Cent. Eur. For. J. 66 (2020) 49–59 sciendo DOI: 10.2478/forj-2020-0001 CENTRAL EUROPEAN Forestry Journal
http://www.nlcsk.sk/fj/

ORIGINAL PAPER

Beetles and nematodes associated with wither Scots pines

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Abstract
This study focused on the occurrence of xylophagous beetles and nematodes in the different parts of *Pinus sylvestris* L. trees of different health condition in the pure stands in Zhytomyr region (Central Polissya). Stem fragments with thin, thick and transitional bark, branches and twigs were examined in each of 12 model trees. Xylophagous beetles were identified by adults or by galleries. Nematodes were isolated from wood samples in the laboratory using the Baermann method and identified by morphometric characteristics. Among 10 species of xylophagous beetles, *Ips acuminatus* (frequency 16.7%; dominance 17.9%) and *I. sexdentatus* (frequency 11.1%, dominance 54.6%) dominated, which prefer the fragments with thin and thick bark respectively. No xylophagous beetle was found in the healthy and slightly weakened trees. Among 15 nematode species, 40% were saproxylic, 33.3% entomophilic, 13.3% phytophagous, and by 6.7% predators and species associated with fungi. An entomophilic nematodes *Cryptaphelenchus macrogaster* f. *acuminati* was common in all parts of stem and branches (frequency of occurrence 25–33.3%). An entomophilic nematodes *Parasitorhabditis acuminati* and a predator *Fuchsia buetschlii acuminati* had the highest frequency of occurrence (41.7%) under the thin bark and in the branches. The frequency of these species in colonized with xylophagous insects stem fragments with thin bark was significantly higher than in respective not colonized fragments.

Key words: *Ips acuminatus*; *Ips sexdentatus*; entomophilic nematodes; phytophagous nematodes; predator; *Bursaphelenchus*; saproxylic nematode

Editor: Jaroslav Holuša

1. Introduction

Health condition of pine stands worsens in last decades promoted by climate change and anthropogenic activity, which increases the vulnerability of trees to different injurious organisms, including xylophagous insects, pathogenic fungi, bacteria, nematodes, etc. (Wood 1982; Sauvard 2007; Pernek et al. 2012; Siitonen 2014; Andreieva et al. 2018; Meshkova et al. 2018).

Xylophagous insects, mainly bark beetles from subfamily Scolytinae (family Curculionidae), longhorn beetles (Cerambycidae), and jewel beetles (Buprestidae) are the most evident and the most studied agents of tree decline (Wood, 1982; Sauvard 2007). They develop, at least the part of their life cycle, inside xylem or phloem tissues of a tree (Lieutier et al. 2016). The xylophagous insects, which inhabit living trees and bring to their mortality at high population density, cause physiological harm, which increases due to their maturation feeding in living trees and pathogen vectoring (Lieutier et al. 2016; Meshkova 2017).

Another type of harm is technical because it brings to losses of timber quality and can be caused by insects inhabiting living, felled, wind-broken or windthrown trees. Its severity depends on larval galleries and pupal chambers width and depth, their distribution in stem parts with thick, transitional or thin bark. Each species of xylophagous insects is evaluated by a certain score of physiological and technical harm, as well as by general harm considering physiological, technical harm and number of generations per year. It was proved that the same insect species can be non-harmful, low, moderately or extremely harmful depending on region, population density and other conditions (Meshkova 2017).

The presence of pathogenic or parasitic organisms can play an important role in the harmful consequences of tree colonization by xylophagous insects. Along with profound researches of blue-stain and wood-decaying fungi vectoring by bark beetles (Linnakoski et al. 2012; Davydenko et al. 2014, 2017), considerable attention is paid to nematodes (d'Errico et al. 2015; Holuša et al.

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2017; Korma & Sigaryova 2017), especially after spread of pine wilt disease caused by pinewood nematode or pine wilt nematode' (PWN) *Bursaphelenchus xylophilus* (Steiner & Buhrer 1934) Nickle 1970 (Nematoda: Aphelenchoididae) (Mota et al. 1999). This nematode inhabits in the resin canal systems of infected pines, spreading across the stem, branches, and roots (Mamiya 1983). This nematode is spread by beetles of the genus *Monochamus* (Coleoptera, Cerambycidae) through maturation feeding on the bark, particularly *Monochamus galloprovincialis* (Olivier 1795) (Coleoptera Cerambycidae), which usually inhabit weakened trees (Meshkova 2017; Dayi & Akbulut 2018).

Some researchers suspect other nematode species dangerous to pine trees, especially *B. mucronatus* (Kulinich & Ryss 2006; Kozlovsky 2016; Ryss et al. 2018). At the same time research of the nematode spread in the forest zone of Ukraine (Polissya) revealed *Bursaphelenchus* species only in drying-up trees. No nematode was found in the trees, weakened by wood-decaying fungi, as well as from the trees, which were not colonized by bark beetles (Davydenko et al. 2015). If nematode species suspected to be harmful are revealed only in the drying up trees, they cannot be the cause of its weakening.

Nematodes associated with trees are subdivided into different ecological groups. Some of them are phytophagous nematodes and are vectored by insects (Dayi & Akbulut 2018). Another group includes the entomophilic nematodes of xylophagous insect-pests of living or recently felled trees (Grucmanová & Holuša 2013; Holuša et al. 2017; Takov et al. 2019) and can be the ground for the production of biological means of tree protection on the base of entomophilic nematodes. Other nematodes are associated with insects, whose larvae develop in decaying wood, with predatory insects living in the galleries of xylophagous insects or living in tree sap or on bark surface (Blinova 1982), can be predators, nematodes associated with fungi and saproxylic species (Korma & Sigaryova 2017). Unlike xylophagous beetles, both phytophagous nematodes and entomophilic nematodes are less studied in forest stands of Ukraine (Korma & Sigaryova 2017) than in agrocenoses (Pylypenko & Kalatur 2015). Therefore, the control measures against the first group and the use of the second group to control insect pests are poorly developed. However, due to the urgency of pine forest decline, it was important to reveal, if nematodes inhabiting trees can be harmful to a tree or for bark beetles. Therefore, the research of the spread of nematodes associated with trees of different ecological groups together with xylophagous beetles can be also the ground for elaboration on the measures of forest protection from phytophagous nematodes.

