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REVIEW PAPER

Occurrence of gypsy moth (*Lymantria dispar* L.) in the Slovak Republic and its outbreaks during 1945–2020

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Abstract

The gypsy moth is one of the most serious pests in forests and fruit tree plantations over prevailing parts of the Northern Hemisphere. This work is based on a literature review, and presents history of gypsy moth *Lymantria dispar* L., observed in Slovak forests within the period 1945–2020. The life cycle, hosts, natural enemies, population dynamics of pests, impact of outbreaks on forests and different management methods used in the past are discussed. Since 1945, there were nine gypsy moth outbreaks in Slovakia. Between 1945 and 2020, a total of 155,034 ha of deciduous forests were touched with varying intensity, representing an average annual damage of 2,040 ha. The strongest outbreak culminated in 2004. Totally 51,479 ha were attacked in the period of 2000–2008. We have found outbreak periods that repeat with frequency of 7.8 ±2.2 years and the average outbreak phase lasts 3.1 ±1.1 years. The period between two subsequent outbreaks seems to be more or less constant and duration of the outbreak phase seems to be gradually shortened during the study period. Several factors influencing the gypsy moth population dynamics in Slovakia are discussed. The role of biological control by using entomopathogenic fungus *Entomophaga maimaiga* is described.

Key words: population dynamics leaf-eating insect; periodic outbreaks; natural enemies; *Entomophaga maimaiga*

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1. Introduction

The gypsy moth *Lymantria dispar* L. (Lepidoptera: Erebidae) is one of the most serious forest insect pests, but also of fruit trees across much of the Northern Hemisphere. Large areas damaged by the gypsy moth are reported from the Northeastern United States and Asia (Schedl 1936; Doane & McManus 1981; McManus & Csóka 2007; Zúbrik et al. 2013). The cyclic gypsy moth outbreaks (Hlásny et al. 2015) resulted in loss of radial growth (Muzika & Liebhold 1999), changes in fruiting (Gottschalk 1990), and if repeated, in tree mortality in subsequent years (Patočka & Novotný 1985; Davidson et al. 1999). In Southeast Europe, outbreaks are more frequent (Pernek et al. 2008) and more intense (McManus & Csóka 2007) than in Central Europe (Hlásny et al. 2015).

Over the period of 1945–2016, more than 0.5 million hectares was damaged in different ways by leaf-eating insects in Slovakia (Zúbrik et al. 2017a). The gypsy moth ranked among key insect pests that feed on leaves in forests of Slovakia during the study period, along with *Operophtera brumata* L., *Erannis defoliaria* Clerck, *Agriopis leucophaearia* Denis & Schiffermüller, *Tortrix viridana* L., *Orthosia* spp., *Choristoneura murinana* Hübner, *Epinotia nigricana* Herrich-Schäffer, *Diprion pini* L., *Diprion* spp., *Melolontha* spp., and some other species (Turček 1956; Charvát & Patočka 1960; Čapek 1961; Patočka 1955, 1963a, b, 1967a, 1973; Leontovyč et al. 1980; Surovec et al. 1989; Zúbrik 2006; Zúbrik et al. 2015, 2017a, b; Vakula et al. 2015; Sarvašová et al. 2020). Some species, such as *Pristiphora laricis* Hartig, *Rhyacionia buoliana* Denis & Schiffermüller or *Coleophora laricella* Hübner, have caused damage to trees only occasionally and only in relatively restricted areas (Leontovyč et al. 1980; Surovec et al. 1989; Zúbrik et al. 2017b).

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Despite direct effect of defoliation on the tree mortality is questionable, often defoliated drought-stressed trees increase their secondary mortality caused by other pests such as *Scolytus intricatus* Ratzeburg (Coleoptera: Scolytinae), *Agrilus* spp. as well as other species of jewel beetles (Coleoptera: Buprestidae) and long-horned beetles (Cerambycidae), etc., developing frequently and abundantly in weakened trees (Novotný 1986; Zach 1994; Patočka et al. 1999). In the 70s and 80s of the last century, oak stands in Slovakia, were heavily affected by tracheomycosis disease (Leontovyč 1980; Surovec et al. 1989). Tracheomycosis disease and also armillaria root disease (*Armillaria* spp.) significantly reduce the ability of oak stands to resist defoliation caused by gypsy moth.

The aim of this study was to summarise the more detailed information on the gypsy moth in Slovakia, in particular: to reconstruct its population dynamics, analyse data about life cycle, hosts, natural enemies, impacts on forests and evaluate other significant aspects related to its biology and ecology over the period of 1945–2020. All these aspects are discussed in the context of theoretical knowledge, and practical expertise obtained from sources in Slovakia and others being external. Data from Slovakia are discussed with those, known from other areas of gypsy moth occurrence.

2. Methods

2.1. Data sources

To obtain the data we used three main sources for this work.

A) The primary source was the data present in different scientific and expert publications. Accurate information about the gypsy moth presence from the period 1945–1960 is lacking. Most of the data were available on the country level. We also used information about local gradations, determined by a place, or certain locality. These data are based on published estimations. Recent data, in papers published over the last 50 years are of higher accuracy.

B) For the period since 1961, we used official statistical records reported by forest managers. Damages were recorded on the accuracy level of the forest districts or country district (Zúbrik et al. 1999).

C) The most accurate data were obtained through monitoring conducted by forest managers and supervised by the Forest Protection Service in the frame of relevant projects, as our observations and experience.

We analyzed data from all the above-mentioned sources to reconstruct the long-term trend of the gypsy moth population occurrence in Slovakia. After analysing the data from all these three sources (A, B, C), we constructed a table with the estimated area, damaged by gypsy moth per years.

To define temperature and precipitation trends, we analysed the data from the Slovak Hydrometeorological Institute (SHMÚ) in Bratislava. Average monthly temperatures and precipitation data recorded at 26 weather stations (Gabčíkovo, Bratislava Airport, Bratislava-Koliba, Dolné Plachtince, Dubník, Dudince, Holíč, Hurbanovo, Komárno, Kráľová pri Senci, Kuchyňa, Leles, Lučenec, Malacky, Malé Ripňany, Moldava nad Bodovu, Nitra, Nový Tekov, Podhájska, Rimavská Sobota, Somotor, Štúrovo, Tesárske Mlyňany, Trnava, Žihárec) over the period of 1931–2015 were used. The presence of the gypsy moth in the area was the criterion for including a particular weather station in the analysis. Data were smooth using Local Polynomial Regression Fitting.

2.2. Terms

In order to be as precise as possible and to avoid confusion due to unclear terms, we provide a short explanation of some of the most commonly used terms (Fig. 1). We defined ‘outbreak’, ‘outbreak length’ or ‘outbreak period length’ respectively, as a period, during which gypsy moth caused certain damages in the forest (higher than zero). During building and declining phase of outbreak, gypsy moth population density often remains on a very low level for a long time. For that reason we also introduce a term “outbreak phase“. We defined it as a period, during which more than 1,000 ha of forest stands was damaged annually. This rule was not applied to the period 2013–2014 and 2017–2020. During this period, only minor areas were damaged, but we nevertheless, for certain reasons mentioned in the article, decided to label the events as outbreaks of the gypsy moth. The ‘outbreak peak’ is considered the year, with the highest registered damage during the outbreak period (Fig. 1). If we speak about “outbreak frequency“, we mean the period between two outbreak peaks.

2.3. Statistics and data presentations

For common statistics and data interpretation, we used Microsoft Excel 2016. We used mean, standard deviation (mean ± standard deviation) and coefficient of determination calculated in this program. Trend lines were calculated using the method of least squares in the same program. For picture processing, and figure elaborating we used Adobe Photoshop® (2016) and R Studio, version 1.3.1093, package ggplot2 Wickham (2016).

3. Gypsy moth life cycle

In Slovakia, the gypsy moth prefers older forest stands that are under warmer conditions in the southwestern and southern regions of the country, as well as in the eastern lowlands. It can also be found in dry localities, on steep slopes, in sparse and in wet-mesic floodplain forests along rivers (Turček 1956; Stolina 1985; Novotný & Turčáni 1992; Patočka et al. 1999).

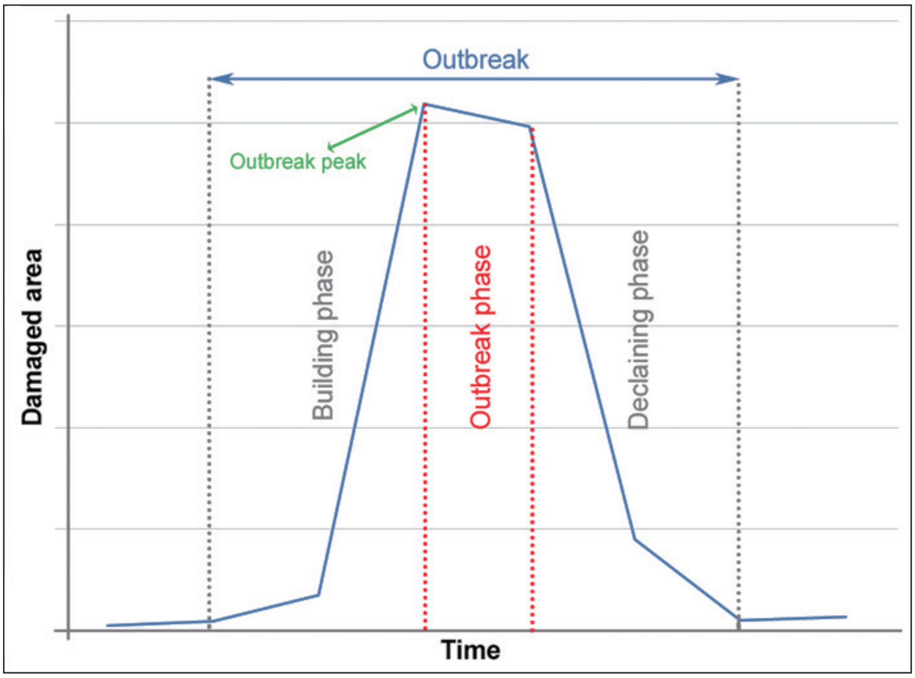

Fig. 1. Gypsy moth outbreak phases.

The gypsy moth has one generation per year. Adults (Fig. 2) are on the wings from July to August (from middle June to early September in recent years). Females lay eggs (Fig. 2) preferably at the base of the tree trunk although, during an outbreak, they do it also high in the crowns and even on thin branches (Turček 1956; Patočka 1961, 1973; Novotný 1986; Zúbrik 2006; Vakula et al. 2015). For assessing pest density on a plot, the Turček's method (based on egg masses count) is commonly used (Turček 1956), later this being changed slightly in forest documents that are officials (STN 43 2715). During an outbreak period, average number of egg masses per tree can reach 20 to 30 clusters (Hoch et al. 2001; Zúbrik, 2006), but it can be in some exceptional cases even more, 30 to 70 in heavily infested stands (Patočka 1973; Novotný 1986; Novotný & Turčáni 1992; Zúbrik & Novotný 1997). Patočka (1973) counted as many as 250 egg masses on one tree. If the number of masses per tree average two or more, the pest outbreak can be expected the following year (Turček 1956; Vakula et al. 2015), but critical numbers should be considered based on the forest age and health status (Patočka 1961).

The number of eggs per egg mass varied from 343–491 (Novotný, 1986) to 500–1000 (Turček 1949a; 1956). Turček (1949a; 1956) assumed 500–600 eggs are an average value. Hoch et al. (2001) reported similar numbers (averaging 534 eggs per egg mass) from Klingenbach (Austria, 60 km southwest of the Slovakia's border).

The gypsy moth overwinters as eggs. Larvae hatch from the middle of April (Novotný 1986; Vakula et al. 2015; Zúbrik et al. 2020a). After hatching, they rest several days on the surface of egg mass and then crawl to the crown on sunny days or balloon if the weather is windy (Patočka 1961). Airborne spread of larvae can be up to 15 km by wind (Novotný 1986). The larva (Fig. 2) has 5–6 instars, depending on sexes, that are relatively easy to determine by external morphological characters (Patočka 1954; Gogola 1969). Larvae start to feed in the crown at the beginning of May, defoliation culminates in mid-June (Fig. 3). They co-occur with other abundant species of leaf feeding caterpillars, such as *Archips xylosteana* L. and *Orthosia* spp. ("dispar-xylosteana complex") (Kulfan et al. 2018). Larvae pupate (Fig. 2) in late June and early July. The pupal stage lasts two weeks (Novotný 1986; Vakula et al. 2015; Zúbrik et al. 2020a). Swarming starts in the earlier part of July and culminates in its second half and eggs are laid in early August. At the beginning of September, no living adults are seen in forests (Turček 1949a; Novotný 1986).

4. Hosts to the gypsy moth

Gypsy moth hosts in Europe vary somewhat depending on how its distribution corresponds to the predominant vegetation in various geographical regions. There are slight differences in the gypsy moth food preferences between North, Central and southern Europe (Schopf et al. 1999; Švestka 1993, 1994, 2004; Hirka 2006; Csóka & Hirka 2009; McManus & Csóka 2007).

The gypsy moth is a highly polyphagous species, not only in Slovakia, but also in the whole territory of its occurrence (Kurir 1953; Janković 1958; Jahn & Sinreich 1957; Fuester et al. 1983; Novotný 1986; Zúbrik et al. 2013). In Eurasia, gypsy moth larvae are able to consume about 90 different tree species, while in the United States

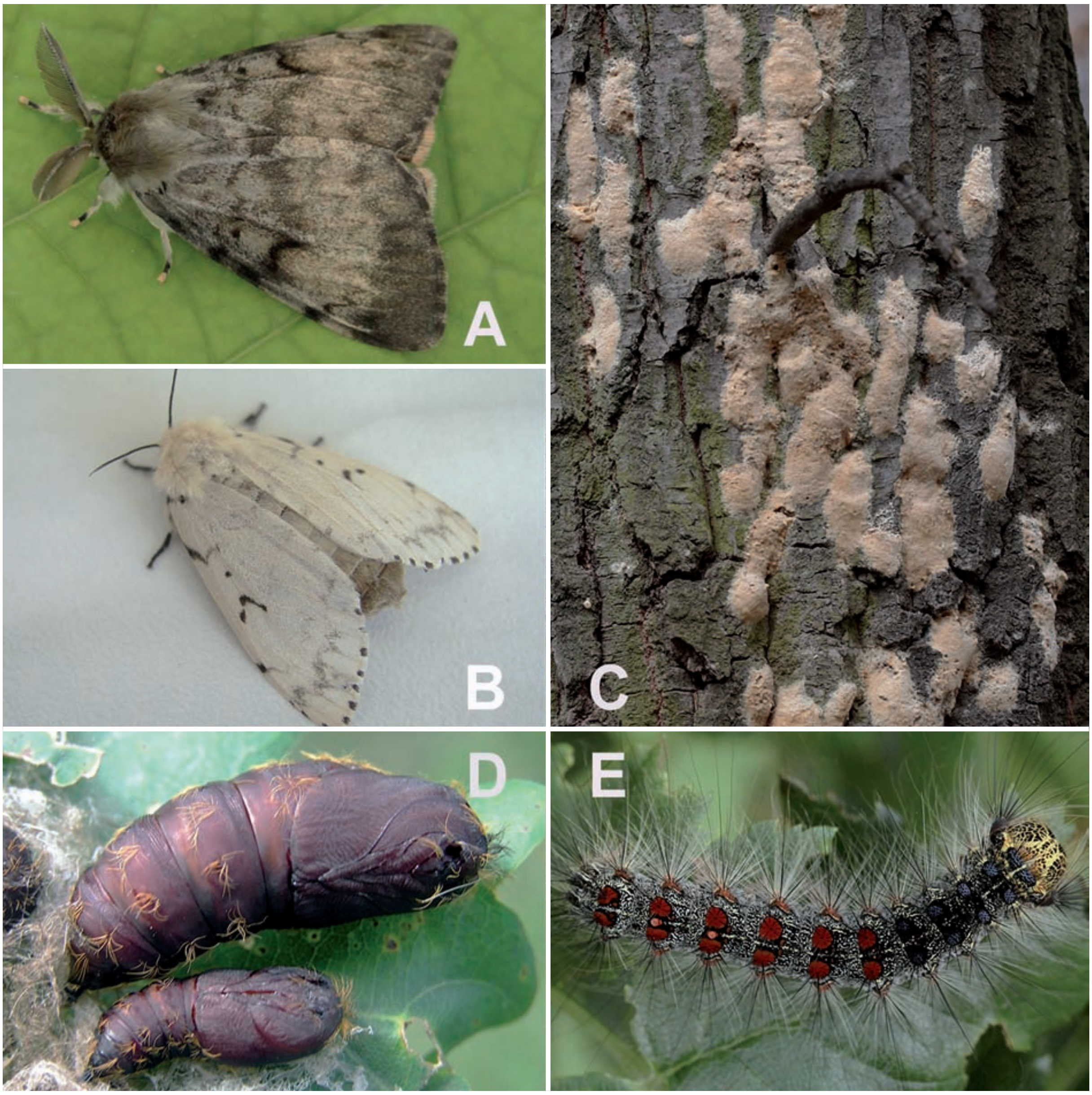

Fig. 2. Gypsy moth stages A) male, B) female, C) egg masses, D) pupae, E) larva in a final instar.

it is about 85 species (Schedl 1936; Doane & McManus 1981). Wellenstein & Schwenke (1978) state that up to 300 host plants have been recorded worldwide. It seems that younger larvae prefer mainly oak, possibly other trees with soft leaves, and only older instars are more polyphagous (Patočka 1970; Novotný 1986).

In Slovakia, larvae cause defoliation mostly in oaks (*Quercus cerris* L., *Q. robur* L., *Q. petraea* (Matt.) Liebl., *Q. pubescens* Willd.) (Tab. 1), but they can also feed on other trees and shrubs such as *Carpinus betulus* L., *Acer* spp., *Robinia pseudoacacia* L., *Prunus* spp., *Crataegus* spp., *Malus sylvestris* Mill., *Pyrus communis* L., *Tilia* spp., *Populus* spp., *Betula* spp. as well as others (Turček 1956; Patočka 1973; Stolina 1985; Novotný 1986) (Table 1).

