching Staff, Scientists, and Post-Graduate Students of the Kharkiv National Agrarian University named after V. V. Dokuchayev. (10–13 January, 2012). Ch. 1. Kharkiv, KhNAU, p. 257–259 (in Ukrainian).

Oblinger, B. W., Smith, D. R., Stanosz, G. R. 2013. Diplodia shoot blight damage to understory red pine seedlings. Northern Journal of Applied Forestry, 30(1): 23–27.

Ploetz, R. C., Hulcr, J., Wingfield, M. J., De Beer, Z. W. 2013. Destructive tree diseases associated with ambrosia and bark beetles: black swan events in tree pathology? Plant Disease, 97(7): 856–872.

Stanosz, G. R., Smith, D. R., Leisso, R. 2007. Diplodia shoot blight and asymptomatic persistence of *Diplodia pinea* on or in stems of jack pine nursery seedlings. Forest Pathology, 37(3): 145–154.

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

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

Sphaeropsis sapinea (Sphaeropsis blight). 2020. [Electronic resource]. Invasive Species Compendium. CABI. Available from: https://www.cabi.org/isc/datasheet/19160 (last accessed date 19.05.2020).

Давиденко К. В.[1], Батуркін Д. О.[1,2]

ВЕРХІВКОВИЙ КОРОЇД *IPS ACUMINATUS* ЯК ПОТЕНЦІЙНИЙ ПЕРЕНОСНИК ПАТОГЕНА *SPHAEROPSIS SAPINEA*

[1]*Український науково-дослідний інститут лісового господарства та агролісомеліорації ім. Г. М. Висоцького*

[2]*Державне спеціалізоване лісозахисне підприємство «Харківлісозахист»*

Визначено згідно з постулатами Ліча, що верхівковий короїд *Ips acuminatus* здатний бути переносником патогена *Sphaeropsis sapinea,*. Зібрані жуки *I. acuminatus* проаналізовано за допомогою молекулярних методів. Виявлено зв'язок жуків із численними видами грибів, зокрема патогенних, а саме *Sphaeropsis sapinea* та групою офіостомових грибів. Підтверджено всі чотири постулати Ліча, що ідентифікують перенесення збудника діплодіозу. Перенесення гриба *Sphaeropsis sapinea* за допомогою *I. acuminatus* підтверджено експериментально для 62.9 % усіх гілок (44% проб хвої та 82 % проб деревини). Наявність *S. sapinea* в ходах та на поверхні тіла жука *I. acuminatus* свідчить, що він може переносити збудника діплодіозу та пізніше інфікувати ним здорові дерева під час додаткового живлення на пагонах крон сосни та під час заселення гілок і стовбура.

К л ю ч о в і с л о в а : діплодіоз, , взаємодія грибів і комах, сосна звичайна,.

Давиденко К. В.[1], Батуркин Д. А.[1,2]

ВЕРШИННЫЙ КОРОЕД *IPS ACUMINATUS* КАК ПОТЕНЦИАЛЬНЫЙ ПЕРЕНОСЧИК ПАТОГЕНА *SPHAEROPSIS SAPINEA*

[1]*Украинский научно-исследовательский институт лесного хозяйства и агролесомелиорации им. Г. Н. Высоцкого*

[2]*Государственное специализированное лесозащитное предприятие «Харьковлесозащита»*

По результатам исследований согласно постулатам Лича определено, что вершинный короед *Ips acuminatus* способен быть потенциальным переносчиком патогена *Sphaeropsis sapinea*. Собранные жуки *I. acuminatus* проанализированы с помощью молекулярных методов. Установлена прямая связь жуков с многочисленными видами грибов, в т. ч. патогенными, а именно *Sphaeropsis sapinea* и группой офиостомовых грибов. Подтверждены все четыре постулата Лича, идентифицирующие перенос возбудителя диплодиоза. Перенос гриба *Sphaeropsis sapinea* с помощью *I. acuminatus* подтвержден экспериментально для 62,9% веток (44 % проб хвои и 82 % проб древесины). Наличие *S. sapinea* в ходах и на поверхности тела жука свидетельствует о том, что *I. acuminatus* может переносить возбудителя диплодиоза и позже инфицировать им здоровые деревья во время дополнительного питания на побегах крон сосны и во время заселения ветвей и ствола.

К л ю ч е в ы е с л о в а : диплодиоз, , взаимодействие грибов и насекомых, сосна обыкновенная.

E-mail: kateryna.davydenko74@gmail.com; baturkin.denis@ukr.net