Contrary to most other pine species, Scots pine possesses in its youth stage a light-brown smooth bark on the full stem length. With increasing tree age on the lower part of stem the bark gradually becomes thick, scaly dark grey-brown, with deep cracks; flakes of bark form irregularly shaped plates. The bark remains thin, flaky and orange on the upper stem and branches. Such differentiation goes on during all lifespan of pine. Bark shape and thickness depends on race and provenance as well as influenced by forest sites (Schultze-Dewitz & Koch 2008).

Host selection by xylophagous beetles is based on visual and olfactory signals from host trees (Wood 1982), as well as host physical properties like bark thickness (Paine et al. 1981; Amezaga & Rodrıguez 1998; Borkowski & Skrzecz 2016). Different bark beetles colonize stem parts depending on bark thickness, which correlates with phloem humidity and quality as feed resource as well as provides certain defense in winter (Saarenmaa 1983). Therefore, a study of stem insects and other organisms' distribution along the stem provides indirect confirmation of the associations on pine trees.

The aim of this study was to reveal the spread features of xylophagous beetles and nematodes associated with trees in the different parts of Scots pine trees of different health condition.

2. Material and methods

The research was carried out in the pure Scots pine (*Pinus sylvestris* L.) forests of 70 and 100 years old in Stanyshivske and Levkivske forestries of the State Zhytomyr Forest Enterprise (Zhytomyr region, so-called Central Polissya of Ukraine).

The long-term climate characteristics of the region are following: the length of the growing season is 205 days; annual air temperature is +6.8 °C, minimum –38 °C; annual rainfall is 552 mm, among which 60% of precipitation falls during the growing season. The average freezing depth of soil is 56 cm, maximum 120 cm. In average permanent snow cover is set on December 15 and melts on March 5th. Prevailing types of soils are fresh and humid soddy podzolic loams and sandy loams (Buzun et al. 2018).

Prevailing types of forest site conditions in the region are fresh relatively poor forest site conditions and humid relatively poor forest site conditions – so-called 'fresh soubor' and 'humid soubor' according to Ukrainian forest typology (Migunova 1993).

In two forests, four foci of Scots pine forest decline were chosen as sample plots (Table 1). Three trees of different health condition were felled on May 29, 2018, in each sample plot. Trees of the 1st (healthy), 3rd (severely weakened), and 4th (drying up) category of health condition were felled in SP 1 and SP 2. Trees of the 2nd (weakened), 3rd (severely weakened), and 5th (recently died) category of health condition were felled in SP 3. Trees of the 1st (healthy) and 2nd (weakened) category of health condition were felled in SP 4. Total of 12 trees felled.

Category of tree health condition was evaluated on a range of visual characteristics (crown density and color,

Table 1. Characteristics of sample plots.

Sample plots (SP)	Locality	Plot; subcompartment	Latitude, Longitude	Forest site conditions*	Age [years]	Height [m]	DBH [cm]
SP 1	Stanyshivske	33; 8.6	50° 23' N 28° 85' E	humid	70	24	26
SP 2	Stanyshivske	33; 43.4	50° 23' N 28° 85' E	fresh	70	24	26
SP 3	Levkivske	30: 15	50° 22' N 28° 71' E	fresh	70	24	28
SP 4	Levkivske	28; 1	50° 21' N 28° 72' E	humid	100	22	32

*Fresh or humid relatively poor forest site conditions (Migunova 1993).

the presence and proportion of dead branches in the crown, etc.) according to "Sanitary rules in the forests of Ukraine" (Anonymous 1995).

Parts of a stem with thin, thick and transitional bark, as well as branches and twigs were examined in each model tree. In our model trees, the height from the soil of stem part with thick bark was 5 – 9 m, with thin bark 2.5 – 12 m, and with transitional bark 9 – 17.5 m. The width of thick bark was 20 – 30 mm, of transitional bark 5 – 15 mm, and of thin bark 2 – 4 mm.

For each felled tree in the central part of the region with a thick, transitional, and thin bark, a fragment 1 m long was selected for examination. The diameter of fragments with thick bark was 26 – 32 cm, with transition bark 16 – 24 cm, with thin bark 8 – 14 cm. The length of examined fragments of branches and twigs was 0.5 m, and their diameter was 2 – 4 cm and 5 – 8 cm, respectively.

From each of 12 trees, 5 fragments were taken: from parts of a stem with thin, thick and transitional bark, from a branch and a twig, in total 60 fragments.

In these fragments, xylophagous beetles were identified directly by adults or by characteristic galleries, and wood samples were taken from each fragment for nematodes isolation.

Wood samples for nematodes isolation were taken as saw cuts with a saw to a depth of 2 – 5 cm in two places at a distance of 10 cm from each other and then chipping off with a chisel and a hammer. The obtained sample was split with an ax and then was crushed with pruners into fragments 1–2 cm in size. The twig and branch samples were sawed and then crushed.

Nematodes were isolated from wood samples in the laboratory using the Baermann method (Southey 1970). The crushed wood sample was placed in a funnel with a diameter of 12 – 15 cm and a tilt angle of 50°. A rubber tube of the appropriate diameter was put on the narrow end of the funnel, and a vial was inserted into the free end of the tube. In the middle of the funnel brass or synthetic sieve was placed with a hole size of 0.1 mm. The crushed sample was put onto the sieve and added water so that the water level was 1 – 2 cm higher.

Active nematodes in water come out of wood and, due to a greater specific gravity than water, are lowered to the bottom of the test tube. After 24 hours, the test tube was disconnected from the hose. The contents of the test tube were heated in a water bath at a temperature of 50 – 60 °C for 2 – 4 minutes and then fixed with a solution of TAF (7 ml of 40% formalin, 2 ml of triethanolamine, 91 ml of distilled water) or 4 – 6% solution of formalin.

Nematodes were identified by morphometric characteristics (Korma & Sigaryova 2017). Their distribution in different parts of a tree was evaluated as the proportion of respective samples with the presence of certain species. The population density of each nematode species was evaluated as an individuals' number per sample.