In Slovakia, *Q. cerris* (Fig. 3) seems to be the most preferred food source (Turček 1953; Patočka 1961; Stolina 1985; Novotný 1986). Laboratory experiments showed that gypsy moth populations developed faster, and its larvae and pupae gained more weight on *Q. cerris* than on *Q. petraea*. *Q. cerris* females also laid significantly more eggs than those from *Q. petraea*. Field studies demon-

Table 1. Area defoliated by the gypsy moth in years 2002, and 2004–2006, by tree species, according to the official statistics (Varínsky et al. 2003; Kunca et al. 2005, 2006, 2007, 2008).

Tree species	Area damaged (in hectares)	%
Quercus spp.	38,099	91.09
Carpinus betulus	2,492	5.96
Robinia pseudoacacia	743	1.78
Fagus sylvatica	417	1.00
Populus spp.	72	0.17
Tilia spp.	2	0.00
Acer spp.	1	0.00
Alnus spp.	1	0.00
Total	41,827	100.00

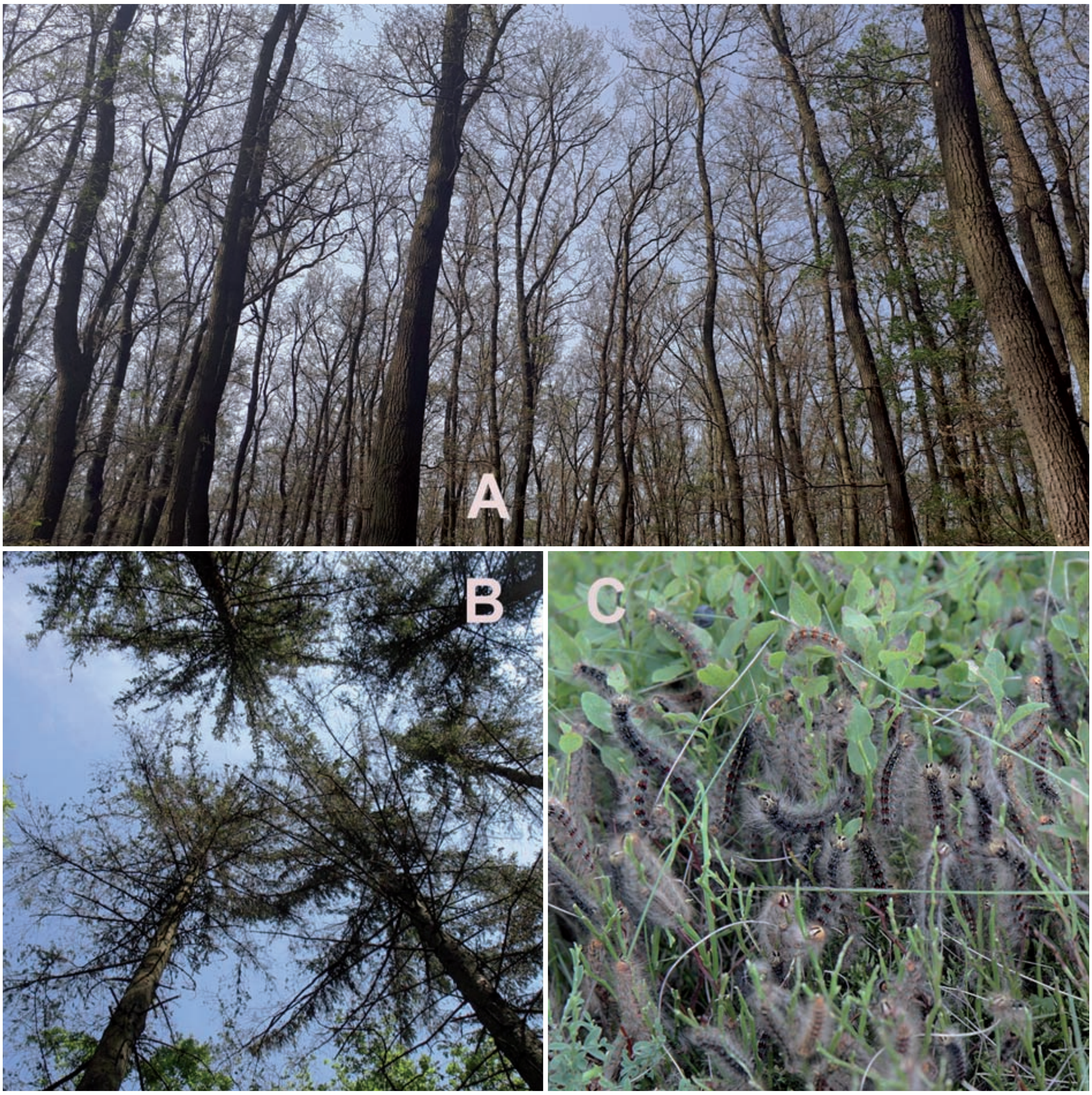

Fig. 3. A) Defoliation of *Q. cerris* stands by the gypsy moth are often severe; Šenkvice, June 2019. B) Defoliation of the spruce trees in 2018 (Vraná nad Vltavou, Czechia) documents gypsy moth is a highly polyphagous species. C) Unusual phenomenon in Slovakia: defoliation of *Vaccinium myrtillus* by the gypsy moth in a meadow habitat in 2011–2012 and 2020.

strated as well, a much faster development with less mortality of the caged gypsy moth larvae feeding on *Q. cerris* compared to those on *Q. petraea* (Schopf et al. 1999). In Slovakia, there is about 174,500 hectares of forests with prevalence of oak (Green report 2019) (Fig. 5).

Some tree species remain completely or partially untouched during gypsy moth outbreaks, like *Loranthus europaeus* Jacq., *Fraxinus excelsior* L., *Fraxinus ornus* L., *Ligustrum vulgare* L. and *Morus alba* L. (Turček 1956; Novotný 1986). This is not the same everywhere, as some local insect pest populations may have different feeding preferences (Patočka 1973).

Coniferous trees are also usually untouched; however, taken individually in oak forests, they can be heavily defoliated too (Fig. 3). Novotný (1986) reported defoliation of *Picea abies* L., *Abies alba* Mill., *Larix decidua* Mill., *Pinus sylvestris* L., *Pseudotsuga menziesii* Mirb. and *Pinus strobus* L. Some conifers, as *P. abies* or *P. silvestris*, occurred in mixed forests were heavily defoliated by the gypsy moth in 2018 and 2020 in Slovakia and Czechia (Zúbrik, Liška, personal observation). In Slovakia, beech (*Fagus sylvatica* L.) was defoliated only on a relatively restricted area (Patočka 1967b; Novotný 1986), unlike neighbouring Hungary, where forests of it had heavy defoliation on large areas in the past (Csóka et al. 2015).

In 2011–2012, and again in 2020, approximately 0.25 ha of meadows (600 m a.s.l.) near Banská Štiavnica in Slovakia covered by *Vaccinium myrtillus* L. (Fig. 3) was completely defoliated, along with *Sorbus* spp., *Salix* spp., *Malus sylvestris* as well as *P. abies* and *P. sylvestris* growing solitary in that area (Zúbrik, personal observation). Damages to fruit tree plantations, vineyards and even agricultural crops (*Zea mays* L.) were also reported in the past from Slovakia (Turček 1949a, 1956; Patočka 1973; Leontovyč et al. 1980; Novotný 1986; Alford 2010).

5. Natural enemy complex of the gypsy moth

In spring, some egg masses eaten by unknown predators are often seen in forests (personal experiences). It seems that predation may play a meaningful role, especially during the latency period. Inversely, density-dependent relationship (between the gypsy moth egg mass density and the predation) was found, but this was not significant (Turčáni et al. 2003). About 30% mortality in eggs is caused by birds as predators, such as *Certhia familiaris* L., *Sitta europaea* L., *Parus major* L., *Cyanistes caeruleus* L. and *Aegithalos caudatus* L. (Turček 1949a). The gypsy moth larvae may fall into the diet of several bird species in forests. However, they appear to be barely able to reduce the population density of this pest (Krištín 1999).

There are two egg parasitoids present on the territory of Slovakia, native *Anastatus disparis* Ruschka and *Ooencyrtus kuwanae* How (Hymenoptera: Eupelmidae), originating in Asia. Both species were released in that country at certain sites between 1960 and 1965 (Čapek 1966, 1971). They were successfully recorded again between 1985 and 1988 (Novotný & Čapek 1989) and also later, from 1992 until 1995 (Zúbrik & Novotný 1997), although, parasitisation rates were low, varying from 1 to 3%.

The natural enemy complex is much broader in the larval and pupal stages than in the egg stage. In total, 28 insect species from the orders Hymenoptera and Diptera and one nematode were recorded during the investigation in 1991–1996. Species like tachinids *Parasetigena silvestris* R.–D. and *Blepharipa pratensis* Meig., braconids *Cotesia melanoscelus* Ratz., *Phobocampe* spp., and *Glyptapanteles liparidis* Bouché were the most important (Hoch et al. 2001). A broad range of insect parasitoids was also confirmed by other authors (Čapek et al. 1969; Čapek 1988; Zúbrik 1997; Turčáni et al. 2001). At latency sites, *C. melanoscelus* was the dominant species, followed by *Phobocampe* spp. and *P. sylvestris*. The oligophagous tachinids *P. silvestris*, *B. pratensis* and braconid *G. liparidis* were the dominant parasitoids at sites of outbreaks and pro-outbreaks (Zúbrik 1997; Hoch et al. 2001). About 20% of larvae investigated were killed by parasitoids. Mortality by pathogens was higher more than 30% (Hoch et al. 2001). That of larvae by pathogens during the culmination stage can reach even 60% (Novotný 1989). The most frequently occurring pathogen, which has been present in Slovakia, is the *Lymantria dispar multicapsid nuclear polyhedrosis virus* (LdMNPV). LdMNPV is considered the main reason for the collapse of the gypsy moth outbreak in 1949 (Charvát 1967). Other pathogens, such as *Bacillus thuringiensis* Berliner, *Beauveria bassiana* (Bals.-Criv.) Vuill., *Nosema serbica* Weiser and *Nosema lymantriae* Weiser were also recorded (Novotný 1989; Zúbrik 1997; Hoch et al. 2001). The exotic entomopathogenic fungus *Entomophaga maimaiga* Humber, Shimazu & Soper was found in Slovakia for the first time in 2013 (Zúbrik et al. 2014). Further study revealed that the fungus was relatively widely spread here (Zúbrik et al. 2018b). It was suggested as well that it is spreading from the Balkan Peninsula (Zúbrik et al. 2016). Field study conducted in Slovakia during the years of 2014–2017 documented a narrow host range of *E. maimaiga*. Therefore, significant negative effects on the native lepidopteran fauna are not expected to occur (Zúbrik et al. 2018a).

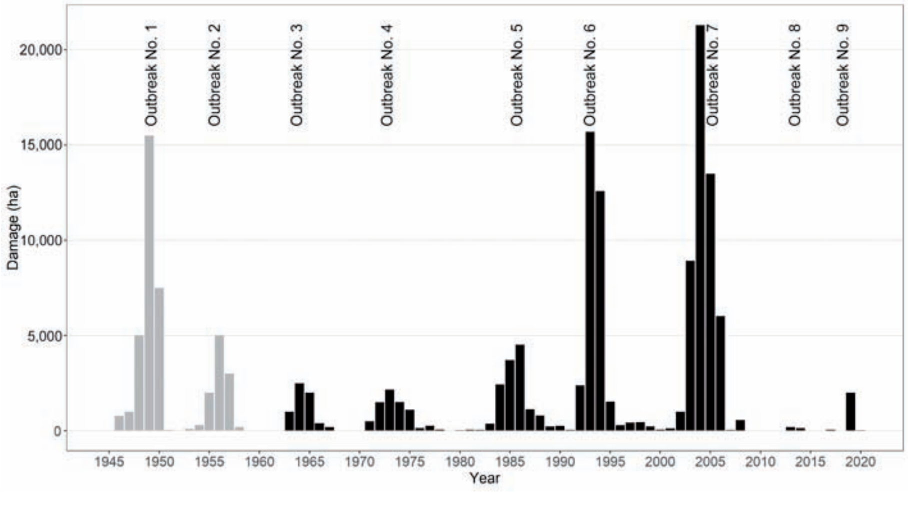

Fig. 4. Area damaged by the gypsy moth during the period from 1945 to 2020. Period from 1945 to 1960 is coloured in different shade. In this way we wanted to point out, that information we had available about gypsy moth population density in the period up to 1960 is less accurate and not very detailed. While information after 1960 is more accurate and quite comprehensive.

Predation on pupae was also investigated. It shows that small mammals may play an important role in pupal mortality (Turčáni et al. 2001). Invertebrate predation on gypsy moth caterpillars and pupae can also be a major mortality factor, mainly *Calosoma sycophanta* L. and *Calosoma inquisitor* L. (Coleoptera: Carabidae) frequently seen in forests during an outbreak period (Zúbrik, personal observations), but they have not yet been systematically studied as predators of larvae in Slovakia.

Outbreak situation influences in a different way forest ecosystems. Structure of natural enemies has changed (Hoch et al. 2001). Impact of defoliation on the presence of plant diseases was also confirmed (Patočka & Novotný 1985). The number of birds increased 1.5 to 2 times during the outbreak period (Turček 1956).

6. Dynamics of the gypsy moth population

According to our analyses, there were nine gypsy moth outbreaks on the territory of Slovakia since 1945 (Table 2, Fig. 4). Over the period of 1945–2020, 155,034 ha of deciduous forests were touched with varying intensity, representing an average annual damage of 2,040 ha. The strongest outbreak was recorded in 2000–2008. Totally 51,479 ha were attacked during that period (Tab. 2). The year with the greatest damage intensity was in 2004, when 21,304 ha were attacked. On average, 17,226 ha were affected per one outbreak. We have found outbreak periods in Slovakia that repeat with frequency of 7.8 ± 2.2 years and the average outbreak phase lasts 3.1 ± 1.1 years overall. This value agrees with previous studies of Patočka (1954), Novotný (1986). Novotný (1986) was speaking about an outbreak period of three years. Sometimes local outbreaks collapse faster from different reasons, after one or two years (Patočka 1973; Zúbrik et al. 2020a). Only 4 outbreaks were included into the data analyse in Hlásny et al. (2015), and also outbreak period, not outbreak phase was used in analyse. In our study, 9 outbreaks and outbreak phase was used. This difference caused, our results slightly differ from those published by Hlásny et al. (2015).

We tried to construct a trend for the length of the outbreak phase and the length of the period between two outbreak peaks (outbreak frequency) (Fig. 6). The trend indicates the period between two subsequent outbreaks seems to be more or less constant and duration of the outbreak phase seems to be gradually shortened during the study period. However, the coefficient of determination is very low in both cases. The shortening of the length of the outbreak phase may be related to the appearance of the fungus *E. maimaiga*. Several circumstances suggest that *E. maimaiga* has affected the length of gradation in 2013–2014 and 2017–2020 significantly (Zúbrik, personal observation).

There are some traditional areas for gypsy moth outbreaks on Slovakia's territory that do not change too much over time (Fig. 5). These regions are, such as the following: 1) the western part of the country, around Pezinok, Modra, Šenkvice, Bratislava; 2) around Nitra, Čifáre, Levice and Nové Zámky; 3) around Veľký Krtíš, Rimavská Sobota and Lučenec; and 4) eastern Slovakia, around Michalovce, Ortov and Sobrance (Pfeffer 1961; Patočka 1961, 1973; Charvát 1967; Kunca et al. 2005, 2006, 2007; Zúbrik et al. 2019). Gypsy moth outbreaks usually start in the western and central parts of the country, and then slowly shift to the East (Turček 1956; Charvát 1967; Zúbrik et al. 2019). We tried to confirm this statement on an example of the outbreak in 2002–2007 (Fig. 7). Defoliation in 2002 was reported only from western part of Slovakia and defoliation in eastern part clearly occurs first only after 3 year in 2005 and continued to 2006.

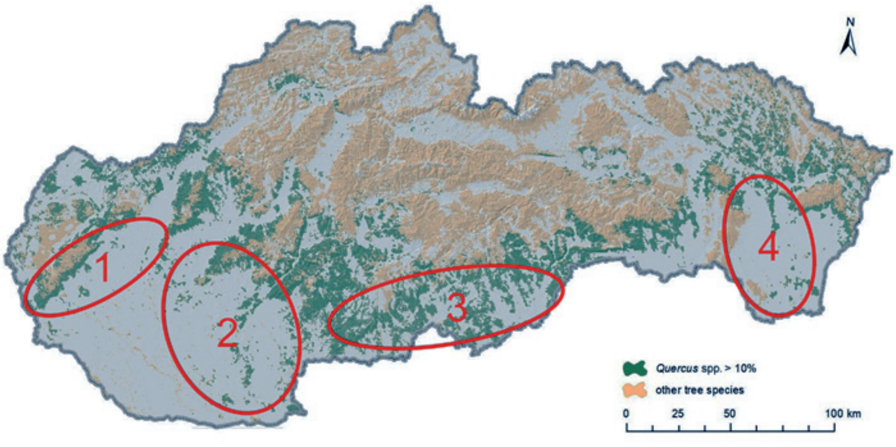

Fig. 5. Map showing the distribution of all oak forest types in Slovakia (only those with more than 10% of oak in composition were considered as oak forest). Very schematic indication of traditional areas for gypsy moth outbreaks on Slovakia's territory (see description in text).

Outbreak No. 1:

About this first, it was a very strong gypsy moth outbreak after World War II, reported Hendrych (1959). Patočka (1961) mentioned that there was damage to 30,000–50,000 hectares during two outbreaks in 1946–1949 and then in 1954–1957. Charvát (1967) confirmed that about 30,000 ha were defoliated and it culminated in 1949 during the outbreak period of 1946–1949. He was speaking about a "large outbreak" in 1946–1949, comparing it with the one in 1953–1958, which he commented as a "smaller outbreak of local importance". Patočka (1973) described outbreaks in 1946–1948 as "massive" and the subsequent two in 1954–1956 and 1964–1966 as "smaller ones". Turček (1950) and Konôpka (1978) also reported about a large, area-wide defoliation during this outbreak period. It was preceded by extremely dry and hot weather in 1947. The defoliation started in western Slovakia and moved to eastern Slovak lowlands in subsequent years (Charvát 1967). Forests were especially damaged near Levice, Nitra, Lučenec, Šahy, Rimavská Sobota and Sobrance (Patočka 1953; Charvát 1967). Turček (1949a) described outbreaks at the local level, reporting total defoliation in 1946–1948. In 1949, after all primary food sources were consumed; hungry caterpillars caused damages even to agricultural crops (Patočka 1961).

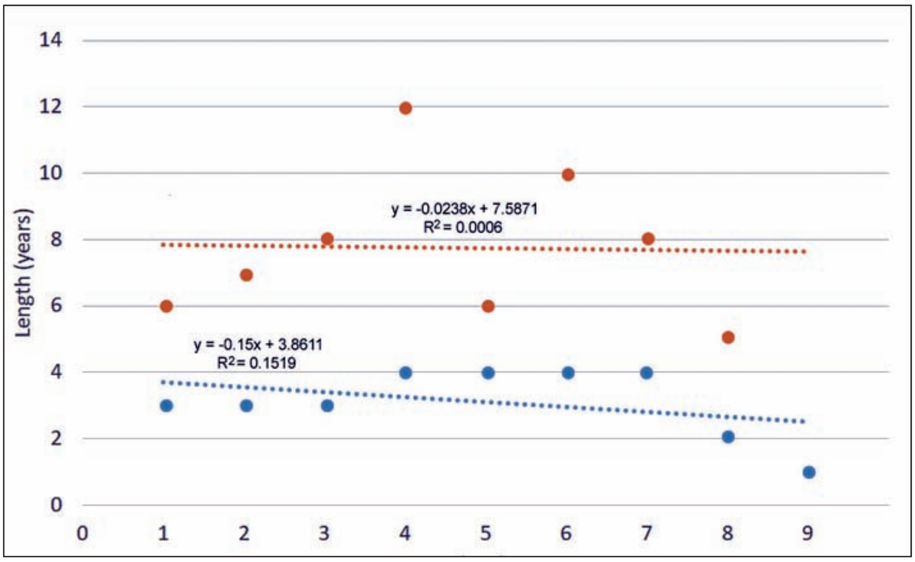

Fig. 6. The length of the outbreak phase (blue dots, 1-1948–1950, 2-1955–1957, 3-1963–1965, 4-1972–1975, 5-1984–1987, 6-1992–1995, 7-2003–2006, 8-2013–2014, 9-2019) and the length of the period between two outbreak peaks (red dots, 1-1949–1956, 2-1956–1964, 3-1964–1973, 4-1973–1986, 5-1986–1993, 6-1993–2004, 7-2004–2013, 8-2013–2019) with linear trends (duration of the outbreak phase – blue dotted line, duration of the period between two outbreak peaks – red dotted line). Trend lines were calculated using the method of least squares.

Outbreak No. 2

Shortly about this outbreak, reported Pfeffer (1961), largest-scale defoliation was observed in 1955, near Šaštín-Stráže and Smolenice, as well as in 1956, near Pezinok and Palárikovo. Also, Patočka (1961) mentioned this outbreak and dated it as 1954–1957. Years with very mild weather in 1952, 1954, 1955 and 1956 could have initiated this, which started in stands of *Fageto-Quercetum* and *Carpineto-Quercetum* under warmer climate. Outbreak culminated in 1956 and then the gypsy moth density was reduced, probably due to the unsuitable weather in 1957 and 1958. Charvát (1967) dated this oubreak approximately to the years 1953–1958.

Outbreak No. 3

Leontovyč et al. (1980) deeply discussed this period of 1963–1967. They reported complete defoliation near Pezinok, Palárikovo, Levice, Šahy, Lučenec and other areas. It is estimated that there was damage to about 2,000 ha annually during this one. Patočka (1967b) and Čapek et al. (1969) also noted this outbreak and dated it as 1963–1965, with an abundance reaching culmination in 1964. Čapek et al. (1969) studied larval parasitoids in the gypsy moth population and he therefore independently monitored the density of the latter on seven study plots. The results show that the number of gypsy moth egg masses has already increased in 1963 and their abundance culminated in 1964. Population was locally high even in 1965, but in 1966, it falls down to a low level, apparently also affected by cold spring weather (Patočka 1967b). In 1968, egg masses only occurred sporadically (Charvát 1969).

Outbreak No. 4

Leontovyč et al. (1980) first dated this to the years 1971–1974. They mentioned a situation where 300 hectares, even of coniferous trees (*Pinus* spp., *P. abies*, *Pseudotsuga* spp.), were damaged. Patočka et al. (1999) recorded an increase in the gypsy moth density, also in that period, and confirmed the outbreak culminating in 1973–1975, with local occurrences in 1976 and 1977.) A very high pest density in the whole zone was observed in 1975, but in 1976 and 1977, only small outbreaks were reported and the damaged area was increasing from 145 ha in 1976 to 247 ha in 1977 (Surovec et al. 1989).

Outbreak No. 5

The exact data about this outbreak are provided by Surovec et al. (1989). It speaks about an outbreak where approximately 2,400–4,500 hectares were damaged on an annual basis, especially in Bratislava, and slightly less in the Banská Bystrica administrative district. First defoliations were already reported in 1983 (near Tesárske Mlyňany). In 1984, local occurrences were more frequent in every primary affected zone, nearby specifically Čifáre, Podhájska, Nitra and Žitavany. In 1985 and 1986, there was area-wide defoliation reaching impressive intensity. Significant reduction in abundance was found in 1986 and local outbreaks became scarce in 1987. Outbreak period had a low-range peak in 1986. These data were also confirmed by analysing the official statistical evidence from the state forest (Zúbrik et al. 2013). Patočka et al. (1999) dated this outbreak to the years 1984–1987 too.

Outbreak No. 6

The period that was warm and dry at the beginning of the nineties of the last century (especially the year 1992) contributed to the rapid progress of the gypsy moth outbreak in 1993 and 1994 (Zúbrik 1998). Novotný & Turčáni (1993) reported previously in 1992 some changes in the population density. The infested area already reached more than 2,000 hectares in 1992, with a tendency to further increase. Outbreak culminated in 1993, and very high level of infestation remains in 1994. The most damaged areas were close to Nitra, Levice, Malé Karpaty, Košice and Prešov. In 1995, damages were not so extensive. In 1996, the gypsy moth population was in latency; a local outbreak in the area of about 200 ha was recognised (Zúbrik & Turčáni 1997).

Outbreak No. 7

The pest population density rose slightly in 2002 followed by a very fast increase in 2003. The outbreak started in the western part of the country and progress to the East (Fig. 7). In 2004, already 21,304 ha were defoliated by the gypsy moth, mainly around Nitra and Levice (Kunca et al. 2005). As for 2005, 13,498 ha were damaged, primarily in the vicinity of Veľký Krtíš, Krupina, Lučenec as well as Bratislava and Nové Zámky (Kunca et al. 2006, 2007, 2008; Zúbrik 2006). Aerial applications of insecticides were realised on a territory of 29,831 ha in 2004–2006. Approximately 7,000 hectares were defoliated (officially 6,025 ha) in 2006 (Kunca et al. 2006). Kunca et al. (2007) were speaking about the end of the outbreak in 2007, when only a very restricted area was damaged (45 ha) and no application was made. They concluded that this was the largest gypsy moth outbreak during the last 50 years. It was exceptionally severe not only in Slovakia, but also in many other EU countries. Hungary, for example, reported record-breaking damages to 212,000 hectares starting from 2005 (Csóka & Hirka 2009), while in Croatia the gypsy moth affected 33,000 ha in this particular year (Hrašovec et al. 2008). Švestka (2004) observed a large gypsy moth outbreak in the neighbouring Czechia in 2003 and 2004. He expected that defoliation will continue in 2005.

Outbreak No. 8

This is very unusual outbreak and after consideration, we decided to keep it here, despite it does not fulfil all criteria for a typical outbreak period. In 2011, in certain areas, a high number of gypsy moth larvae and adults was found in the oak forests. During autumn 2011, high amount of gypsy moth egg masses in oak stands was confirmed. A further significant rise in population density was expected in 2012. However, this did not occur (Zúbrik et al. 2013). In 2013, defoliation was recognised on about 200 ha in some isolated "spots" (for example, near Ortov in the eastern part of Slovakia) (Kunca et al. 2014). In 2014, about 150 ha was defoliated (Kunca et al. 2015, 2016). A detailed monitoring of the gypsy moth population density confirmed these observations; it slightly increased in 2011–2013. As for 2014, there was a fast decline into a

Table 2. Outbreaks overview in the period 1945–2020.

No. of outbreak	Outbreak peak	Outbreak	Hectares defoliated during outbreak	Outbreak phase	Hectares defoliated during outbreak phase	Control methods used
1	1949	1946–1951	29,822	1948–1950	28,000	Dynocid (DDT)
2	1956	1953–1958	10,600	1955–1957	10,000	Dynocid (DDT)
3	1964	1963–1967	6,100	1963–1965	5,500	no data available
4	1973	1971–1978	7,268	1972–1975	6,278	no data available
5	1986	1979–1990	13,588	1984–1987	11,799	*B. thuringiensis* + growth regulators
6	1993	1991–1999	33,639	1992–1995	32,210	*B. thuringiensis* + growth regulators
7	2004	2000–2008	51,579	2003–2006	49,758	*B. thuringiensis* + growth regulators
8	2013	2013–2014	350	2013–2014	350	*B. thuringiensis*
9	2019	2017–2020	2,053	2019	2,000	no control realised
Total (ha)			155,034		145,925	

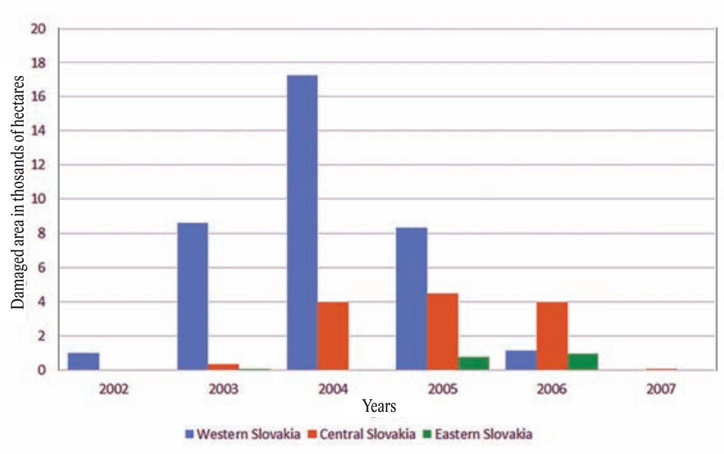

Fig 7. Area damaged by the gypsy moth from 2002 to 2007 in different geographical parts of Slovakia, demonstrating, that the defoliation started in the western part of the country and progress to the East.

latency stage (Zúbrik, unpublished data). Despite these changes were only occurring at low levels and were not recognised so significantly at a "macro" one, we do consider them as a gypsy moth outbreak. In the same years, quite strong-evidence outbreaks were also reported in some other European countries (Tabaković–Tošić 2015).

Outbreak No. 9

In 2018, the gypsy moth population density again has increased significantly in many places and a new outbreak started (Zúbrik et al. 2019). Data from a detailed monitoring confirmed, that gypsy moth population density was very high in 2018 in an area of 2,418 ha, although no significant damage was reported (Kunca et al. 2019a, 2019b). Zúbrik et al. (2019) expected that about 2,000 to 4,000 ha were going to be damaged in 2019, the outbreak will continue to reach a peak in 2020, and gradually decline in 2021. Despite high defoliation occurring in 2019 in some areas, most of the gypsy moth larvae died at the beginning of June 2019 infected by *E. maimaiga*. Very cold and wet conditions in May can be a predisposing factor for strong activity of fungi (Zúbrik et al. 2020a, 2020b). This situation resulted in only 91 ha which were officially reported as damaged in 2019, but the area defoliated by the gypsy moth was in reality definitely larger, estimated at a level of 2000 ha (Zúbrik et al. 2020b). Only very few local areas of the country are staying with high gypsy moth population density in 2020. One of these was a forest near Párovské Háje (Nitra region). Field observations confirmed almost no defoliation in Slovakia caused by the gypsy moth in 2020 (Zúbrik, personal observation). In neighbouring Czechia about gypsy moth outbreak in this period reports Liška (2018).

7. Causes of the origin and the collapse of gypsy moth outbreaks

There are generally several factors determining outbreaks of leaf-eating insects. Some are pest´s density-dependent (food source, natural enemies, etc.) and some are pest´s density-independent (temperature, wind, humidity, rainfall, etc.) (Clark et al. 1967; Barbosa & Schultz 1987).

Gypsy moth outbreaks come, according to some authors, mostly after years of warm weather, as well as balanced climate in May and without late frost during spring months in Slovakia (Patočka 1973; Novotný 1986; Kunca et al. 2013). We can also discuss how much was the decline in pest population density, affected with a heavy, late frost occurring in southern Slovakia – on April 18 2012, was measured −9.4 °C (Kunca et al. 2013). Patočka (1967b) stated that spring weather in April and May could have a very significant impact on pest abundance. However, it seems that most of these statements were just expert estimations and were not seriously supported by field experiments. Not any deeper research was done on the territory of Slovakia to assess the impact intensity of individual climate factors on gypsy moth population dynamics.

We addressed temperature and precipitation trends, analysing data from 26 meteorological stations in the area of gypsy moth outbreaks (Fig. 8). So far, we have not made a statistical comparison between the trend in population dynamics and that of precipitation and temperature. It is likely that some deeper analysis is needed to estimate more precisely this influence. It seems that another factor or more than one could deteriorate the population dynamics besides these two. Definitely more research and investigation is required in this area.

Natural enemy complex plays an important role in population dynamics of pests (Novotný 1989; Hoch et al. 2001). In 2013, *E. maimaiga* was found for the first time in Slovakia (Zúbrik et al. 2014). The impact of this fungus on the *L. dispar* population should not be underestimated since that time, especially when certain indications are suggesting it was introduced a few years earlier, as was finally recognised (Zúbrik et al. 2014, 2016, 2018b). Zúbrik et al. (2016) stated that interactions between *E. maimaiga* and gypsy moth population dynamics can be very strong. This entomopathogenic fungus can even lead to the collapse of its outbreak. Further observation documented that local outbreaks in the country were very heavily attacked by *E. maimaiga* (near Šenkvice, Párovské Háje, Čifáre, etc.) in 2019 as in 2020, and it was clearly responsible for fast collapse of outbreaks occurring locally (Zúbrik et al. 2020a; Zúbrik personal observation). It can be assumed that due to *E. maimaiga*, gypsy moth outbreaks should reach a lesser intensity in the future and be more local compared to the past.

Genetic predisposition and food quality are also important (Patočka 1973). Patočka (1973) concluded that outbreaks in Slovakia will occur only during particularly favourable years and in the most suitable areas. In experiments with manipulated water availability, gypsy moth larvae consumed much more leaves (birch *Betula pendula*) and the food conversion efficiency was lower, if food comes from plots with no watering (Castagneyrol et al. 2018). However, the growth rate of these larvae was the same for both types of experimental plots, watered and non-watered. These results suggest that larvae compensated the low quality of leaves from areas with no watering by consuming larger amounts of them. Adaptation to lower food quality in drought conditions generally leads to greater consumption, which is accompanied with more tree damage (Jactel et al. 2012).

8. Impacts of defoliation on forests

Generally, direct defoliation-induced tree mortality is ever lower, even in sensitive forests, compared to that caused by wind, snow or bark beetles (Kunca et al. 2019b). Some authors pointed out its impacts on radial growth, presence of secondary pests (*Agrilus* spp., *S. intricatus*, *Armillaria* spp., etc.) and the one on coniferous trees, which is typically negative (Turček 1950; Patočka 1974; Novotný 1986; Novotný & Surovec 1992; Csóka & Hirka 2009).

Novotný (1986) divided the tree species into several categories regarding their responses to the defoliation caused by the gypsy moth. The most resistant ones, such as *Q. cerris, Tilia* spp. or *Prunus* spp., can completely replace their foliage within about 60 days. Impacts of defoliation on the health of trees used to be more severe if repeated for two or three consecutive years and if it is combined with dry and hot weather conditions.

Defoliated trees have reduced annual stem growth; they are less tolerant to water stress and easily attacked by secondary pests (Patočka et al. 1999; Nakajima 2015; Camarero et al. 2018, 2019). The Turkey oak evidently had the best recovery potential (Csóka et al. 2015). It almost totally replaced its lost foliage in four months after severe defoliation by gypsy moth caterpillars in western Hungary. The pedunculate oak and beech needed two years to reach the same level of recovery. This first species used to suffer from a heavy infection of powdery mildew *Erysiphe alphitoides*, following defoliation, which may slow down tree recovery (Csóka et al. 2015; Zúbrik et al. 2020a). Defoliation can be fatal in case of seedlings (Patočka 1973).

Impact of defoliation on plant production can also be very important, mainly in areas where natural regeneration is expected. There was almost no crop output occurring in heavily defoliated stands (Turček 1956; Novotný 1986). Similar impact of it on acorn production is known in North America (McConnell 1988).

Patočka (1973) was also speaking about reducing the recreational value of forests during gypsy moth outbreaks. The invasion of large, hairy caterpillars in the recreation area of Pata, during the pest outbreak of 1972–1973, caused problems to forest visitors and residents in the area.

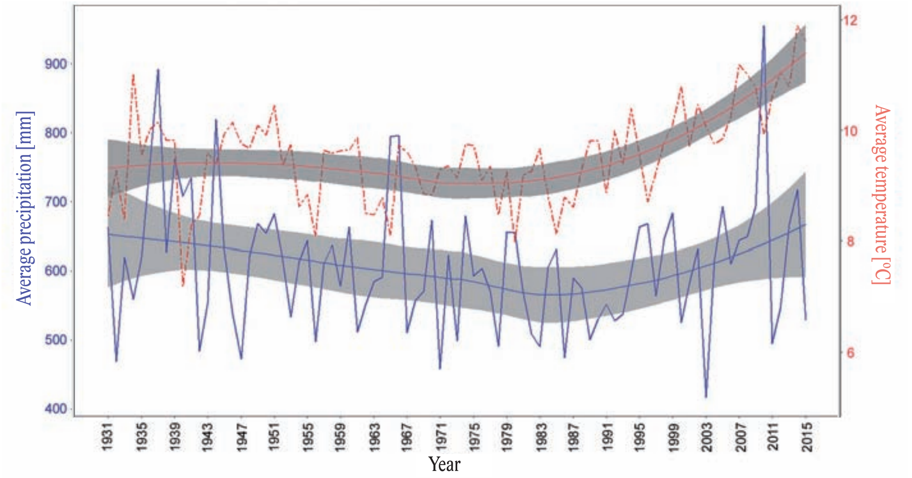

Fig. 8. Development of mean annual temperatures (°C) and annual precipitation (mm) in the period of 1931–2015 calculated as an average values from 26 weather stations of Slovak Hydrometeorological Institute (SHMI). Mean annual temperatures and precipitations were smoothed using smoothed conditional means – local polynomial regression fitting. Grey background behind smooth represents standard error with 95% confidence intervals.