The frequency of occurrence of nematode species was evaluated as the proportion of samples with the presence of particular species from all 60 examined samples.

As no beetles and their galleries except *Ips acuminatus* (Gyllenhal 1827) galleries were found in twigs and branches, and only this species was found in the fragments with thin bark, we calculated the frequency of beetles as the proportion of tree fragments with the presence of particular species in 36 fragments from 12 trees (12 stem fragments with thin, 12 stem fragments with transitional bark and 12 stem fragments with thick bark).

Dominance of each beetle species was calculated as the proportion of stem fragments with the presence of this species of the total number of populated fragments.

The dominance of each nematode species was calculated as the proportion of tree fragments with the presence of this species of the total number of populated fragments.

Normality tests, summary statistics, one-way analysis of variance (ANOVA), Tukey HSD test with a significance level of $p < 0.05$ and Welch F test were performed. Microsoft Excel software and statistical software package PAST: Paleontological Statistics Software Package for Education and Data Analysis (Hammer et al. 2001) were used.

3. Results

3.1. Xylophagous beetles

Not a single stem fragment populated by any xylophagous beetle was detected in the sample areas SP 3 and SP 4. The significance of the frequency of xylophagous beetles in SP 1 and SP 2 was not proved (Welch F test: $F = 0.0829$; $df = 13.4$, $p = 0.7778$). In total, 10 species of xylophagous beetles were identified (Table 2, Fig. 1).

Table 2. Frequency (%) (±SE) of xylophagous beetles in different parts of Scots pine trees.

Species	Parts of stem		
	thin bark	transitional bark	thick bark
	(N=12)		
Cerambycidae			
Acanthocinus griseus (Fabricius, 1792)	0.0	8.3±7.98	0.0
Acanthocinus aedilis (Linnaeus, 1758)	0.0	0.0	8.3±7.98
Arhopalus rusticus (Linnaeus, 1758)	0.0	0.0	16.7±10.76
Curculionidae: Scolytinae			
Ips acuminatus (Gyllenhal, 1827)	25.0±12.50	25.0±12.50	0.0
I. sexdentatus (Boerner, 1767)	0.0	8.3±7.98	25.0±12.50
Tomicus piniperda (Linnaeus, 1758)	0.0	16.7±10.76	0.0
T. minor (Hartig, 1834)	0.0	16.7±10.76	0.0
Orthotomicus proximus (Eichhoff, 1867)	0.0	0.0	16.7±10.76
Trypodendron lineatum (Olivier, 1795)	0.0	0.0	8.3±7.98
Crypturgus pusillus (Gyllenhal, 1813)	0.0	8.3±7.98	0.0
All species	25.0±12.50	83.3±10.76	75.0±12.50

N – number of stem fragments.

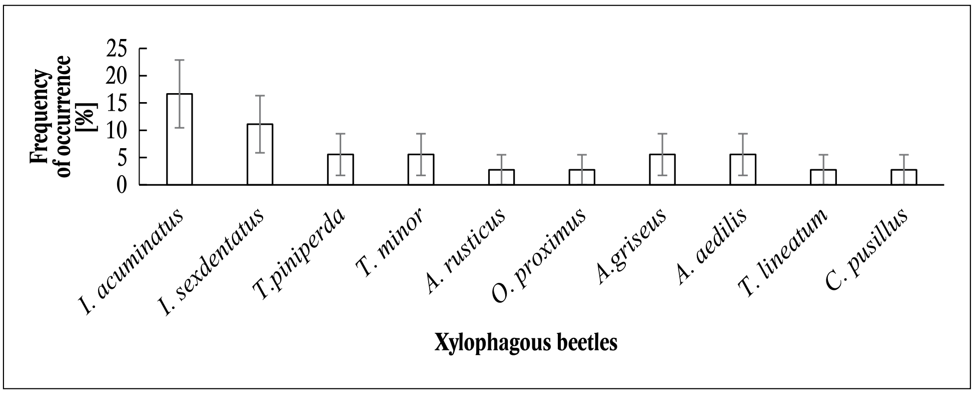

Fig. 1. Frequency of occurrence of xylophagous beetles in analyzed Scots pine trees (±SE) (the species are arranged in decreasing order of frequency of occurrence; N = 36; stem fragments with different bark).

The highest frequency was found in *I. acuminatus* (16.7% of stem fragments) and *I. sexdentatus* (11.1% of stem fragments) (Fig. 1).

The frequency of occurrence of *T. piniperda, T. minor, O. proximus,* and *A. rusticus* was 5.6%. The rest 4 species were found only in 2.8% of stem fragments (see Fig. 1).

Each xylophagous beetle species was confined to a specific part of the tree (see Table 2). Only *I. acuminatus* was found in the fragments with thin bark (frequency 25%). However, it has the same frequency in the fragments with transitional bark (25%). *I. sexdentatus* occurred in 25% of fragments with thick bark and in 8.3% fragments with transitional bark. The both *Tomicus* spp., *A. griseus,* and *C. pusillus* were found only under transitional bark. Longhorn beetles *A. aedilis*, *A. rusticus*, and bark beetles *O. proximus* and *T. lineatum* were found only under thick bark (see Table 2).

Significant differences were absent in the frequency of occurrence of *Ips sexdentatus* in the stem parts with thick and transitional bark (Welch F test: $F = 1.158$; $df = 18.69$; $p = 0.2956$). The frequency of occurrence of xylophagous beetles in the total samples from fresh relatively poor and humid relatively poor forest site conditions was 72.2 and 55.6% respectively, however, the difference was not statistically significant (Welch F test: $F = 1.663E–06$; $df = 17.92$; $p = 0.999$). The frequency of most common species *I. acuminatus* and *I. sexdentatus* was 1.3 and 3 times higher in the fresh relatively poor than in the humid relatively poor forest site conditions (Fig. 2), however, the difference was not statistically significant (Welch F test: $F = 0.7727$; $df = 31.65$; $p = 0.386$ for *I. acuminatus*; $F = 0$; $df = 34$; $p = 1$ for *I. sexdentatus*). Species *A. aedilis*, *T. lineatum*, and *C. pusillus* were found only in the stem fragments from fresh relatively poor forest site conditions. The longhorn beetles *A. rusticus* and *A. griseus* were found only in the fragments from humid relatively poor forest site conditions.