9. Previous and current methods of control

Intense applications of Dynocid (DDT) against gypsy moth larvae were realised in the past in Slovakia. Several authors reported about large aerial applications during the outbreak of 1946–1949 (Turček et al. 1950; Patočka 1973; Konôpka 1978). Later on, forests were treated by insecticide (DDT), about 5,000 ha were in 1955, 4,000 in 1956, and 6,000 in 1957 (Kudler et al. 1958). Some authors observed negative effects of DDT on natural enemies and environment (Turček 1949b; Patočka 1973).

Patočka (1961) discussed a possible control method based on collecting and destroying egg masses. This is more ecologically friendly but less effective, compared to chemical applications against larvae. As a disadvantage, this procedure is considering that part of these masses can be overlooked and many are in inaccessible areas in the crown.

Since the 60's and the 70's of the last century, intense experimentation with biological agents against gypsy moth larvae was realised in Slovakia. As the most promising one, *B. thuringiensis* was tested in several trials (Hešková 1978; Novotný 1985, 1988a, 1988b; Novotný & Švestka 1986, Turčáni 2001). Larval mortality was high and full efficiency was reached after 2–8 days of treatment, depending on the dose (Novotný 1988b, 1989). Besides *B. thuringiensis*, also LdMNPV (Novotný 1985, 1989), *B. bassiana* (Novotný 1989), viruses (Švestka & Pultar 1997) and microsporidia (Weiser & Novotný 1987; Hoch et al. 2008; Solter et al. 2010) have been tested against the gypsy moth on the territory of Slovakia.

As a result of this intense research in the field of biomonitoring, gypsy moth outbreaks were almost exclusively managed with biological and biotechnical preparation since 1984–1987. Insecticide formulations based on *B. thuringiensis* were used on 1,126 hectares (24% of total 5,878 ha sprayed) during those years (Novotný 1988a). In order to prevent damage in 1992–1995, extensive aerial applications were also realised. *B. thuringiensis*-based bioinsecticides and insect growth inhibitors (e.g. Dimilin) were applied on a territory over 15,000 hectares (Turčáni & Zúbrik 1997; Zúbrik 1998). In the period of 2004–2006 (three years) applications from the air were even more intense. From the total treated area (29,831 hectares; 8,298 ha in 2004, 15,955 in 2005, 5,538 in 2006), the biological preparation based on *B. thuringiensis* (Biobit XL) was used on 6,637 ha (22.2%). Growth regulators were for the remaining 23,194 ha (77.8%). Aerial applications were financially covered by the Ministry of Agriculture and co-financed by State Forest Enterprises. Their cost reached c. 365,000 EUR in 2004, c. 740,000 EUR in 2005, and c. 170,000 EUR in 2006 (Zúbrik & Kovalčík 2005; Zúbrik 2006; Zúbrik et al. 2006). In 2019, applications from the air against the gypsy moth on a territory of about 3,000 ha were planned, while *B. thuringiensis* Biobit XL was proposed exclusively (Zúbrik et al. 2019). They were not realised yet, partially due to a restriction from the State Nature Conservancy officials and also to high mortality of the larvae by *E. maimaiga* (Zúbrik et al. 2020a). The applications were usually taken place in the spring, between May 5 and 20 (Zúbrik 2004).

10. Conclusions

This is the most complete and most detailed reconstruction of the gypsy moth population dynamics on the territory of Slovakia, although some partial reconstruction was already compiled and published (Zúbrik et al. 2013; Kunca et al. 2013, 2014; Hlásny et al. 2015; Zúbrik et al. 2017a). We have included these works in our assessment, and they have been filled with new, until now unanalysed data.

- There were nine gypsy moth outbreaks since 1945. In the period of 1945–2020, 155,036 ha were damaged with varying intensity. The strongest outbreak was in 2000–2008 when 51,579 ha were attacked. On average, 17,226 ha were affected per one outbreak period. We have found outbreak periods that repeat with frequency of 7.8 ± 2,2 years and the average outbreak phase lasts 3.1 ± 1,1 years. The outbreak started in the western part of the country and progress to the East. The period between two subsequent outbreaks seems to be more or less constant and the duration of the outbreak seems to be gradually shortened during the study period.
- There are several factors influencing the gypsy moth population density in Slovakia. For example, weather in May, late frosts, genetic predisposition, natural enemy complex and food quality are considered by many authors as key ones. Unfortunately, most published statements were just an expert estimation and were not sufficiently supported by field experiments.
- Gypsy moth is extremely polyphagous species in the country; however, *Q. cerris* seems to be the most preferred host.
- Defoliated trees have reduced annual stem growth; they are less tolerant to water stress and easily attacked by secondary pests.
- Intense applications of Dynocid (DDT) against gypsy moth larvae were realised in the past. Gypsy moth outbreaks were almost exclusively managed with biological and biotechnical preparation since 1984.
- The exotic entomopathogenic fungus *E. maimaiga* was found in Slovakia for the first time in 2013. Certain circumstances suggest that, due to *E. maimaiga*, gypsy moth outbreaks should reach a lesser intensity in the future and will be more local compared to the past.

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REVIEW PAPER

Infectious and parasitic diseases of phytophagous insect pests in the context of extreme environmental conditions

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Abstract

The density of phytophagous insect pest populations is related (directly and indirectly) to several groups of factors that can be broadly divided into: abiotic, biotic and anthropogenic. Each extreme in the abiotic environment at a macro-level leads to a series of consecutive extremes in the biotic environment, which eventually results in micro-level responses in the individual organisms. The manifestation of factors acts in aggregate or in a sequence, creating a chain of processes around us. Insects very efficiently use the abundance of nutritional resources, resulting in a tremendous increase in their population density, and triggering control mechanisms through the emergence of parasitic and pathogenic infections (viruses, bacteria, fungi, microsporidia, protozoa and nematodes). The development of entomopathogenic infections in host populations is directly dependent on the characteristics of both the antagonist and the insect. It is associated with the lifestyle and life cycle of the insect, with features encoded in the mechanism of pathogen action, and limited by the pathogen's virulence and pathogenicity.

Key words: entomopathogenic infections; environmental conditions; extreme; insect pests; epizootic

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1. Introduction

Among animals, insects are the most numerous taxonomic group. They have occupied almost every ecological niche on the Earth and, therefore, studies of their ecological strategies, but also of control mechanisms is becoming increasingly important (Leibhold 2012). The advancement of industrialization and development of technology on one hand and increased consumption of goods and services by the world's population and at the same time accelerated mobility on other hand influence different ecosystems and their species composition and health. The high level of development in our societies and the need of minimizing the negative effects of human activity on nature as a whole, are becoming more important (Schowalter 2012). The global climate change is a fact and is increasingly manifested as extreme weather phenomena that affect large areas of the planet with direct impacts on the lives of large numbers of people (Vitousek et al. 1997; Thomas et al. 2004; Burney & Flannery 2005). The study of impacts of these factors on forest communities condition, which are habitats of various group of insects, is a major task for scientists. Sustainable management of natural resources involves implementation of measures which are environmentally friendly in all spheres of life.

Like in any organism, disease-causing agents are involved in the regulation of insect populations, and include viruses, bacteria, protozoans, microsporidia, fungi, and nematodes. Despite extensive research worldwide, there are still many unknowns about biological control mechanisms in insect populations. This review presents pathogen groups causing infection and parasite deseases in different forest and other insect pests in the context of extreme environmental conditions, cascade effect of the factors (abiotic, biotic and anthropogenic) and epizootic which finally can results as natural mechanisms of insect regulation.

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2. Environmental factors are the basis for triggering biological mechanisms

The abiotic factors (physico-geographical and atmospheric-climatic) are fundamental for developing of biotic process and natural responses in the ecosystems.

Physico-geographical factors are all characteristics connected to parameters specific to a given geographical area. Such physical parameters are the landscape, soil composition, the altitude, the geographical orientation, the presence or lack of water basins affecting the development of the various groups of living organisms, including insects.

Atmospheric-climatic factors include the humidity, the solar radiation and temperature, the frequency and strength of winds and storms, fires, the amount of rainfall and snowfall, etc. The drastic deviations of these characteristics lead to changes in the environment and to changes in the volume of food sources, in our case – plants.

Biotic factors are related to the natural response. The presence and abundance of plant and animal resources are in direct correlation with climatic factors and changes. The plant species diversity and vegetation structure are nutrient base for the phytophagous entomofauna. The peculiarities in the age structure of the natural biocoenosis like the tree and shrub vegetation, the presence of enemies (predators, diseases) as well as ecological factors such as interspecific and intraspecific competition are among the main biotic factors.

Anthropogenic factors include fires, the result of human negligence or deliberate action, illegal logging, industrial and domestic pollution, uncontrolled use of pesticides which is increasingly reaching the scale of a disaster with irreversible natural consequences, deforestation – a worldwide problem reaching enormous proportions. Soil erosion is a result of deforestation and inefficient forest management, not least the replacement of forest in agricultural territories which changes also the whole entomofauna. The steady increase of mobility of people, raw materials and goods is a prerequisite for the most intensive spread of new, invasive species in certain areas, which are displacing native species and often, due to lack of natural enemies, high levels of population density are achieved (Brockerhoff et al. 2006).

3. Cascade effect provoked by the environmental factors

One extreme leads to the next one, which leads to a third one, resulting in a chain process, unlocked by an event in the non-living nature and reaching the final manifestations at population level, resp. individual. Climate crises and disasters resulting in damage, such as wind, snow, flooding, drought, fire are of the utmost importance. The extreme variations of certain climatic factors have a direct impact on the development and abundance of insect populations as well as organisms in general. We can confidently conclude that in every physical and climatic extreme the living nature shows a reaction. This can be manifested either in high numbers of one species or vice versa, in the mass extinction of another (Cahill et al. 2012).

A typical example in this regard is snowfall and windthrow in coniferous forests. Mass felling and death of large numbers of trees, caused by strong wind or heavy snowfall, form a favorable environment leading to a massive reproduction of some insects, for example bark beetles that find excellent conditions for their development, as well as insects feeding on decaying plant material (saproxylic species) (Lindgren & Raffa 2013). It is argued that the atmospheric and climatic extremes, which we are witnessing, cause biological extremes that alter the environmental factors, thus directly affecting the organisms that inhabit these areas.

Many phytophagous insects (e.g. xylophagous, phylophagous, saproxylic species) accelerate their life cycles and quickly reach high numbers when an abundant food base and favorable weather conditions are available. The numbers can continue growing in the presence of sufficient food supplies and can lead to outbreaks (White & Pickett 1985; Walker & Willig 1999). While the nutrient base of lepidopterans is mainly the leaves of trees and shrubs, it is more diverse for beetles and includes stem, fruits, seeds, roots and decaying parts. The most important pests in the coniferous forests are scolytids that attack stressed (e.g. draught) or recently killed trees (Lindgren & Raffa 2013). Sometimes, plantation of pine/spruce trees on large areas contributes to bark beetle outbreaks. The concentration of a high numbers of insect individuals in turn is also an extreme process in response to the extreme conditions of environment (Pilarska et al. 2016) (Fig. 1).

The occurrence of insect outbreaks unlocks mechanisms for the emergence of natural enemies including infectious agents such as viruses, bacteria, protozoa, microsporidia, fungi and nematodes. They are involved as a response to an already triggered chain of processes that follow one after the other in a stepwise mode. The significance of pathogenic infections depend, on one hand, on the specifics determined by the life cycle of the target insect, and on the other hand on the biological characteristics of the pathogen. These specifics determine the possibilities for successful infection initiation in insect populations. The increased frequency of infection facilitates the distribution of pathogens. Infections can cause different effects on the host – from low damages to the host to a lethal effect. The virulence degree is very important for the outcome of the infection caused by the pathogen (Lacey et al. 2001).

In holometabolous insects the larvae primarily aim at rapid growth and accumulation of reserve nutrients process to reach the imaginal stage. There are many species in which the number of generations per year is directly related to the abundance of food and the presence of favorable climatic conditions, and this can often vary.

4. Insect populations and their infections (pathogenic and parasitic)

Economically important phytophagous insects are mainly from the groups of coleopterans, lepidopterans, orthopterans and some others. The search for environmentally friendly approaches to control these pests is preceded by the study of their pathogenic and parasitic complex. The development of insect overpopulations is a prerequisite for the unlocking of natural regulatory mechanisms (pathogens, parasites, parasitoids and predators), some of which cause disease in the host organisms. Some of them can reach epizootics (Fig. 1) significantly affecting the density of pests in severe pathogenicity. So here we will look at the main groups of them and some of the successfully used microbial and parasite agents in biological control.

4.1. Viral infections

Many insects are attacked by viruses and some of them are used as biological control agents. Insect viruses are very host specific and therefore their use is in favour for an environmentally friendly biological control. There is a diversity of insect viruses, but the more often observed are those of the families Ascoviridae, Iridoviridae, Polydnaviridae, Baculoviridae, Cypoviridae, and Entomopoxviridae (Vega & Kaya 2012). The first viral disease called jaundice o grasserie (a nucleopolyhedrosis caused by a baculovirus) was reported in the silkworm (*Bombyx mori* L.) in 1524 by Vida (Ibarra & Del Rincón-Castro 2009). In 1926, Paillot described the granuloviruses (also baculoviruses) for the first time in the larvae of cabbage butterfly *Pieris brassicae* L. (Rashidan et al. 2008). The first field application of a nucleopolyhedrovirus for control of the alfalfa caterpillar (*Colias eurytheme* Boisduval) was performed by Steinhaus and colleagues (Ibarra & Del Rincón-Castro 2009).

The most common used viruses as viral bioinsecticides are those of Baculoviridae. Of the four genera of the family Alpha-, Beta-, Gamma-, and Deltabaculoviruses, the lepidopteran nucleopolyhedroviruses (NPV;

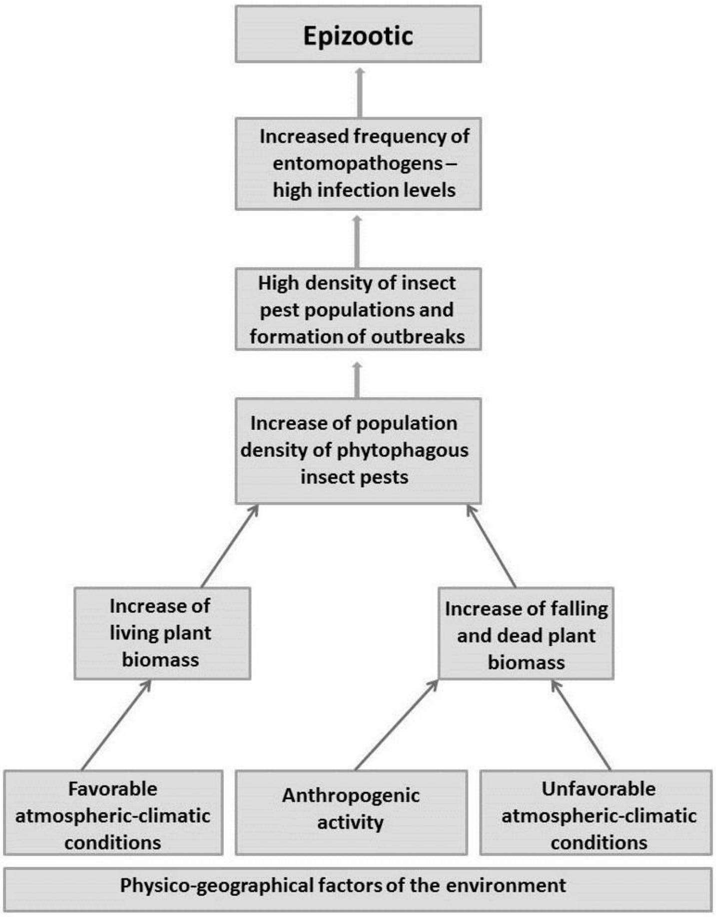

Fig. 1. Relationship between abiotic and biotic factors of nature, the insect populations, and influence of their bioagents as regulators in development of epizootic process.

Alphabaculovirus spp.) and granuloviruses (GV; *Betabaculovirus* spp.) have been commercially developed (Lacey et al. 2015). The first commercial viral bioinsecticide was developed and produced in 1975 under the name Elcar by the company Sandoz. It was used to control the *Heliothis/Helicoverpa* complex (Ibarra & Del Rincon-Castro 2009). The infective stage of baculoviruses has circular double stranded DNA within rod shaped nucleocapsids that are encased within occlusion bodies of crystalline protein. Baculoviruses play important role in controlling some globally important lepidopteran pest species such as *Helicoverpa* spp. (Rowley et al. 2011) and *Spodoptera* spp. Recently, in China, nine baculoviris products are commercially available and there are at Chinese manufacturers of *Helicoverpa armigera* NPV (HearNPV) and several of *Spodoptera litura* NPV (SpltNPV), *Autographa californica* NPV (AucaMNPV), *Plutella xylostella* GV (PlxyGV) and *Spodoptera exigua* NPV (SeMNPV) (Lacey et al. 2015). In Brazil there is a well-established program for production and use of *Anticarsia gemmatalis* NPV (AngeMNPV) for control of velvet bean caterpillar (*Anticarsia gemmatalis* Hübner) on soybean. One of the most widely used commercially developed viruses is the codling moth, *Cydia pomonella* granulovirus (CpGV) which is used recently worldwide. The use of baculoviruses in forest insect pest control in North America and Europe is still limited. *Lymantria dispar* multicapsid nuclear polyhedrosis virus (LdMNPV) is very often the main reason of collapse of natural outbreaks of this important forest pest. It is artificially produced by the U.S. Forest Service and used as biological insecticide under the registered name Gypchek (D'Amico et al. 2012). Commercial production of sawfly *Neodiprion abietis* Harris is also under development (Lacey et al. 2015).

4.2. Bacterial infections

Bacteria of genus *Bacillus* (especially *Bacillus thuringiensis* Berliner) are the most frequently used bacteria for controlling insect pest populations. They are rod-shaped and chain-forming. Under adverse atmospheric conditions they produce spores that, in some species, form parasporal bodies. The spore forming bacterium *B. thuringiensis* Berliner was isolated in 1901 by Shigetane Ishiwata from the larvae of the silkworm *Bombyx mori* (Ishiwata 1901). It was later isolated from the Mediterranean flour moth *Ephestia kuehniella* Zeller from Germany by Ernst Berliner (Milner 1994). *B. thuringiensis* (*Bt*) has over 90 subspecies. The bacterium kills through its specific toxins, of which there are over 30 types, specific to different groups of insects. Traditionally, *B. thuringiensis* strains have been classified into pathological types with activity against various lepidopterans, coleopterans and dipterans. *Bt* strains are isolated as effective agents against insect orders Blattaria, Hemiptera, Hymenoptera, Isoptera, and Orthoptera. The bacterial biopesticides are currently the largest part of the global microbial pesticide market. There are over 225 bacterial pesticides. Currently, the most important are pesticides based on *B. thuringiensis* – 80% of all pesticides. Interestingly, insects can tolerate *Bt* toxins, but genetically modified strains are being developed that are effective for biological control. The host specific pathogenicity of different *Bt* strains is encoded by genes that distinguish host recognition by the pathogen.

Another species of entomopathogenic bacteria, *Paenibacillus larvae* White*,* is an important pathogen of the European honeybees, *Apis mellifera* L. and also causes so-called milky disease in the larvae of the Japanese beetle *Popillia japonica* Newman. The first bacterial pesticide was based on *Paenibacillus popiliae* Dutky. It attacks the Japanese beetle *P. japonica.* Other important gram negative bacterial species are from *Enterobacteriaceae*, species of genus *Yersinia, Pseudomonas, Rikettsiella*. Present studies consider Gram-negative *Chromobaterium*, *Yersinia*, *Pseudomonas* as potential regulatory agents. Two species of *Photorhabdus* and *Xenorhabdus* use entomopathogenic nematodes (genus *Steinernema*) as vectors to enter the insects. *Wolbachia* is an example of a reproductive parasite. Some *Wolbachia* strains are capable of turning genetically male animals into functional females – this is the case with the Asian butterfly *Ostrinia furnacalis* Guenée. The characteristics of *Wolbachia* determine its high potential as a means of controlling arthropod-borne diseases.

4.3. Fungal infections

Entomopathogenic fungi are a group of phylogenetically diverse heterotrophic microorganisms that can act as insect parasites and can utilize insect tissues as a nutritional source to develop their life cycle. At present, there are estimated over 750 recognized species of insect-pathogenic fungi in over 100 genera (Vega et al. 2012). However, based on the number of cryptic species revealed by recent molecular studies (e.g. Rehner 2009; Rehner et al. 2011), it is evident that this number of species is underestimated. A majority of economically important species belongs to the phyla Ascomycota (order Hypocreales) and Entomophthoromycota (orders Entomophthorales and Neozygitales). While hypocrealean fungi are more general insect pathogens with a constant but low prevalence level in insect populations, Entomophthorales and Neozygitales are relatively host specific and prone to epizootic outbreaks. The latter two groups of fungi are well known for causing dramatic epizootics in host populations. In particular, *Entomophaga maimaiga* Humber, Shimazu et R. S. Soper (Entomophthoromycota: Entomophthorales) regularly causes epizootics in populations of gypsy moth caterpillars in the United States (Hajek 1999). Entomopathogenic fungi are considered excellent candidates for biological control of various insect pests in agriculture and forestry (Charnley & Collins 2007; Roy et al. 2010; Patil 2011; Humber 2016). Hypocrealean fungi such as *Beauveria* spp., *Isaria* spp., *Metarhizium* spp., and *Lecanicillium* spp. can be easily mass-produced for application as mycoinsecticides and numerous commercial formulations for inoculative and inundative biocontrol are available (de Faria & Wraight 2007; Ravensberg 2011; Reddy et al. 2013; Lacey et al. 2015). On the other hand, a mass production of entomophthoralean fungi is not possible for a majority of species, due mostly to difficulties with *in vitro* growth and inoculum stability, therefore these fungi, such as *E. maimaiga*, are suitable for strategies of classical biological control (Hajek 1999).

In a general view, the pathogenesis by entomopathogenic fungi starts with attaching of mitosporic spores (conidia) to the external body surface of insects. Under suitable temperature and humidity conditions, the conidia germinate on the host cuticle and form germ tubes that penetrate through the insect integument to colonize the hosts‘ body cavity (haemocoel) (St. Leger 1993; Hajek & St. Leger 1994). The fungi proliferate in the haemocoel, usually as walled hyphae/hyphal bodies or in the form of wall-less protoplasts. During this process, which involve the production of secondary metabolites, the internal organs of the hosts are gradually degraded and ultimately the insects die. Then the fungi grow out of the cadavers, produce conidia in suitable conditions and disseminate in the environment. High humidity is usually prerequisite for conidiogenesis. Conidia are short-lived (up to several days) propagules that are responsible for horizontal transmission of disease in populations. Several species can also form durable, but non-infective, resting spores inside host bodies. They are capable to persist a prolonged period (several years) of adverse conditions in the environment and produce infective conidia when environmental conditions become again favourable (Hajek & St. Leger 1994; Hajek 1999; Vega et al. 2012).

The introduction of entomopathogenic fungus *E. maimaiga* from Asia to the United States during the 20th century is an excellent example of successful story for classical microbial control of forest pests. *Entomophaga maimaiga* is strictly host-specific and the most important entomopathogen of gypsy moth, *Lymantria dispar* L. (Lepidoptera: Erebidae) (Hajek et al. 1996; Hajek 1999). The successful introduction of *E. maimaiga* into the North America inspired Bulgarian insect pathologists, and in 1999 the fungus was introduced into Bulgaria (Pilarska et al. 2000). Surveys conducted at release sites during subsequent years confirmed successful transmission of the pathogen to the Bulgarian populations of *L. dispar*. The first epizootics by this fungus in gypsy moth populations were observed in 2005 (Pilarska et al. 2006 2013; Georgiev et al. 2013). Since 2011, the fungus has been recorded in several countries of Central and Southeastern Europe (Kereselidze et al. 2011; Georgiev et al. 2012; Tabaković-Tošić et al. 2012; Hrašovec et al. 2013; Csóka et al. 2014; Zúbrik et al. 2014; 2016; Hoch et al. 2019).

Hypocrealean entomopathogenic fungi are among important natural mortality factors of forest pests worldwide. Although natural epizootics by these fungi are not usual in host populations, numerous laboratory experiments have already shown their high efficacy against variety of insect pests. In Europe, this group of fungi has been widely studied in outbreaks of the most destructive pest of conifer forests, the Eurasian spruce bark beetle, *Ips typographus* [L.] (Coleoptera: Curculionidae). The bark beetle attacks stressed and dying spruce trees (*Picea abies* [L.] H. Karst.), but it is capable of destroying healthy trees as soon as pest population exceeds critical numbers (Wermelinger 2004). Windstorms and severe drought periods are recognized the key triggers for a gradation of *I. typographus* (Økland & Bjørnstad 2006). Several species of hypocrealean entomopathogenic fungi can be identified from field-collected bark beetles and *Beauveria* species are usually predominant ones (Landa et al. 2001; Kreutz et al. 2004a; Wegensteiner 2007; Draganova et al. 2010, 2017; Mudrončeková et al. 2013; Wegensteiner et al. 2015a; Wegensteiner et al. 2015b; Barta et al. 2018a). For example, in Slovakia a majority (89%) of infected *I. typographus* adults collected in forests were killed with *Beauveria* and only a minor part was infected with *Isaria* (8%) or *Lecanicillium* (3%) (Barta et al. 2018a). Out of the *Beauveria* genus, *B. bassiana* (Bals.-Criv.) Vuill. is the most common species identified in bark beetle populations, but *B. pseudobassiana* S. A. Rehner et R. A. Humber, *B. caledonica* Bissett et Widden and *B. brongniartii* (Sacc.) Petch can also be detected (Takov et al. 2012; Barta et al. 2018a). Pathogenicity of *Isaria* species against bark beetles in laboratory bioassays is significantly lower when compared with *B. bassiana* (e.g. Markova 2000; Draganova et al. 2007; Herrmann & Wegensteiner 2010). *B. bassiana* is therefore tested against bark beetles in laboratory and field trials applying different approaches of inoculum introduction into the bark beetle environment. Generally, results of laboratory assays suggest its high potential for effective biocontrol, however field applications do not demonstrate adequate efficacy. This indicates a need for selecting highly effective strains and improvement of inoculum formulation or application (Hallet et al. 1994; Vaupel and Zimmermann 1996; Kreutz et al. 2004a, 2004b; Jakuš & Blaženec 2011; Vakula et al. 2012; Mudrončeková et al. 2013; Grodzki & Kosibowicz 2015). Recently, a highly-virulent *B. bassiana* strain has been selected against the bark beetle in Slovakia (Barta et al. 2018a, 2018b) and its field efficacy currently undergo a series of experiments.

4.4. Microsporidian infections

Microsporidia are obligate, intracellular pathogens which cause infections of all animal taxa. They belong to Opisthosporidia, a sister group of the true Fungi (Karpov et al. 2014) and are mostly found in insect hosts. There are more than 90 insect species recorded as type hosts of different microsporidian genera (Solter et al. 2012). Generally, entomopathogenic microsporidia are known to produce chronic effects in their hosts leading to low or moderate mortality and that there are often no external signs of infection (Yaman 2020). However they can reduce the reproduction and feeding of their hosts, which cause a decrease of the insect populations. Thus microsporidia are also good candidates for use as classical biological control agents (Hajek & Delalibera 2010; Yaman et al. 2019).

So far, there is only one commercial product available, based on *Paranosema* (*Nosema*) *locustae* Canning, for control of grasshoppers and crickets (Bjornson & Oi 2014). A good example for research on the possibilities for using microsporidia as classical biological control agents is the study on the microsporidia of the gypsy moth, *L. dispar*. A group of insect pathologists from Europe (Bulgaria, Germany, Austria, Slovakia, Czech Republic) and the United States started to screen *L. dispar* populations in Europe. As a result of their cooperation, four species of microsporidia were recovered from European and Bulgarian populations of the gypsy moth (Pilarska et al. 1998; Solter et al. 2000; Vavra et al. 2006; Pilarska et al. 2010). The life history, morphology, host tissue specificity, virulence and persistence, and biology of several of these microsporidia have been extensively studied to elucidate interactions with the host and to facilitate decisions regarding use in classical biological control programs (Solter at al. 2000, 2002; Goertz et al. 2004; Vavra et al. 2006; Hoch et al. 2008; Pilarska et al. 2010). A release of two microsporidian species *Vairimorpha disparis* Timofejeva and *Nosema lymantriae* Weiser isolated from Bulgaria was conducted in 2007 and 2008 in two Bulgarian gypsy moth populations and it was shown that *N. lymantriae* persisted in the test population within three years (Pilarska et al. 2010).

4.5. Protozoan infections

Protozoans exhibit a full range of relationships with insects - from mutualism to parasitism. Apicomplexa (Sporozoa) is the main phylum whose representatives develop intracellularly. Most of the eugregarines are commensal while others, such as neogregarins and coccidia are highly pathogenic. Their host specificity varies greatly. There are several obvious outward signs of symptoms, which are typically chronic and occult, with no apparent discoloration or appearance on the body surface of the species affected. Insect larvae that are severely infected with neogregarins and coccidia show swelling and sluggishness in their movements. These signs and behavior can easily be confused with the process of pupation.

Amoebae, for example, are eukaryotes that are in the Amebozoa. The presence of pseudopods and cytoplasm movement during locomotion and feeding is a characteristic feature of the group. Postmortem diagnosis, after the death caused by protozoan infections, is easy. Because of their larger size they are clearly visible under a light microscope and have a characteristic shape and morphology. Over six species of amoebae (mainly the genus *Malamoeba*) of three genera are found mainly in grasshoppers, beetles (bark beetles and other curculionids), bees and apterygots.

The eugregarines are larger-sized unicellular obligate parasites, mainly found in the gut of dipterans, beetles, cockroaches, odonates and orthopterans, mainly of the genera *Gregarina, Ascogregarina, Haplorhynchus*. Several reviews (Brooks 1988; Boucias & Pendland 1998; Vega & Kaya 2012) concluded that eugregarins are symbionts that fluctuate between mutualism, commensalism and parasitism depending on a number of factors. Some of the eugregarines – Aseptata, such as Ascogregarina, would have potential as biological agents. Studies have shown that there are geographic variations in pathogenicity, for example, some strains of *Ascogregarina culicis* Ross are pathogenic to *Aedes aegypti* L. (Sulaiman 1992).

Neogregarines, mostly known as schizogregarins, are found almost exclusively in insects. They form pellicle-coated movable sporozoites and merozoites that are characteristic of the type. Oocysts of the neogregarines that form gametocysts are typically ovate or lemon-shaped. The main genera are *Matessia* and *Farinocystis*, all of which infect pests, feeding on fruits, seeds and nuts. The genus *Cauleryella* infects dipterans – mainly mosquitoes. *Ophryocystis* is another genus mainly found in the Malpighian tubes of beetles.

Some coccidia, such as *Adelina,* are found in Coleoptera, Lepidoptera, Orthoptera, Diptera, Embioptera and Blattaria. Infection occurs via food, with some species inhabiting the gut. It begins by penetrating the ingestion of oocysts and sporocysts into suitable hosts. Coccidia are mainly vertebrate parasites, with less than 1% infecting insects, and in most cases the insects are vectors for the coccidia to other hosts (Lange & Lord 2012).

4.6. Nematode infections

Nematodes occur often as parasites or are associated mainly with forest coleopterans (Curculionidae, Cerambycidae and other families of the order). Bark beetles are one of the xylophagous insects causing damages in the forest ecosystems and are known as hosts or vectors of nematodes. Among with the nematodes there are many phoretic or mutualistic species that are associated with bark beetles and are common in their galleries (Rühm 1956; Massey 1974). The phoretic nematodes use bark beetles as a means of transport to reach new food plants and their tissues. Very often, phoretic nematode species can enter the haemocoel and cause an enlarged fat body and undeveloped gonads compared to uninfected individuals.

However, for controling populations of beetles, only the parasitic nematodes are of importance. Most important is the role of the Tylenchida (Siddiqui 2000) and Aphelenchida (Poinar 1975) groups. The genera *Cryptaphelenchus, Parasitylenchus* and *Contortylenchus* are primarily found in the bodies of the beetles – in the Malpighian tubules or actively entering the larvae through the oral or anal opening or through the cuticle of the pupae. Female nematodes of the genus *Contortylenchus* can penetrate the beetles directly through the integument, although penetration through the oral and anal openings with subsequent intestinal penetration into the haemocoel is also possible. Once there, the females can be found in the thorax and abdomen. The infected beetle larvae continue to develop, pupate and become adults. There, the female nematodes continue their development with the formation of eggs. But usually eggs do not hatch until mature beetles develop. After four larval instars of nematodes taking place in adult beetles, they migrate to the galleries with the faecal mass of the host, where they reach maturity and the males and females mate. The males then quickly die and the fertilized females reenter the bark beetle larvae and pupae to start the cycle again (Kaya 1984). Wegensteiner et al. (2015b) listed more than 20 species of bark beetles that are associated in one or another degree with nematodes, emphasizing that genera such as *Parasitylenchus*, *Sulphuretylenchus* and *Neoparasitylenchus* are capable of killing bark beetles under certain conditions. The number of individuals in a single host can reach hundreds. Classically entomopathogenic nematodes for the control of bark beetles under experimental conditions are those of the family Steinernematidae and Heterorhabditidae (Gaugler & Kaya 1990). In natural populations of hosts, however, they have not been found.

The nematodes related with the insects in the forest are devided into two groups: typical parasites and species associated with the insects to varying degrees. The latter can develop different types of relationships leading to simple phoresis or passage through the intestine or haemocoel of the insect, and can damage insect health. One group of the nematodes in the forest communities inhabits the soil, others are phytoparasitic, developing in the wood, and the third group develops in rotting and decaying parts of dead trees, where certain fungal species have previously developed. There are also typical parasites found in the bodies of some forest insects. Very often, insects are vectors for nematodes, spreading them to forest areas.

A typical example for phoresis is the transportation of the nematodes of genus *Bursaphelenchus* (Parasitaphelenchidae) by the beetles of familes Cerambycidae and Curculionidae. Nematodes are ofter transported below the elytra of the beetles or on the surface of the body and their legs. Many nematode species develop in insect galleries of some bark beetles which cultivate fungi to feed the larval stages (Wang et al. 2013).

The nematodes have chemoreceptors, actively search for their host and enter it as invasive larvae. High rates of infection are often reported, with values reaching 90–100% for a single species of host, and the intensity of infection can reach 7 500 nematode larvae and eggs in the haemocoel of one individual (Kaya 1984). In the case of severe invasion, reduced fertility and sterility of bark beetles are often reported: developmental delay, reduction in the size of the fat body and infestation of the Malpighian tubules and the trachea (Wegensteiner 2004). Damage can range from reduced fertility to death of the host, mostly caused by species of Rhabditida order and some Tylenchida order. The parasitic nematodes of the beetles in most cases do not kill their hosts immediately, even when more than one nematode enters the haemocoel.

5. Basic aspects of the epizootiological process

For the prognosis of epizootic dynamics, the characteristics associated with the particular insect host, the peculiarities of the pathogen and the dynamics of the environment are considered in order to construct models of the epizootic process. Models are theoretical, empirical, and simulative (Onstad & Carruthers 1990).

Different models set out certain temporal and spatial characteristics related to host dynamics. According to some authors (Bailey 1975) a homogeneous pathogen transmission mode is assumed, while others (Getz & Pickering 1983) replace absolute density with the proportion of sensitive specimens, such as those transmitted sexually, where the density threshold does not exist.

In turn, the host population may be homogeneous or heterogeneous. Spatial distribution is simulated by different models. For example, one model simulates mortality caused by NPV *Malacosoma pluviale* (Welington et al. 1975), dividing hosts into eight different classes – from pathogen-free to highly infected nests, with vertical transmission based on fixed rates that depend on the degree of disease in the colony. Horizontal transmission is dynamically modeled and evaluated at eight daily intervals. According to the authors, the progression of the disease is based on spatial proximity to other diseased colonies for spatial network formation, host density and age, and quality characteristics such as genetics and insect stress. Comprehensive modeling of NPV in *L. dispar* populations (Doane 1976) had been performed to study the effects of virulence and virus transmission during epizootic development. Models were based on several features: larval body size, intestinal loading of viral particles, inoculum spread into the environment, natural breakdown of the virus, and amount of infected leaf mass consumed by the larvae (Etter1981; Valenttine 1981; Valentine & Podgwaite 1982). The model predicted density based on baseline density and terminal morbidity levels. The results showed that simulated increases in virus mortality could lead to an overall decrease in *L. dispar* mortality by reducing the effects of the virus.