No xylophagous beetle was found in analyzed stem fragments from the trees of the 1st and 2nd categories of health condition. In the dead trees, only sporadic longhorn beetles *A. rusticus* and *A. aedilis* were presented (one beetle of each species). There was no significant difference (Welch F test: $F = 1.415E–06$; $df = 17.35$, $p = 0.9991$) in the frequency of occurrence of xylophagous beetles in the trees of the 3rd category (severely weakened trees) and of the 4th category (drying up trees according to health condition score) (Fig. 3).

By the proportion in the total number of tree fragments, *I. sexdentatus* dominated (54.6% from all beetles) (Table 3). The proportion of *I. acuminatus*, *T. piniperda*, and *T. minor* was 3.1, 4.7 and 6.7 times less in the total number of samples, than of *I. sexdentatus*. Obvious domination of *I. acuminatus* in the samples with thin bark and

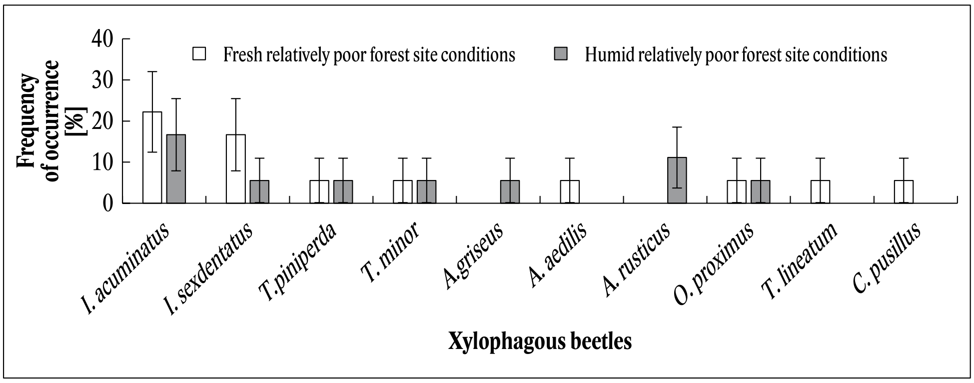

Fig. 2. Frequency of occurrence of xylophagous beetle species in analyzed Scots pine trees in different forest site conditions (±SE) (the species are arranged in decreasing order of frequency of occurrence).

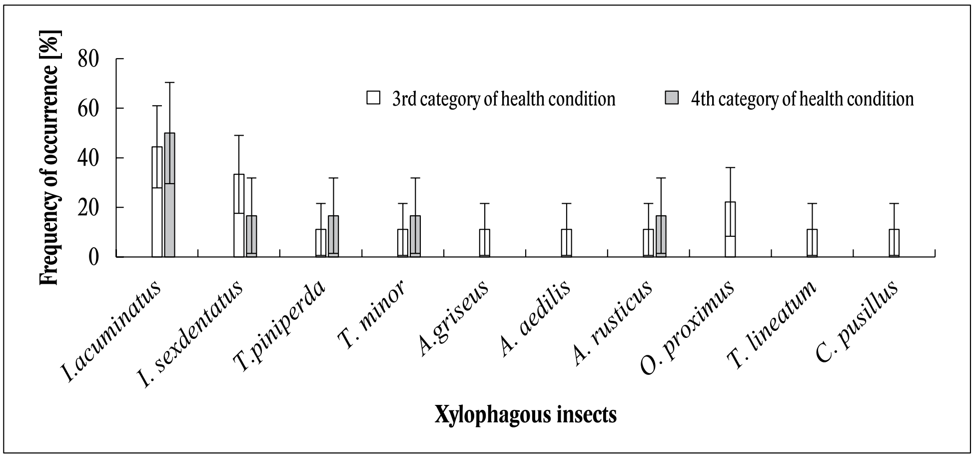

Fig. 3. Frequency of occurrence of xylophagous beetle species in analyzed Scots pine trees of different health condition (±SE) (3rd category – severely weakened trees; 4th category – drying up trees according to health condition score [Anonymous 1995]; the trees of the 1st and 2nd categories of health condition were not inhabited by xylophagous beetles).

Table 3. Proportion of xylophagous beetles in different stem fragments of Scots pine (±SE).

Species	Total (N=36)*	Parts of stem thin bark	 transitional bark (N=12)	 thick bark
I. acuminatus	17.9±3.83b**	100.0	27.9±4.48b	0.0
I. sexdentatus	54.6±4.98bc	0.0	1.6±1.27a	90.6±2.92b
T. piniperda	11.7±3.22b	0.0	37.7±4.85b	0.0
T. minor	8.2±2.74b	0.0	26.2±4.40b	0.0
A. griseus	0.5±0.71a	0.0	1.6±1.27a	0.0
A. aedilis	0.5±0.71a	0.0	0.0	0.9±0.92a
A. rusticus	1.0±1.00a	0.0	0.0	1.7±1.30a
O. proximus	3.6±1.86ab	0.0	0.0	6.0±2.37a
T. lineatum	0.5±0.71a	0.0	0.0	0.9±0.92a
C. pusillus	1.5±1.23a	0.0	4.9±2.16ab	0.0
Berger-Parker index	0.5	1.0	0.4	0.9

Note: * N – number of stem fragments; ** Values followed by the same letter are not significant within column at the 95% confidence level.

I. sexdentatus in the stem fragments with thick bark is supported by the Berger-Parker index (see Table 3).

3.2. Nematodes

Nematodes associated with trees (15 species) were revealed in 56.7% of samples (Table 4).

The most of nematodes were saproxylic (6 species, or 40%) and entomophilic (5 species, or 33.3%). Phytophagous nematodes were in the third place (2 species, or 13.3%). Predators and species associated with fungi were represented by one species each (by 6.7%). The frequency of nematode species in the samples from Scots pine trees from four sample plots didn't differ statistically (Welch F test: $F = 1.686$; $df = 30.11$; $p = 0.1911$). The frequency of certain nematode species in the samples from Scots pine trees was from 1.7 to 36.7% (Fig. 4).