Anderson & May (1981) analyzed the conditions necessary for host regulation and created models describing the pathogen-host interaction. They discuss eight different models, all of which included a number of parameters: horizontal and vertical dynamics of pathogen transmission, host sensitivity, frequency of reproduction and mortality, presence of a latent period, fertility reduction, fertility-related, mortality dependent on the density and presence of infectious agents (spores) in the medium. The authors noted that all these processes (factors) are important for the dynamics of the disease, except for the virulence caused by stress. They concluded that pathogens often regulate their hosts, suggesting that the infectious stages would be homogeneously distributed in the environment and would continuously mix with the susceptible host population. The conclusion was that if a highly virulent pathogen has produced a large number of persistent free-living stages, then stable cyclic changes in host density were likely to occur. According to epidemiological theory, the density threshold is equivalent to herd immunity (collective immunity) (Kermack & McKendrick 1927; Bailey 1975). Anderson & May (1981), for their part, considered that the host threshold level was sufficient to explain the high density and epidemic outbreaks of the disease, and also concluded that when host abundance fell below threshold levels, the pathogen could be, periodically or episodically, maintained at intervals by producing free-living infectious stages transmitted vertically or by occult infection (Regniere 1984).

According to Onstad et al. (1990), who examined the system *Ostrinia nubilalis* Hübner – *Nosema pyrausta* Paillot the threshold changed with the original spread of disease changes, indicating that both the prevalence and the density of susceptible individuals had to be measured in order to predict the future dynamics. In theoretical models, interaction between infected and susceptible hosts is considered to be the determining factor for transmission of infection. The authors assumed that the contamination degree was directly proportional to the densities of the two types of hosts. This is based on the belief that the population is homogeneous and in all respects constantly mixed with the infected individuals of the population, which is crucial for the development and spread of the disease, as well as for their specific interactions in a changing environment. Pathogens that require alternative hosts infect multiple species or produce different spores under different environmental conditions, and they represent an example of behavior that is difficult to predict (Onstad & Carruthers 1990).

6. Conclusion

The relationship between the density of insect populations and their pathogens is natural (or prospective), since the pathogenic process appears to be regulatory for the increased numbers of its hosts. On the other hand, the pathogen-host relationship must be considered at the species level for both groups of organisms. For the host, the particularities of the lifestyle (whether hidden or not, whether in groups or not), the type of food resource they use (leaves, stem, roots, fruits, seeds, decomposing plant and tree organic matter), the degree of contact between individuals and their duration over time, the mode of reproduction (monogamy, polygamy), the number of generations per year, etc. are essential.

For pathogens, most important is the access to the host insect, their virulence and pathogenicity, the mode of infection and transmission (oral-fecal route, surface contact, active parasite entry, transovarial transmission). Maintaining foci of infection in insect populations is also important for a successful spread in the host population.

Fungal pathogens have the greatest potential because of their high virulence and pathogenicity, due to toxic compounds produced. At the same time, they have an effective and rapid spread (through spores, airborne transfer) and an easy way to infect (with superficial contact through the insect cuticle). Infections caused by protozoa, microsporidia and nematodes lead to a chronic process in their host and there is no effective control of the pest host density during mass outbreaks. Often these pathogens are not host specific. A key problem remains to find host-specific pathogens that do not affect non-target insect groups. This is a major task for researchers in the field of biological control.

Since we cannot easily control environmental and anthropogenic factors and thus population growth of insects, the main task should be directed to the efficient-management of natural resources, which includes good expert forecasting of the forthcoming processes of phenomena and their impact in the ecosystems.

In our opinion, when an insect calamity occurrs (due to increased nutrient base and depending on the life cycle of a host, environmental conditions and virulence of a particular pathogen), three main development scenarios are possible:

1. Death of a small number of infected individuals and attenuation of the infection process.
2. Occurrence of a number of individual infection foci (groups of infected individuals) and death of indi-

viduals in them, but without major damage to the host population.

3. Diffuse developing spread of the pathogen with mass infection of individuals and subsequent development of an epizootic with the presence of high mortality in the population of host insects.

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ORIGINAL PAPER

Evaluation of insect pest occurrence in areas of calamitous mortality of Scots pine

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Abstract

Pinus sylvestris is an important production tree. In recent years, there has been a sharp increase in the mortality of pine trees due to insect pests. It is obvious that some pests profit from climatic changes, increase their aggressiveness and spread to new localities. The study aimed to investigate the spectrum of more abundant insect pest species in pine plantations of Czechia. The occurrence of species and intensity of their infestation were studied at 77 localities situated in six regions. Any abundant foliophagous insect species were noticed. Bark beetles and wood-boring insects were found to be prevalent. Namely, *Ips acuminatus*, *Ips sexdentatus*, *Phaenops cyanea* and *Sirex noctilio* seem to be the most dangerous. These species are now better competitors than species previously considered as main pests. Their aggressiveness, expansion to new areas and interspecies co-occurrence are alarming. Due to the advancing climate change, pine mortality due to bark beetles and wood-boring pests will probably continue to increase.

Key words: *Pinus sylvestris*; biotic stressors; Coleoptera; Hymenoptera; Lepidoptera; central Europe

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1. Introduction

Scots pine (*Pinus sylvestris*) has a wide range of applications. Among others, it is used as construction timber and as raw material for pulpwood and paper. Therefore, from the mid-19th century, it began to be artificially cultivated on plantations in central Europe, which greatly expanded its occurrence even outside the original poor soil (Ruotsalainen & Persson 2013). The current proportion of pine trees in the forests of Czechia is 16.1%, while in the recommended composition of forests, their representation is higher by 0.7% (MZe 2020). This remains the recommended proportion despite continuing discussions about the negative impact of coniferous plantations on other components of the ecosystem and proposals for their transformation into deciduous forests (Chiarucci & de Dominicis 1995; Augusto et al. 2002; Poštulka & Bláha 2008; Hartmann et al. 2010). The decline of pine forests may not be the result of a targeted human effort but a consequence of natural processes. Like spruces, pine plantations are susceptible to damage by factors both abiotic (wind, drought, snow) and biotic (herbivorous, bark- and wood-destroying insects). These factors are interconnected by mutually reinforcing relationships (Dobbertin et al. 2007; Sierota et al. 2019).

Climate change and insufficient implementation of pest management can be identified as the main causes of the increase in damage of pine populations (Lindner et al. 2010; Lubojacký et al. 2019). Climate change facilitates the development of pests in several ways. It weakens trees by a lack of rainfall, higher temperatures and changes in the nutrient cycles. At the same time, it accelerates the development of insects and allows the spread of non-native species (see Lindner et al. 2008). A shortening of the gradation cycles of herbivorous insects can be expected in the future, but it has the “advantage” of predictable population collapse after a few years (Berryman 1996; Haynes et al. 2014). However, this is not the case of bark beetles (Biedermann et al. 2019), which is why we can expect the problem of bark beetles and wood-boring insects to worsen in the future. This prediction is corroborated by data comparisons, which show that the damage caused by bark beetles has been gradually increasing in recent years. For example, in 2009, bark beetles caused damage of about 10,000 m^3, while in 2019, damages exceeded 80,000 m^3 of pine wood (Knížek 2010; Knížek & Liška 2020). Furthermore, these official figures are underestimated. Forest managers often do not want to draw attention to their inaction in forest protection and provide distorted statistical data.

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Experts warned that climate change would cause a decline of pine forests more than a decade ago (Dobbertin et al. 2005; Lindner et al. 2008). These expectations have been fulfilled, and in recent years, extensive drying of pine trees started to be observed in Czechia (e.g. Knížek & Liška 2019, 2020).

Widespread mortality of trees associated with the formation of large clearings is undesirable from ecological and socio-economic points of view (Andréassian 2004; Weis et al. 2006; Lindner et al. 2008; Schelker et al. 2014). Rather than creating clearings, it is more advantageous to gradually increase the admixtures of other woody plants (Souček et al. 2018). Therefore, it is necessary to reduce the impact of harmful biotic agents. Appropriate forest protection methods can be planned and applied only with a detailed knowledge of pest agents along with a prognosis of their development (Southwood 1978). For this reason, we tried to assess the current condition of major insect pests in a pine forests and determine the pest distribution in Czechia.

2. Material and methods

The occurrence of beetles was monitored by observing their galleries on dead and dying trees, which were determined by the colour change of their needles. Only trees in a group of at least five dying trees were included in our study. On all trees (max. 15 at one locality), the bark in the trunk part was peeled off at three sections, with each section comprising an area of about 1,000 cm^2. To determine pest presence, galleries, halls or entrances were used. Galleries or entrances in the crown sections of standing trees were determined using high-brightness binoculars. The intensity of infestation was recorded on a scale of 0 (no infestation), 1 – weak (a rare occurrence), 2 – medium (only a few galleries on the peeled plot) and 3 – strong (galleries covering more than 75% of the bark area). In the case of horntails (Siricidae), the number of boreholes was similarly evaluated on a scale of 0 – no hole, 1 – one hole, 2 – two to five holes and 3 – more than five holes. We also looked for the presence of needles damaged by foliophagous insects on the investigated trees as well as on the surrounding ten trees. Observations were made separately at the pole timber stands (23 localities) and mature stands (54 localities). The studied areas were located in central, northern, eastern, western and southern Bohemia, respectively, and western and southern Moravia. Approximately one-third of the trees were monitored in autumn and winter of 2019, the rest in spring and autumn of 2020. We used unconstrained principal correspondence analysis (PCA) for visualisation of species co-occurrence. Both ordination analyses were performed in Canoco 5 (Šmilauer & Lepš 2014). The differences in the abundance of species between forest stands were evaluated by Mann–Whitney tests in Statistica 10.

3. Results

A total of 10 taxa with more than two occurrences were recorded (Table 1). All of these species belonged to wood-boring or bark insects. No strong damage caused by a foliophagous insect was observed. *Ips acuminatus*, *Phaenops cyanea*, *Sirex noctilio* and *Tomicus piniperda* were found in all the examined regions. *I. acuminatus* was the species that most often manifested a strong infestation in both forest types. It was detected in all of pole timber stands and majority of mature stands. It was present mainly in the strong intensity of the attack. In both forest types, it often occurred together with *Ips sexdentatus* (Fig. 1). This species was found only in a few localities, but mainly in the strong intensity of the infestation. The occurrence of both mentioned species in the mature forests was negatively correlated with the occurrence of two other species of bark beetles, which, in contrast, often occur together: *Tomicus minor* and *T. piniperda*. We found *T. piniperda* in 61% of mature stands, most often only in weak or medium infestation. *T. minor* occurred in only 17% of localities, also in weak and medium infestation intensities, hence it was not responsible for any strong attacks. Together with these, two other beetle species, namely *P. cyanea* and *Monochamus galloprovincialis*, occurred. *P. cyanea* was found in 70% of pole timber stands, where the infestation intensities were comparable, and in almost all the studied mature stands, where medium infestation intensities were prevalent. It was significantly more numerous in mature forests ($p = 0.04$).

Table 1. Occurrence and percentage frequency of infestation intensity (0–3) of 10 monitored species. CB – central Bohemia, EB – eastern Bohemia, NEB – north-eastern Bohemia, SM – southern Moravia, WB – western Bohemia, WM – western Moravia.

	Regional species occurrence						Intensity of infestation							
							Mature stands				Pole timber stands			
	SM	CB	NEB	EB	WB	WM	0	1	2	3	0	1	2	3
Chalcophora mariana		×	×				85.2	13.0	1.9	0	95.7	4.3	0	0
Ips acuminatus	×	×	×	×	×	×	3.7	11.1	25.9	59.3	0	30.4	26.1	43.5
Ips sexdentatus	×	×					92.6	1.9	1.9	3.7	82.6	0	4.3	13
Monochamus galloprovincialis		×	×	×			92.6	7.4	0	0	100	0.0	0	0
Orthotomicus longicollis		×					96.3	1.9	0	1.9	100	0.0	0	0
Phaenops cyanea	×	×	×	×	×	×	5.6	24.1	40.7	29.6	30.4	21.7	26.1	21.7
Pissodes spp.		×		×			88.8	1.9	5.6	3.7	82.6	17.4	0	0
Sirex noctilio	×	×	×	×	×	×	20.4	25.9	48.1	5.6	26.1	43.5	30.4	0
Tomicus minor		×	×	×			83.3	9.3	7.4	0	91.3	4.3	4.3	0
Tomicus piniperda	×	×	×	×	×	×	38.9	51.9	7.4	1.9	43.5	47.8	8.7	0

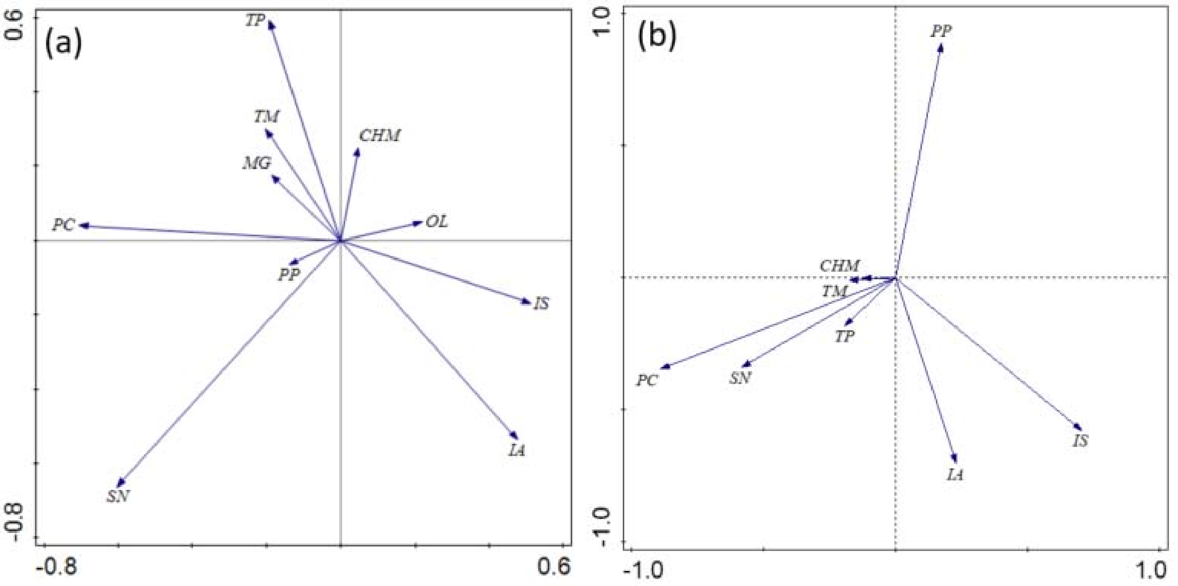

Fig. 1. Unconstrained PCA ordination results for the co-occurrence of 10 species in (a) mature stands and (b) pole timber stands. IA – *Ips acuminatus*, TM – *Tomicus minor*, SN – *Sirex noctilio*, PC – *Phaenops cyanea*, IS – *Ips sexdentatus*, TP – *Tomicus piniperda*, PP – *Pissodes* spp., CHM – *Chalcophora mariana*, MG – *Monochamus galloprovincialis*, OL – *Orthotomicus longicollis*.

Monochamus galloprovincialis was detected in only 7.5% of mature pine forest stands and only in the weak infestation intensity. Also recorded was a mostly weak attack of *Chalcophora mariana*. Representatives of *Pissodes* were found more often in pole timber stands and only in weak infestation. The only representative of the Hymenoptera was *S. noctilio*. It was recorded in 80% of mature stands and in 74% of pole stands, and was more numerous in mature stands ($p = 0.02$). In the mature stands, medium infestation prevailed, while in the pole timber stands, weak infestation was prevalent. Co-occurrence was recorded only with *Pissodes* in mature stands and with *T. piniperda* and *P. cyanea* in pole timber stages.

Other species were detected only in rare occurrences and in low abundances. However, the strong occurrence of the invasive bark beetle *Gnathotrichus materiarius* near Kardašova Řečice (southern Bohemia) is worth mentioning.

4. Discussion

Ips acuminatus was identified as the most common species with strong infestation. A similar incidence in both tree age categories is consistent with the findings of other studies. For example, Siittonen (2014) found it on trees with a diameter of 8–49 cm. During outbreaks, *I. acuminatus* can attack even stronger trees otherwise not prone to infestation (Colombari et al. 2013). This species has long been considered a typically secondary pest with a limited effect on tree mortality (Siittonen 2014). At the beginning of the millennium, it began to grow in aggression. Its harmful effects increase not only in central Europe but also elsewhere (Foit 2007; Grégoire & Evans 2007; Wermelinger et al. 2008; Colombari et al. 2013; Siittonen 2014). In the conditions of central Europe, *I. acuminatus* is now one of the first colonizers of weakened trees (Foit & Čermák 2014).

The change in the harmfulness of *I. acuminatus* can be caused by higher spring temperatures (Chinellato et al. 2014). We observed it mostly in a strong infestation. In this case, the galleries can occupy more than 90% of the bark area in crowns (Siittonen 2014). With respect to population, the recorded incidence of *I. acuminatus* appears as outbreaks. Outbreaks are characteristic by the occurrence of infested trees in foci, while in periods of low abundance, the distances between infested trees are higher (Colombari et al. 2013). The number of killed trees in the studied areas reflects the state of the population well, as the numbers of infested trees in one plot do not increase year-on-year because the beetles fly to new areas up to hundreds of meters away in spring. It is precisely this first generation of beetles that causes the greatest damage (Colombari et al. 2013). In the examined areas, *I. acuminatus* often co-occurred together with *I. sexdentatus*. Both species have much in common: by means of aggregation pheromones, they undertake mass attack, and they are associated with *Ambrosiella* species that are phylogenetically related to *Ophiostoma*, which trigger hypersensitive response in plants. Both these bark beetle species use mycangia for fungal transport (Kirisits 2007; Lieutier 2007). The occurrence of *I. sexdentatus* in the studied areas was relatively rare. Historically, its presence is known especially in south-eastern Moravia (Pfeffer 1955), which corresponds with our results. Our unique findings of this species in Bohemia confirm its expansion (Knížek 2020). The strong intensity of the infestation together with the observed spread in Bohemia ranks both species among the taxa that require attention.

Tomicus minor is also a mycophagous species, but this beetle does not have mycangia, and its mycobionts differ considerably (Francke-Grosmann 1963; Kirisits 2007). This could partly explain the negative dependence on *I. acuminatus* and *I. sexdentatus* that was found in the studied mature forest stands. *Ophiostoma canum*, which is primarily used by *T. minor*, develops relatively slowly and does not benefit from higher temperatures as much as other fungi (Solheim et al. 2001; Kirisits 2007). Species of *Ips* can fly over longer distances (Jactel & Gaillard 1991; Bouhot et al. 1988; Lieutier & Faccoli 2015) and thus better occupy freshly weakened trees. *T. minor* is probably not able to compete sufficiently with the above species. The existing competition among *T. minor* and species of *Ips* is obvious. A comparison of habitat parameters further shows that *T. minor* competes partially with *I. sexdentatus* and strongly with *I. acuminatus* (Sauvard 2007).

The positive correlation found between *T. minor* and *T. piniperda* is not surprising. These species commonly occur together on one tree, as their habitat demands do not overlap (Sauvard 2007). *T. minor* is considered to be the secondary colonizer of the subsequent *T. piniperda* (Långström & Hellqvist 1993). Neither of these species showed strong aggression. This confirms the assumption that species aggressiveness also depends on the virulence of associated blue-stain fungi, and the fungi used by *Tomicus* have low virulence (Krokene & Solheim 1998; Solheim et al. 2001).

Along with *Tomicus* species, also *P. cyanea* was very numerous and recorded in all regions. It occurred in majority of mature forest stands, where the intensity of infestation was most often medium, and in more than two thirds of the studied pole timber stands. It is a common aggressive species, which can successfully attack older and only slightly weakened pines (Wermelinger et al. 2008; Sowiñska et al. 2000; Foit & Čermák 2014). Research conducted in German forests has confirmed that the species responds positively to global warming (Wulf 1995). It is one of the most common pests in Switzerland, where it periodically causes significant pine damages (Dobbertin et al. 2007). During pest monitoring in the second half of the 1980s, Kolk (1992) recorded the presence of *P. cyanea* on more than 20% of trees and predicted an abundance increase in damaged and weakened trees. From our results and the results of the above studies, it is clear that the abundance of this beetle indeed increases. Infested trees show a decrease in xylem flow, reduced content of wood moisture and increased heartwood/sapwood ratio (Apel et al. 2000), thus enabling a successful invasion of other species (Lindner et al. 2008).

The only recorded species in our study not belonging to the beetles is *S. noctilio*. It was detected in approximately three quarters of the studied localities, most often in a weak and medium degree of infestation. It occurred independently from all the other more abundant species. Co-occurrence was recorded with *Pissodes* spp. in mature stands and with *T. piniperda* and *P. cyanea* in pole timber stands. The co-occurrence of *S. noctilio* with *T. piniperda* and *Pissodes* is described in a Swiss study published by Wermelinger et al. (2008). The same authors state that the links to other insect species are weak. The independent occurrence of *S. noctilio* from other species can be explained by its ability to attack almost healthy trees and partly also by the competition between fungal associates of *S. noctilio* and bark beetles (Bordeaux & Dean 2012; Ryan et al. 2012). *S. noctilio* is a major pest in the SouthernHemisphere, where it was introduced, but until recently it was considered an economically insignificant species in Europe (see Hurley et al. 2007; Wermelinger et al. 2008). It benefits from the weakening of pines by drought, as well as their partial regeneration after short rains, which enhances the development of larvae (Madden 1988). The infestation of trees is characterized by a markedly focal character, while the area of foci is constantly increasing (Corley et al. 2007). This wasp has eruptive population dynamics (Madden 1988; Corley & Villacide 2012). Due to its relatively abundant occurrence in our studied areas, it can be concluded that *S. noctilio* is now in the outbreak phase and owing to its excellent flight abilities, we expect rapid spread to new localities.

5. Conclusion

In recent years, there has been an increase in temperatures and a decrease in the amount of precipitation during the growing season (Hanel et al. 2011). Higher temperatures are associated with increased stress on trees and shorter development time of economically undesirable organisms, thereby prolonging the reproductive season and increasing voltinism. Shorter development times also reduce the exposure time of larvae to their natural enemies (Chinellato et al. 2014). The results of this study confirm the significant influence of insect pests, especially *I. amitinus*, *I. sexdentatus*, *P. cyanea* and *S. noctilio*. What is alarming is the abundance and co-occurrence of aggressive species or their expansion to new areas. Due to the advancing climate change, pine mortality due to bark beetles and wood-boring pests will probably continue to increase. Therefore, pest management should become a necessary priority in pine plantations in the face of climate change.

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REVIEW PAPER

Ecology, management and damage by the large pine weevil (*Hylobius abietis*) (Coleoptera: Curculionidae) in coniferous forests within Europe

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Abstract

Coniferous forests in Europe have a considerable number of pests that attack trees of all ages from youngest up to oldest ones. One of them is the large pine weevil *Hylobius abietis*. This species is widespread in Europe and occurs from warm southern areas (Spain) up to northern countries (Sweden, Finland). Larvae of this species do not cause damage, they help in the decomposition process of stumps and roots. Adults are harmful as they consume bark of young coniferous seedlings, above all of spruce and pine. One adult can consume on average 75 mm^2 of bark per day. Individuals can live up to 4 years, and during their lives they can damage and kill several tens of coniferous seedlings. Traditional chemical protection of seedlings against this pest is and will gradually be more and more restricted or forbidden. In this review, we describe this method as well as all other alternative methods including biological protection. We estimate that *H. abietis* causes annual damages in Europe of almost 120 million € and damages several tens of thousands of hectares of young forest stands.

Key words: biocontrol; *Beauveria bassiana*; control; costs; life history; pest

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1. Introduction

The large pine weevil *Hylobius abietis* (Linnaeus 1758) (Coleoptera: Curculionidae) is an important forest pest that is widespread over the whole Europe and Asia (Scott & King, 1974; Långström & Day 2004). It is estimated that in Europe it annually causes damages equal to 140 million € (Långström & Day 2004). Since the beginning of the last century it has been considered a pest of young coniferous forest stands (Trägårdh 1913). Adults are pests of young coniferous seedlings (Stoakley & Heritage 1990; Wallertz et al. 2006; Thorpe & Day 2008; Gradinariu et al. 2012), but can also feed on roots or branches of older coniferous trees (Eidmann 1974; Örlander et al. 2000; Wallertz et al. 2006). Population of *H. abietis* is dependent on the number of fresh stumps (Långström & Day 2004). The number of fresh stumps suitable for the weevil development increases due to wind and bark beetle disturbances (Kunca et al. 2016), which provide more habitats for the reproduction of *H. abietis* (dead and dying trees; Nordlander 1987), and its reproduction is stimulated by logging and subsequent planting, which is a prevailing approach in European forestry (Nilsson et al. 2010). Several seedling protection methods are described below.

2. Bionomics and a life-cycle

Hylobius abietis adults are dark brown with yellow and light-brown spots arranged in irregular rows on their elytra. They are 10 to 14 mm long; their head is extended to a distinct snout with antennae and a mouth opening at its end (Zúbrik et al. 2013).

Larvae develop in stumps and roots of dying and dead coniferous trees. Eggs are laid in small holes in the bark of roots gnawed by adults at the end of spring (Scott & King 1974; Nordenhem & Nordlander 1994; Inward et al. 2012). Larvae develop through four larval stages before pupation. Eggs occur in the bark of roots or stumps just

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*Corresponding author. Michal Lalík, e-mail: michal.lalik@nlcsk.org under the soil surface, and after hatched larvae migrate to roots (Pye & Claesson 1981).

Adults emerge from soil, roots or stumps in spring, when temperatures reach 8 – 9 °C (Munro 1928; Nordenhem 1989). In spring, adults consume bark of coniferous and other tree species, and they mature sexually before mating and egg laying. According to Fedderwitz et al. (2018), *H. abietis* spends 6% of its lifetime for feeding, while they feeds 4 to 5 times a day and consume approximately 13 mm^2 of bark at one feeding. Adults stay at one place after egg laying until the end of summer. Some adults leave their pupal chambers at the end of summer (July to September), but most of them overwinter in the pupal chamber (Nordenhem 1989). Overwintering can occur in the third or fourth instar phase and in maturity. An average super cooling point of larvae is −12.6 °C (Luik & Voolma 1989). This temperature is not very low (Leather et al. 1993), which is probably due to *H. abietis* adaptation to overwintering in soil. Adults can live up to four years (Eidmann 1979; Leather et al. 1999). Sex ratio is around 50:50, although the proportion of trapped female beetles can vary between 20 to 70% depending on the season (Långström 1982; Tilles et al. 1986; Djeddour 1996; Lalík et al. 2019). In the first egg-laying season, young female need two to three weeks to reach their sexual maturity, while females in the second or third year are immediately ready to mate and lay eggs (Fig. 1a) (Bejer-Petersen et al. 1962; Nordenhem 1989). After mating females search for fresh coniferous stumps that are suitable for egg laying. They chew holes in the bark, in which they lay between one and five eggs (Salisbury 1996). The egg-laying period lasts from May to September with the peak between the middle of May and the beginning of June (Bejer-Petersen et al. 1962; Lekander et al. 1985). Egg laying and insect fertility are affected by a number of factors, e.g. size, life span, nutrition of adults and larvae, and plant stimulants (Leather 1987, 1988; Leather et al. 1995). The eggs are laid most frequently within 10 cm part of stumps under ground, although some eggs were found in the depths of more than 40 cm (Pye & Claesson 1981). In Scandinavia (Nordenhem 1989) and at higher elevations of Central Europe (Lalík et al. 2019), stumps can remain suitable for egg laying for three years. Development of eggs and larvae is faster in stump parts closer to soil surface that are warmer than those deeper under the ground. Fast development increases the survival chance because of sibling cannibalism (Henry 1995) and natural enemies (Pye & Claesson 1981).

Larvae are whitish with brown heads, legless, slightly arched, and 12 to 23 mm long (Fig. 1b). Pupae are loose, white, soft, with relatively long and narrow appendages. Body parts of a future adult including its nose are visible on a pupa (Zúbrik et al. 2013).

Depending on the microclimate and the quality of host stumps, larvae can develop from an egginto adult within one year, or larvae can pupate, and adults can develop only in the next year. In colder climatic conditions, larval development usually lasts two years, but can take up to five years. However, 75% of individuals in Europe develop within one year (Bejer-Petersen et al. 1962; Scott & King 1974). Sunny place on stumps also positively affect larval development. Larvae closer to the

Fig. 1. Developmental stages of *Hylobius abietis*: a) egg, b) larva, c) adult, d) adult feeding on the spruce seedling (photo by authors).

soil surface develop faster than those deeper in stumps underground (Fraser 1952; Bakke & Lekander 1965). The higher temperatures of up to 25 °C accelerate larval development and also affect the duration of a diapause period, which lasted from 60 to 220 days at temperatures between 10 °C and 20 °C, but lasted only 17 days at 25 °C (Eidmann 1963). Unlike most insect species (Kingsolver & Huey 2008), the size of adults increases if larvae develop at higher temperatures (Inward et al. 2012). If temperature falls below 8 °C, adult weevils (Fig. 1c) bury themselves underground and start to hibernate (Munro 1928; Nordenhem 1989). They frequently occur at the border between soil and litter of adult forest stands (Leather et al. 1999).

2.2. Damage of tree species

Adults of *H. abietis* are pests of young coniferous seedlings (Fig. 1d), such as *Abies alba* Miller, *Larix decidua* Miller*, Picea* spp.*, Pinus* spp*, Pseudotsuga menziensi* Franco (Stoakley & Heritage 1990; Wallertz et al. 2006; Thorpe & Day 2008; Gradinariu et al. 2012), but can also feed on roots or branches of older coniferous trees (Eidmann 1974; Örlander et al. 2000; Wallertz et al. 2006). Adults can feed on thin bark of coniferous tree species, but also of other host tree species, e.g. hazel (*Corylus avellana* Linnaeus), oak (*Quercus* spp.), or even heather (*Calluna vulgaris* Hull) (Munro 1928; Djeddour 1996).

Adults feed on bark of stems of diameter of 2 mm to 20 mm, but prefer food sources (seedlings, twigs, roots) with a diameter of around 10 mm (Örlander et al. 2000; Wallertz et al. 2005). Adults cause the greatest damage to seedlings, as they can completely consume bark and can subsequently cause seedling death. With no preventive measures, the mortality of seedling can be extremely high, more than 80% (Petersson & Örlander 2003). Several factors affecting damage are known, among them year of planting with regard to time of logging (Örlander & Nilsson 1999), period of planting within the growing season (Wallertz et al. 2016; Nordlander et al. 2017), soil type and presence of vegetation surrounding seedlings (Petersson & Örlander 2003; Örlander & Nordlander 2003; Nordlander et al. 2011), presence of a parent stand (Nordlander et al. 2003), seedling type and size (Thorsén et al. 2001), seedling condition (Wainhouse et al. 2004; Zas et al. 2006), and genetics of planting stock (Zas et al. 2017; Puentes et al. 2018).

It is probable that *Fraxinus excelsior*, *Betula pendula* and *Acer pseudoplatanus* repel or are even toxic for *H. abietis* adults (Leather et al. 1994; Manlove et al. 1997). Results from field experiments show that logs of *P. sylvestris* are more attractive than those of *P. abies* (Långström 1982), although if pine logs are missing, logs of *Picea sitchensis* are more attractive than logs of other coniferous species (Wilson & Day 1995). A simple selection of laboratory tests shows that adults prefer coniferous trees to broadleaved ones, such as birch or maple. All mentioned studies showed that *P. sylvestris* was the most preferred food source followed by *P. abies* (Leather et al. 1994; Djeddour 1996; Manlove et al. 1997). The preference of food source can have a direct relationship with the larval developmental stage. It has been stated that *H. abietis* larvae develop faster in stumps of *Pinus* spp. than in those of *Picea* spp. (Bejer-Petersen et al. 1962). In spite of a large number of works dealing with olfactory reactions of *H. abietis* to host volatile compounds (Selander et al. 1973, 1974, 1976; Nordlander 1990, 1991; Nordenhem & Eidmann 1991; Mansson & Schlyter 2004; Voolma & Sibul 2006; Moreira et al. 2008; Olenici et al. 2016; Lalík et al. 2019), there are only a few papers focusing on the effect of a host plant on survival of imagoes, fertility, and a selection of a place for laying eggs (e.g. Leather 1987, 1988; Leather et al. 1995).

3. Methods of seedling protection

Seedling protection against *H. abietis* can be chemical and mechanical. Mechanical protection is performed by applying sand, wax, glue or other barriers on stems. Adults can also be collected with pitfall traps that contain either natural or synthetic baits or using trap barks. Biological methods are based on natural insect enemies or fungal pathogens.

3.1. Chemical protection of seedlings

Chemical protection of seedlings has gradually become the main preventive measure against *H. abietis*. In 1950s and 1960s, insecticides based on long-acting chlorinated carbohydrates with dichlorodiphenyltrichloroethane (DDT) as an active substance were intensively used in Europe and world. Due to its negative impacts on people, e.g. affecting the nervous systems, metabolism disruption, carcinogenic effects (liver cancer), affecting the reproductive system and foetus development (Harte et al. 1991), Norway and Sweden were among the first countries to ban DDT in 1970. Its worldwide ban on use was not issued until 2001 by the Stockholm Convention on Persistent Organic Pollutants (Milton 2011). DDT based agents were gradually restricted and later forbidden. In the former Czechoslovakia DDT was banned in 1974 (Baránek & Petrlík 2005). In the year of 1967, six groups of pyrethroids with different active substances were developed (bioallethrin, permethrin, cypermethrin, deltamethrin, cyhalothrin, alfamethrin) (Elliott 1977). *H. abietis* adults, which fed on seedlings treated with deltamethrin, had various physiological effects, but only a few of them died during the feeding period. Main symptoms were paralysis or cessation of feeding. After being exposed to deltamethrin, *H. abietis* did not die at all or were dying slowly. Five-day-long feeding was too short to detect mortality or other serious toxic symptoms. It is however stated that LD50 value of deltamethrin was 0.71 mg/g for adults after 10 days of exposure, and LD90 was 2.63 mg/g (Dobrowolski 2000). Lempérière & Julien (2003) used insecticide based on carbosulfan in the form of granules, which was put into soil during planting. They found that seedlings protected in this way are sufficiently protected for at least three years after planting. Rose et al. (2005) showed that the application of pyrethroid and neonicotinoid insecticide on young trees (*Pinus sylvestris*) prevented attacks of *H. abietis*. Rose et al. (2006) tested insecticide containing lambda-cyhalothrin and found that treated spruce twigs were damaged 5–7 times less than untreated control. Recently, more works comparing chemical protection with physical barriers have been published (Hardy et al. 2020; Willoughby et al. 2020; Lalík et al. 2020a). These works used various insecticides in different concentrations, which contained different active substances. All applied insecticides reduced seedling mortality in comparison to control by more than a half.

Currently, the usage of insecticides in forestry is progressively reduced. In Slovakia, several agents for protecting seedlings against *H. abietis* (Vaztak Active, Karate Zeon 5 CS, Lambdol, Fury 10 EW, Greenfury, Zetta) (UKSUP 2019) are currently registered and allowed to be used. Similarly, several agents for seedlings protection against the large pine weevil are permitted in the Czech Republic (Alfametrin ME, Decis Mega, Decis Protech, Forester, Vaztak Active, Vaztak Les) (Zahradník & Zahradníková 2019).

Seedlings can be treated in three different ways: treatment of seedlings in the nursery directly on the flowerbed before picking, dipping the aboveground part of bundles in insecticidal solution before planting, spraying the seedlings with insecticide after planting (Torstensson et al. 1999; Varínsky 2011).

The usage of these insecticides is being progressively forbidden in the EU due to the environmental impact concerns, although agents with permethrin can be used until October 31, 2026 (https://ec. europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event= activesubstance.detail&language=EN&selectedID=937).