Entomophilic nematodes had the highest frequency of occurrence and the highest species proportion (see Table 4, Fig. 4).

Table 4. Nematodes and their belonging to trophic groups in total sample from Scots pine trees.

Family	Species	Trophic group
Bunonematidae	*Bunonema poligraphi* (Fuchs, 1930) Sachs, 1949	saproxylic
Rhabditidae	*Parasitorhabditis acuminati* (Fuchs, 1915) Sobolev et Paramonov, 1954 (syn. *Rhabditis obtusa acuminati* Fuchs, 1915; *Parasitorhabditis obtusa acuminati* (Fuchs, 1915) Fuchs, 1937)	entomophilic
Diplogasteroididae	*Diplogasteroides sexdentati* Vosylyte, 1979	saproxylic
Diplogasteroididae	*Rhabdontolaimus carinthiacus* Fuchs, 1931	saproxylic
Diplogasteridae	*Tridontus longicaudata* Khera, 1965	saproxylic
Diplogasteridae	*Fuchsia buetschlii acuminati* Ruhm, 1956	predator
Cephalobidae	*Anguilluloides procera* Weingartner, 1954 (syn. *Alloionema procerum* Weingartner, 1954)	saproxylic
Cephalobidae	*Cephalobus persegnis* Bastian, 1865	saproxylic
Contortylenchidae	*Contortylenchus acuminati* Ruhm, 1956	entomophilic
Tylenchidae	*Ditylenchus striatus* Fuchs, 1938 (syn. *Anguillonema striata* Fuchs 1938)	associated with fungi
Aphelenchoididae	*Laimaphelenchus penardi* (Steiner, 1914) Filipjev et Sch.-Stekhoven, 1941	entomophilic
Ektaphelenchidae	*Cryptaphelenchus macrogaster f. acuminati* Ruhm, 1956	entomophilic
Parasitaphelenchidae	*Parasitaphelenchus acuminati* Ruhm, 1956	entomophilic
Parasitaphelenchidae	*Bursaphelenchus sexdentati* Ruhm, 1960	phytophagous
Parasitaphelenchidae	*Bursaphelenchus eggersi* Ruhm, 1956	phytophagous

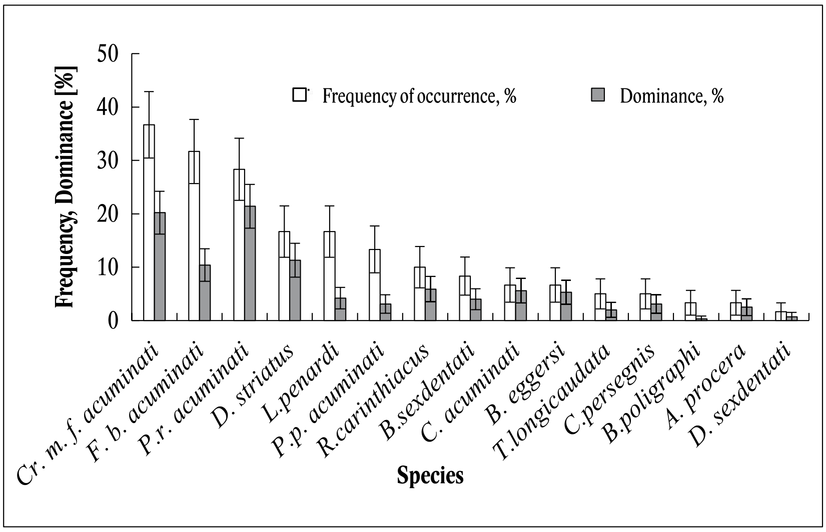

Fig. 4. Frequency of occurrence and abundance of nematode species in Scots pine trees (±SE)
(*Cr. m. f. acuminati – Cryptaphelenchus macrogaster f. acuminati; F. b. acuminati – Fuchsia buetschlii acuminati; P.r. acuminati – Parasitorhabditis acuminati; D. striatus – Ditylenchus striatus; L. penardi – Laimaphelenchus penardi; P.p.acuminati – Parasitaphelenchus acuminati; R. carinthiacus – Rhabdontolaimus carinthiacus; B. sexdentati – Bursaphelenchus sexdentati; C. acuminati – Contortylenchus acuminati; B. eggersi – Bursaphelenchus eggersi; T. longicaudata – Tridontus longicaudata; C. persegnis – Cephalobus persegnis; B. poligraphi – Bunonema poligraphi; A. procera – Anguilluloides procera; D. sexdentati – Diplogasteroides sexdentati.*

Nematodes were found in twigs, branches, and stem parts with thin, transitional and thick bark (Fig. 5). Significant differences were not proved both in species number (Welch F test: $F = 0.1197$; $df = 27.44$; $p = 0.9743$) and their abundance (Welch F test: $F = 0.07535$; $df = 27.46$; $p = 0.9891$).

The frequency of two nematodes was the highest in all parts of a tree (Table 5). Entomophilic nematode *Cryptaphelenchus macrogaster* f. *acuminati* had the minimal frequency of occurrence 25% under the thick bark and maximal frequency of occurrence under the thin bark and in branches. Its frequency of occurrence under transitional bark and in the twigs was 33.3%, which is the highest for these parts of a tree. The second species with high occurrence in all parts of a tree was predator *Fuchsia buetschlii acuminati* with the frequency of occurrence 41.7% under the thin bark and in the branches and the frequency of occurrence 25% in other parts of a tree. An entomophilic nematode *Parasitorhabditis acuminati* had a high frequency of occurrence (41.7%) under the thin bark and in the branches, frequency of occurrence 25% in twigs and under transitional bark and only 8.3% under the thick bark.

In general, the frequency of occurrence of entomophilic nematodes was the highest among trophic groups. In average it was 25% under the thin bark, 23.3 and 20% in branches and twigs, 18.3% under transitional bark and 15% under the thick bark. Nematodes *Bursaphelenchus sexdentati* and *Bursaphelenchus eggersi* are considered as phytophagous nematodes like other members of this genus. Their average frequency of occurrence was rather low in all parts of a tree (4.2 – 8.4%), except stem part with thick bark (15% on average). The frequency of occurrence of saproxyllic nematodes was 4.2 – 6.9% in twigs, branches and under thin bark, and only 2.8% under thick bark.

The number of nematode species was significantly higher in the trees of the 3rd category of health condition

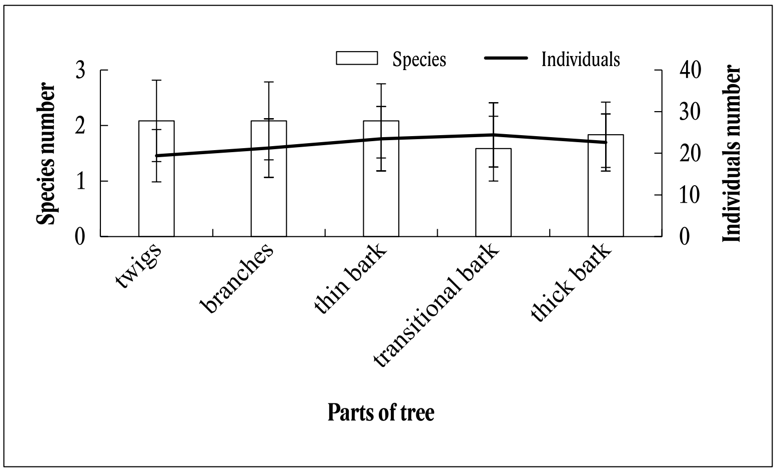

Fig. 5. Frequency of occurrence and abundance of all nematode species in certain fragments of Scots pine trees (±SE) (12 samples of each fragment: twigs, branches, thin bark, transitional bark, and thick bark, 60 samples in total).

Table 5. Frequency of nematodes in parts of Scots pine trees (±SE).

Nematode species	Parts of tree				
	twig	branch	thin bark	transitional bark	thick bark
Bunonema poligraphi	8.3± 7.96a	0	8.3± 7.96a	0	0
Parasitorhabditis acuminati	25.0± 12.50b	41.7± 14.23b	41.7± 14.23b	25.0± 12.50b	8.3± 7.96a
Diplogasteroides sexdentati	8.3± 7.96a	0	0	0	0
Rhabdontolaimus carinthiacus	8.3± 7.96a	16.7± 10.77ab	8.3± 7.96a	8.3± 7.96a	8.3± 7.96a
Tridontus longicaudata	8.3± 7.96a	0	16.7± 10.77ab	0	0
Cephalobus persegnis	8.3± 7.96a	8.3± 7.96a	8.3± 7.96a	0	0
Anguilluloides procera	0	0	0	8.3± 7.96a	8.3± 7.96a
Fuchsia buetschlii acuminati	25.0± 12.50b	41.7± 14.23b	41.7± 14.23b	25.0± 12.50b	25.0± 12.50b
Ditylenchus striatus	25.0± 12.50b	16.7± 10.77ab	16.7± 10.77ab	8.3± 7.96a	16.7± 10.77ab
Contortylenchus acuminati	0	8.3± 7.96a	0	8.3± 7.96a	16.7± 10.77ab
Laimaphelenchus penardi	25.0± 12.50b	8.3± 7.96a	16.7± 10.77ab	16.7± 10.77ab	16.7± 10.77ab
Cryptaphelenchus macrogaster f. acuminati	33.3± 13.60b	41.7± 14.23b	50.0± 14.43b	33.3± 13.60b	25.0± 12.50b
Parasitaphelenchus acuminati	16.7± 10.77ab	16.7± 10.77ab	16.7± 10.77ab	8.3± 7.96a	8.3± 7.96a
Bursaphelenchus sexdentati	8.3± 7.96a	8.3± 7.96a	8.3± 7.96a	0	16.7± 10.77ab
Bursaphelenchus eggersi	8.3± 7.96a	0	0	16.7± 10.77ab	8.3± 7.96a

Note: Values followed by the same letter are not significant within column at the 95% confidence level.

than in the trees of the 2nd category (Welch F test: F = 11.16; df = 24.99; p = 0.002627). Nematodes number gradually decreased from the trees of the 3rd to the 5th category of health condition (Fig. 6).

At the same time, nematode abundance (number of nematode individuals) continued to increase from the 2nd to the 4th category of health condition and decreased in the dead trees (of the 5th category of health condition). The differences between nematode abundance in the trees of the 2nd and the 3rd categories (Welch F test: F = 14.91; df = 25.5; p = 0.000688) as well as in the trees of the 3rd and the 5th categories (Welch F test: F = 6.343; df = 12.13; p= 0.0268) are significant.

Only four nematode species were found in the trees of the 2nd category of health condition (Table 6). The frequency of occurrence of *Fuchsia buetschlii acuminati* and *Cryptaphelenchus macrogaster* f. *acuminati* was the highest in all living trees, however, these species were absent in dead trees.

The highest frequency of occurrence of nematodes of all trophic groups was found in the trees of the 3rd category of health condition. Phytophagous nematodes and

Table 6. Frequency of occurrence of different nematode species in Scots pine trees of different health condition (±SE).

Species	Health condition category			
	2nd	3rd	4th	5th
Bunonema poligraphi	0	0	1.67±4.05a	1.67±6.41a
Parasitorhabditis acuminati	0	16.7±9.63a	5.01±6.90a	0
Diplogasteroides sexdentati	0	0	1.67±4.05a	0
Rhabdontolaimus carinthiacus	0	1.67±3.31a	0	3.34±8.98a
Tridontus longicaudata	0	0	3.34±5.68a	1.67±6.41a
Cephalobus persegnis	0	3.34±4.64a	1.67±4.05a	0
Anguilluloides procera	0	3.34±4.64a	0	0
Fuchsia buetschlii acuminati	5.01±8.25a	13.36±8.78a	3.34±5.68a	0
Ditylenchus striatus	1.67±4.84a	8.35±7.14a	5.01±6.90a	0
Contortylenchus acuminati	0	1.67±3.31a	3.34±5.68a	1.67±6.41a
Laimaphelenchus penardi	3.34±6.79a	3.34±4.64a	1.67±4.05a	3.34±8.98a
Cryptaphelenchus macrogaster f. acuminati	5.01±8.25a	16.7±9.63a	5.01±6.90a	0
Parasitaphelenchus acuminati	0	8.35±7.14a	5.01±6.90a	0
Bursaphelenchus sexdentati	0	1.67±3.31a	3.34±5.68a	1.67±6.41a
Bursaphelenchus eggersi	0	5.01±5.63a	1.67±4.05a	0

Note: Values followed by the same letter are not significant within column at the 95 % confidence level.

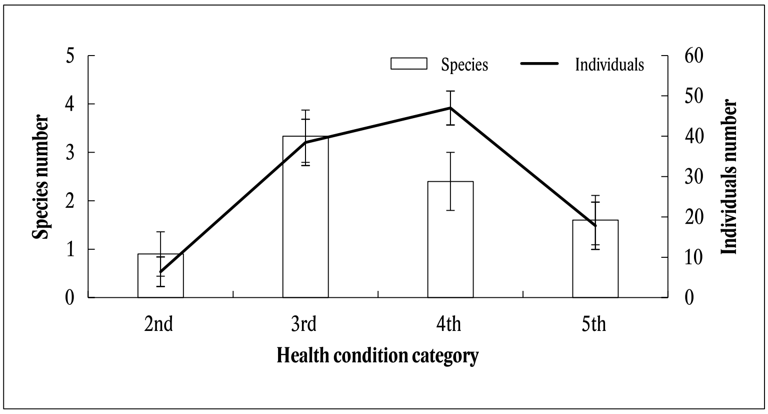

Fig. 6. Frequency of occurrence and abundance of nematode species in Scots pine trees of different health condition (±SE).

saproxylic species were absent in the trees of the 2nd category of health condition, whereas predators and associated with fungi species were absent in the dead trees (see Table 4).

The most common entomophilic nematode – *Parasitorhabditis acuminati* was absent in the stem fragments with thick and transitional bark which were not colonized with xylophagous insects (Table 7). The frequency of *Parasitorhabditis acuminati* in colonized stem fragments with thin bark was significantly higher than in respective not colonized fragments.

The frequency of another high spread entomophilic nematode – *Cryptaphelenchus macrogaster f. acuminati* didn't differ significantly in colonized and not colonized stem samples. The predator nematode – *Fuchsia buetschlii acuminati* was found in both colonized and not colonized stem samples, however, its frequency was significantly higher in colonized stem fragments with thin bark (see Table 7).

4. Discussion

Ten species of xylophagous beetles were revealed in the sample trees (see Table 2), and their distribution for the most part of Europe (Davydenko et al. 2015; Lieutier

Table 7. Frequency of occurrence of most common nematode species in Scots pine trees inhabited with xylophagous insects (±SE).

Species	Stem fragment		Welch F test		
	colonized with xylophagous insects	not colonized with xylophagous insects	F	df	p
Stem fragments with thick bark					
Parasitorhabditis acuminati	33.3±19.24	0.0	—	—	—
Fuchsia buetschlii acuminati	50.0±20.41	16.7±15.23	1.429	9.245	0.2618
Cryptaphelenchus macrogaster f. acuminati	33.3±19.24	33.3±19.24	0	10	1
Stem fragments with transitional bark					
Parasitorhabditis acuminati	33.3±19.24	0.0	—	—	—
Fuchsia buetschlii acuminati	33.3±19.24	16.7±15.23	0.3846	9.494	0.5497
Cryptaphelenchus macrogaster f. acuminati	33.3±19.24	16.7±15.23	0.3846	9.494	0.5497
Stem fragments with thin bark					
Parasitorhabditis acuminati	66.7±19.24	16.7±15.23	3.462	9.494	0.094
Fuchsia buetschlii acuminati	66.7±19.24	16.7±15.23	3.462	9.494	0.094
Cryptaphelenchus macrogaster f. acuminati	66.7±19.24	33.3±19.24	1.25	10	0.2897

et al. 2016; Korma & Sigaryova 2017; Andreieva et al. 2018). Dominance of *I. acuminatus* and *I. sexdentatus* is characteristic for bark beetles' outbreaks of last years (Siitonen 2014; Meshkova et al. 2018), because they have advantages like multivoltinous species (Leutier et al. 2016; Meshkova 2017).

An absence of *Monochamus galloprovincialis* (Olivier 1795), which is responsible for vectoring of phytophagous nematodes (d'Errico et al. 2015; Mamiya 1983; Ryss et al. 2018), in our sample trees can be explained by sampling more early (end of May) than the swarming of this longhorn beetle in the region (Meshkova et al. 2017).

Not a single stem fragment was populated by xylophagous beetles in the sample areas SP3 and SP4. It may be explained by the fact that the trees of the 1st, 2nd and 5th category of health condition (healthy, slightly weakened and dead) dominated in the sample from these plots. Usually, the trees of the 1st and 2nd categories of health condition are not attractive for xylophagous insects, and the dead trees are usually abandoned by these insects or were not colonized in the case of quick drying up (Meshkova 2017).

Confinement of each bark beetle species to the stem parts with particular bark thickness is connected with insect adaptation to certain humidity and temperature level (Sauvard 2007; Borkowski & Skrzecz 2016; Lieutier et al. 2016). For example, *I. acuminatus* and *T. minor* prefer the upper part of the stem with thin bark, and *I. sexdentatus* and *T. piniperda* prefer the lower part of the stem with thin bark. Recent years *I. acuminatus* and *I. sexdentatus* had the highest frequency of occurrence and proportion (see Table 2, 3), which is a result of its outbreak in studied regions (Siitonen 2014; Lieutier et al. 2016; Meshkova et al. 2018). Due to multivoltinism, the above-mentioned species replaced *T. piniperda* and *T. minor* both in the forest and in the stem level (Meshkova 2017).

Usually, at high density, the bark beetles concentrate not only in the preferable part of the tree but also in the other parts of the tree (Amezaga & Rodrigues 1998; Meshkova 2017). In our research, *I. acuminatus* dominates in the stem fragments with thin bark (Berger-Parker index=1), and *I. sexdentatus* dominates in the stem fragments with thick bark (Berger-Parker index = 0.9). The both species together with other xylophagous insects present in the stem part with transitional bark, where the Berger-Parker index is 0.4 (see Table 3).

Forest site conditions did not affect significantly the frequency of occurrence of xylophagous beetles (see Fig. 2), because at high population density they inhabit all susceptible trees (Wood 1982; Sauvard 2007). Both fresh and humid forest site conditions are favorable for Scots pine growth in Ukraine (Migunova 1993), although in the case of drought the fresh forest site conditions can be more attractive for xylophagous insects (Meshkova et al. 2018).

Fifteen nematode species were found in analyzed tree fragments, no new species for the fauna of Ukraine and Europe were detected for the first time in this study.

Some species from genera *Contortylenchus, Parasitylenchus, Parasitorhabditis, Parasitaphelenchus*, and *Cryptaphelenchus* are mentioned as entomophilic (parasitic) nematodes of bark beetles in Europe (Nedelchev et al. 2008).

However, the distribution of nematodes by ecological trophic grouping is somewhat conditional (Korma & Sigaryova 2017). The larval stages of parasitic nematodes may be obligatory or optional parasites of insects, with a free-living stage under the bark of an affected tree. At the same time, they can be associated with fungi or be saprobionts. Conversely, phytophagous nematodes with a pronounced pathological effect on larval stages may partially parasitize on the surfaces of insect-vectors or in the abdominal cavity. Some *Bursaphelenchus* species only in certain environmental conditions deal as phytophagous nematodes. They penetrate into plant tissues and destroy the epithelium of the resin ducts and can kill the tree (Mamiya 1987; Zhao et al. 2009).

Two identified species of the genus *Bursaphelenchus* are considered as phytophagous nematodes (Kulinich & Ryss 2006). However, they were identified only in the trees of the 2nd category of health condition (see Table 5), which makes one doubt that all members of the genus *Bursaphelenchus* are phytophagous nematodes.

In our study, five identified nematodes have species name "*acuminati*" and two nematodes have species name "*sexdentati*", which respects their association with the most abundant xylophagous beetles – *I. acuminatus* and *I. sexdentatus* and is agree with other authors (Blinova 1982; Grucmanová & Holuša 2013; Yaman et al. 2016; Slonim et al. 2018). The most abundant entomophilic nematodes *Cryptaphelenchus macrogaster* f. *acuminati* and *Parasitorhabditis acuminati*, as well as a predator *Fuchsia buetschlii acuminati* are associated with Ips acuminatus. Usually, the high frequency of occurrence and abundance of any entomophagous or entomopathogenic organism is characteristic of the decline phase of the bark beetle outbreak (Sauvard 2007). As we know, that an outbreak of *I. acuminatus* in the region started over 5 years ago (Davydenko et al. 2015; Meshkova et al. 2018), we can assume that in the investigated forest the outbreak of this bark beetle has also the trend to collapse.

The preferences of bark beetles to the parts of pine stem and forest health condition are rather well-known (Amezaga & Rodrigues 1998; Sauvard 2007) and are supported in this study. However, within-tree distribution of nematodes is studied only for pinewood nematode (PWN), *Bursaphelenchus xylophilus*, a serious invasive and destructive species, is listed as a quarantine pest in the legislation of more than 40 countries (Zhao et al., 2009).

The highest frequency of occurrence of nematodes in the trees of the 3rd category of health condition and abundance in the trees of the 4th category of health condition (see Fig. 6) is explained by domination of entomophilic nematodes (see Table 4), which follow their hosts (Holuša et al. 2017). However, the phytophagous nematodes also follow bark beetles, which facilitate their penetration into the tree (Grucmanova & Holuša 2013; Korma & Sigaryova 2017).

Usually, xylophagous beetles do not attack healthy trees, however, they can weaken them during maturation feeding and then attack successfully (Lieutier et al. 2016; Meshkova 2017). If the beetles colonize the weakened trees (of the 2nd category of health condition) in April, then to the end of May (the time of our assessment) these trees had the 3rd or the 4th category of health condition. Entomophilic nematodes could penetrate the tree simultaneously with bark beetles.

In our study, nematodes were found in all parts of the stem, branches, and twigs (see Fig. 5). The frequency of *Parasitorhabditis acuminati* and *Fuchsia buetschlii acuminati* in stem fragments with thin bark colonized with xylophagous insects was significantly higher than in respective not colonized fragments (see Table 6). However, even these nematodes were sometimes found in the samples without xylophagous insects.

5. Conclusion

Among 10 species of xylophagous beetles, *Ips acuminatus* and *I. sexdentatus* dominated. Only *I. acuminatus* was found in the stem fragments with thin bark and much less in the fragments with transitional bark. *I. sexdentatus* occurred in the fragments with thick bark and much less with transitional bark. The both *Tomicus* spp., *A. griseus,* and *C. pusillus* were found only under transitional bark. Longhorn beetles *A. aedilis*, *A. rusticus*, and bark beetles *O. proximus* and *T. lineatum* were found only under the thick bark. No xylophagous insect was found in analyzed samples from the trees of the 1st and 2nd category of health condition.

Among 15 nematode species 40% were saproxylic, 33.3% entomophilic nematodes, 13.3% phytophagous nematodes, and by 6.7% predators and species associated with fungi. An average frequency of occurrence of phytophagous nematodes *Bursaphelenchus sexdentati* and *Bursaphelenchus eggersi* was low in all parts of a tree, except stem part with thick bark.

The frequency of saproxylic nematodes was 4.2–6.9%in twigs, branches and under thin bark, and only 2.8% under thick bark. Predators and species associated with fungi were absent in the dead trees. The absence of *Bursaphelenchus sexdentati* and *Bursaphelenchus eggersi* in the trees of the 2nd category of health condition makes one doubt that all members of the genus *Bursaphelenchus* are phytophagous nematodes. Confinement of these nematodes the lower parts of the stem supports its association with *I. sexdentatus.*

An entomophilic nematode *Cryptaphelenchus macrogaster* f. *acuminati* was common in all parts of stem and branches. The frequency of *Parasitorhabditis acuminati* and *Fuchsia buetschlii acuminati* in colonized stem fragments with thin bark was significantly higher than in respective not colonized fragments.

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