3.2. Mechanical protection of seedlings

3.2.1 Waxing of seedlings

Since 1992, Norsk Wax company has developed KVAAE (Ecovax) wax that should protect seedlings´ stems against *H. abietis* attacks. A thin layer of wax covers stems for two or more growing seasons. KVAAE wax has been used, tested and included in the studies of plant physiology and growth characteristics for a number of years. White pigment added to wax protects seedlings against sun rays. Bright appearance of seedling stems after the wax application also acts as a deterrent for *H. abietis*. Previous experiments showed problems with wax cracking as plants grew and increased their stem diameters. In 2010, new environmentally friendly additives were added to the wax, thanks to which the wax is now more elastic and has correct physical properties that allow the wax to keep up with seedling´s growth (https://www.norsk-wax.no/forestry).

Fig. 2. Wax machine (photo by authors).

The simplest way, how to wax seedling is using "fountain machine" with two fountains. This simple working machine is filled with stiff wax, while the machine can take up approximately 300 kg of wax (Fig. 2). The wax is melted in the machine at a temperature of 85 °C so that it can circulate via a so called "fountain". Seedlings are treated by inserting them into the fountain and by applying the wax on their stems approximately 10–15 cm from the root collar. About 2,000 seedlings can be treated per hour, while about 5–10 g of wax is applied to one seedling (or 4 g in the case of container seedlings). Since wax is hot, seedlings are cooled in cold water immediately after its application on a seedling, and wax instantaneously hardens. To reduce costs and to simplify the operation with the waxing machine, cooling of seedlings is coupled with the treatment against the drying of the root system (Ondruš et al. 2014).

Several works compared wax with insecticides or glue. Modlinger et al. (2018) compared treatments of two tree species (Norway spruce and Douglas fir) with wax and insecticide with untreated control. Wax protection was as effective as insecticide regardless of tree species. Similar research was performed by Lalík et al. (2020a), who revealed that wax could protect seedlings to the same degree as insecticide or glue. Galko et al. (2015a) compared wax protection of spruce seedlings with untreated control at various numbers of adults attacking seedlings. They found that waxed seedlings are damaged by 2/3 less than untreated seedlings. Several works in Sweden compared various treatments of seedling protection against the large pine weevil attacks (Table 1).

Table 1. Seedling mortality at the end of experiments (after three years of testing).

Reference	Merit Forest WG	Kvaae Wax	Type of Wax	Glue Bayer
Petersson et al. 2006	43.0	43.0	Bugwax 103	—
Härlin and Eriksson 2016	29.3	40.7	C	—
Eriksson et al. 2017	20.0	13.3 and 10.7	C and D	38.0
Eriksson et al. 2018	8.7	10.7	F	27.3

3.2.2 Sand coating of seedlings Conniflex®

Conniflex can be simply described as a sand barrier on seedlings' stems (Nordlander et al. 2009). This method can be applied only on container seedlings. Seedlings in growing trays are first cleaned of the dirt with nozzles, and afterwards water-based glue is applied (http://www.bccab.com). After glue application, seedlings are turned into a horizontal position and coated with fine sand from two sides (0.2 mm particle size) (Nordlander et al. 2009).

The process is finished in a drying unit where glue hardens. The whole process lasts 30 - 35 minutes. The coating is elastic and expands as the seedling grows (http://www.bccab.com).

The results clearly demonstrated similar levels of protection with Conniflex as with insecticides (Nordlander et al. 2009; Nordlander et al. 2011). Unlike insecticides, seedling treatment with Conniflex has no impact on the environment and is safe for the nursery staff. Seedlings are treated with Conniflex in the nursery before they are packed and sent to forest owners. Only one treatment in nursery is necessary for effective two-year-long protection. Conniflex can be applied to all types of seedlings in containers.

Sand coating protection method was developed in Sweden and it has been used in practice since 2003 (http://www.bccab.com).

3.2.3 Gluing of seedlings

Tree protection with glue in the form of adhesive tapes is mainly used on fruit trees in gardens or in forestry. The tapes reduce movements of larvae or non-flying females from tree stems to tree crowns (Webb et al. 1995; Berlinger et al. 1997; Mayo 2003). Seedling protection with glue is in the testing stage. These tests have been performed by applying glue to protect other trees. We presume that seedlings with glue coating are less attractive to the large pine weevil. Glue makes the movement of adults on seedlings' stems more difficult, or if adults attempt to feed on bark treated with glue, glue gets into their gnaws, and thus discourages adults from feeding such bark (Lalík & Galko personal observation).

Seedlings treated with Vermifix glue were protected against *H. abietis* to the same extent as seedlings treated with wax or insecticides (Lalík et al. 2020a). Only a half of seedlings treated with Pellacol was damaged in comparison to untreated control (Rell et al. 2018) and seedlings treated with Vermifix glue were only minimally damaged (Lalík et al. 2020a). Similar experiments with glue were performed by Eriksson et al. (2017, 2018), who found that mortality of glued seedling was higher than those treated with insecticide or wax.

3.2.4 Protection collars

Protection collars to protect seedlings were developed at the end of 1970s after the retreat of the DDT insecticide (Lindstöm et al. 1986). At the end of 1980s and the beginning of 1990s, a number of other feeding barriers were developed and tested, e.g. "stockings" (Eidmann & von Sydow 1989), "BEMA" plastic fibre wrapping (Hagner and Jonsson 1995). Further development resulted in several types of feeding barriers (Fig. 3).

Helast bio is a 125 mm high plastic (polypropylene) cylinder with four thin stripes of the same material that surround the root substrate. Cylinder surface is smooth, which makes the climbing of *H. abietis* difficult (Petersson et al. 2004).

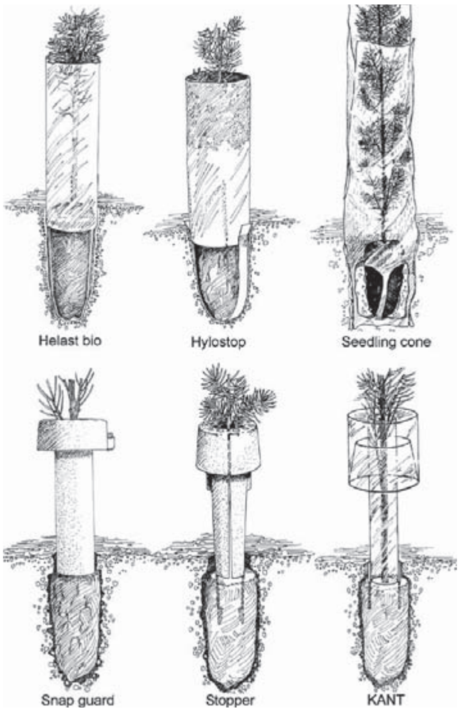

Fig. 3. Different types of protection collars (Petersson et al. 2004).

Hylostop is a 140 mm high cylinder made of paper board coated with polyvinyl chloride (PVC) (Fig. 3). The root substrate is surrounded by two paper stripes without PVC. The outer upper barrier par is coated with Fluon (small particles of polytetrafluoroethylene), which disables climbing the barrier. Fluon is slippery for adults and they are not able to stay on this surface (Eidmann et al. 1996).

The seedling cone is a cone for seedlings that is 280 mm high and is made of thin soft transparent perforated plastic to reduce heating by solar radiation (Petersson et al. 2004).

The root substrate is surrounded by four stripes of the same material (Fig. 3) (Petersson et al. 2004).

Snap guard is a protection shield made from polypropylene with a collar in the upper part that makes climbing of adults more difficult (Fig. 3). The collar is installed by opening the lock, placing it around seedlings from the side and subsequent closing the lock. The collar is pushed to 1 cm soil depth to prevent adults from climbing under it (Petersson et al. 2004).

A stopper is a narrow 65- or 95-mm high tunnel made of polypropylene (Fig. 3). The reverse collar in the upper part complicates its overcoming. This shield has a similar design as the Snap protective cover, but it should be installed at the time of sowing, and seedlings grow directly inside the collar. The cover is attached to the root substrate with two pegs (Petersson et al. 2004).

KANT is a 150 mm high shield, which consists of the inner tube attached to the root and a collar preventing the weevil to get to the seedlings (Fig. 3). The collar has a reverse collar designed to return the weevils back. The used version is an early prototype made of the transparent starch-based plastic (Petersson et al. 2004).

Currently, the snap guard type of the protection collar is available on the market. This collar is available in two versions, one made of reusable polypropylene, and the other one of biodegradable plastic, which decomposes after 4 years (www.grube.sk). The purchase prices are a disadvantage of protection collars. The locks on collars do not join well, in some cases it is difficult to close them. The biggest disadvantage is that if *H. abietis* overcomes the protection collar and gets to the seedling. The collar provides ideal conditions for the weevil from the point of the shelter and food availability, due to which it stays there for a longer time and consumes seedling bark until the seedling dies (pers. obs.).

3.3. Direct field trapping of adults

3.3.1 Trap barks

Mass trapping of *H. abietis* with attractive material was applied before seedling protection. Mass trapping has been mentioned in the literature since the year since 1839 (Ratzeburg 1839).

Other works that describe the usage of trap barks were published at the end of the 19th century (Altum 1889) and the beginning of the 20th century (Burket 1905). Escherich (1923) also stresses the importance of collecting beetles and using trap barks as a protection method. However, already Komárek (1924) noted that methods of beetle trapping are of a control character, which was confirmed by extensive research of Schmidt (1934), who concluded that to avoid outbreaks 96% of adults would have to be caught. Material used for attracting *H. abietis* was fresh bark, fresh branches, or fresh logs. In all cases, bark, logs, or branches (obtained from host species) were distributed over the cleared area, and after some time they were removed together with attracted beetles and must be manually collected and killed. Trap barks were made attractive by pine twigs freely placed between two pieces of spruce bark (Pfeffer et al. 1961). In the 1980s, trap barks were still used in Czechoslovakia as a defence method to reduce the abundance of *H. abietis* on cleared areas (Novák 1965). To meet these expectations, it was however necessary to use more than 100 pieces of trap barks per hectare. The work required for collecting *H. abietis* from trap barks can be reduced by processing bark with synthetic pyrethroid or another insecticide, which kill adults. However, this method is currently used only in some countries on very restricted area (Långstöm et al. 2004; Modlinger & Knížek 2009; Galko et al. 2012). The main reason of reduced usage of this seedling protection method is the complexity of trap bark preparation, the need of frequent inspection and replacement and the associated high operational costs (Galko et al. 2015b; Lalík et al. 2020b).

Trap barks are prepared from plates of fresh spruce bark with sapwood, in which a pine twig that can be needle-less is placed. The use of poisoned trap barks with twigs soaked in insecticides was very frequent. It is generally known that pyrethroids have a repellent effect on insects (Rose et al. 2005). This characteristic is appropriate for the curative treatment of seedling stem base, but it is not desirable for trap barks, which should attract *H. abietis*.

Trap barks are no longer used in practice to reduce the abundance of adults. This method remains appropriate to signalise the presence of adults (monitoring) before the planned curative spraying. Slovak technical standard STN 48 27 12 (Galko et al. 2016) states to use at least 20 to 30 pcs of trap barks per ha. The recommended bark size is 50 × 25 cm. The bark is folded into halves, which means that the final size is 25 × 25 cm. The best place for the bark is on the soil between root swellings of fresh stumps. The inspection needs to be performed every 3 to 5 days.

3.3.2 Pitfall traps

Pitfall traps exist, e.g. in Poland they developed a trap of IBL-4 type, which is, in fact a PVC tube closed from both sides with drilled holes, in which special funnels are placed that are used by adults to enter the trap (Fig. 4a). The lower edge of the entrance funnels is at the level of the terrain to enable easy entering of adults (Skrzecz 2003). The most frequently used traps are simple plastic buckets with a volume of 1.2 l, which have 10 holes drilled in the upper part under the lid, each with a diameter of 10 mm. The trap is buried in the ground in such a way that the lower edge of the entrance holes are at the level of the ground to allow easy entering of adults. The same type of traps (Fig. 4b) was used by several authors (Tilles et al. 1986; Nordlander 1987; Nordlander 1990; Zumr & Starý 1992; Voolma et al. 2001; Erbilgin et al. 2001; Lalík et al. 2019). Olenici et al. (2016) used a funnel type of traps with the bottle of 2 dcl volume placed in the ground (Fig. 4c). A funnel was placed above the bottle to concentrate beetles to traps and the funnel is covered with a lid, in which holes with a diameter of 10 mm were drilled and through which adults fell into the trap.

It is possible to catch living insect adults with all types of traps, i.e. to perform a dry type of trapping. However, with this type, there is a risk that adults escape from traps. The risk can be eliminated by applying teflon (Fluon) on trap walls. Insects cannot climb such a surface, and thus, they do not escape from the traps. This type of trapping is suitable if we want to get adults for further research (Lalík et al. 2021). The second type of trapping is the so-called wet trapping (wet option), when liquid is poured into traps. For further determination, it is suitable to use propylene glycol with water in a ratio 1:1 or a cheaper variant composed of water with 5% NaCl (personal observation). Pitfall traps are not useful if an appropriate attractant that attracts *H. abietis* adults is not placed in them. Many different attractants including wooden cylinders and discs were used (Nordenhem 1989; Skłodowski & Gadziński 2001; Moreira et al. 2008; Kuźmiński & Bilon 2009), but the original bait from trap bark, i.e. pine twigs (Pfeffer et al., 1961) remained forgotten. This type of baits is rarely used in the works (Zumr & Stary 1992; Lalík et al. 2019). Lalík et al. (2019) used a pine twig as a bait in their pitfall traps, which was attached to the bottle with 70% ethanol. This was a more successful bait for trapping than chemical attractants.

Alpha-Pinene combined with ethanol is very attractive for *H. abietis*, which is understandable because alpha-Pinene is one of the resin compounds of coniferous trees (Tilles et al. 1986). Traps containing alpha-Pinene with ethanol are more effective than traps containing only alpha-Pinene or ethanol (Nordlander 1987). Attractants are placed in two “evaporators” (containers with attractants that release their odours), one for alpha-Pinene and the second one for 70% ethanol (Tilles et al. 1986; Nordlander 1990; Erbilgin et al. 2001; Olenici et al. 2016). These chemicals are used to increase the attractiveness of bark and other natural materials (Moreira et al. 2008).

Traps with attractants can be used to monitor or control pest population (Nordenhem 1989; Kuźmiński & Bilon 2009). Such traps together with chemical protection of seedlings are frequently used in Poland (Stocki 2000). IBL-4 traps containing Hylodor, an aggregation pheromone of *H. abietis* (Skrzecz 2003), caught significantly more *H. abietis* individuals than natural baits (branches, discs) (Kuźmiński & Bilon 2009).

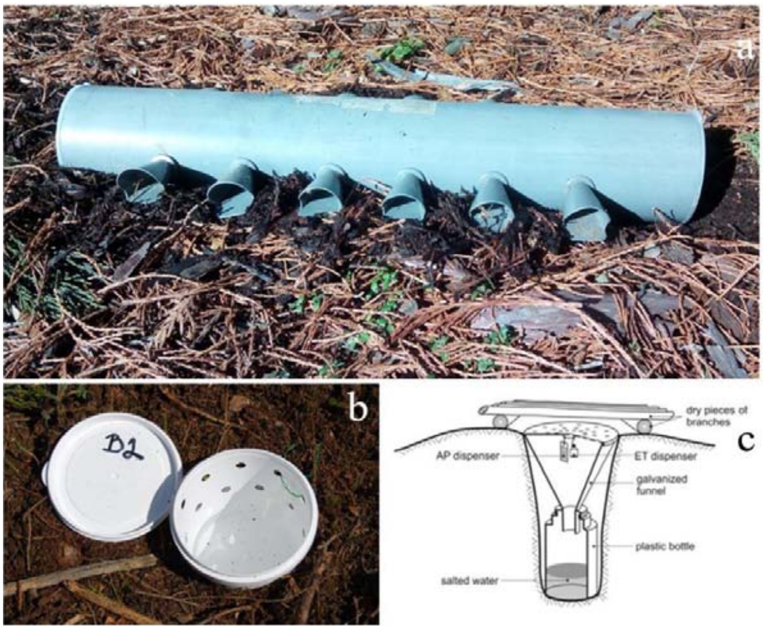

Fig. 4. Different types of pitfall traps: a) trap IBL-4, b) plastic buckets with a volume of 1.2 l, c) a funnel type of traps with the bottle of 2dcl. (photo a), b) by authors, c) Olenici et al. 2016).

3.4. Biological method against the large pine weevil

3.4.1 Entomopathogenic fungi

Entomopathogenic fungi (EPF) are natural antagonists of arthropods and can help to control the host population and to prevent the of outbreaks (Vega et al. 2012). Extensive research focusing on the usage of EPF as prospective biocontrol substances of insect pests has been performed in agriculture and forestry (Lacey et al. 2015), and some strains have been successfully licensed and commercialised (De Faria 2007; Reddy et al. 2013). These fungi are considered environmentally safe (Zimmermann 2007) and can be mass-produced (Jaronski 2014). They show considerable potential in controlling different forest pests (Augustyniuk-Kram & Kram 2012). EPH are known to infect almost all insect species and developmental stages, above all Hemiptera, Lepidoptera, Diptera, Coleoptera, Orthoptera and Hymenoptera (Ramanujam et al. 2014; Khan & Ahmad 2015). It is assumed that *Beauveria bassiana* alone can kill more than 750 different insect species (Ghikas et al. 2010). Environmental factors are known to affect the survival of the entomopathogenic fungus of *B. bassiana*. Solar radiation is the most limiting factor affecting the survival of spores (Gardner et al. 1977; Huang & Feng 2009; Posadas et al. 2012). Tests of several authors in laboratory conditions confirmed that even short exposure to UV-C can kill 99% of conidia within 16 minutes, and UV-A and UV-B after 31 minutes (Krieg et al. 1981).

Temperature is another factor limiting the survival of conidia. Optimal temperatures for *B. bassiana* are between 23 and 28 °C, minimum temperatures are from 5 to 10 °C, and maximum is approximately 30–38 °C depending on tested isolates (Müller-Kögler 1965; Roberts & Campbell 1977; Hywel-Jones & Gillespie 1990; Fargues et al. 1997; Hallsworth & Magan 1999). The thermal death point for spores is at a temperature of 50 °C for a period of 10 minutes (Walstad et al. 1970).

Several experiments in Europe focused on the occurrence of EPF in *H. abietis* populations, and on species of *Beauveria* a *Metarhizium* genera, which were found in all weevil stages from larvae up to adults. In general, fungi occur in *H. abietis* population at a constant but relatively low level (Gerdin 1977; Williams et al. 2013; Wegensteiner et al. 2015; Barta et al. 2019).

The results of previous recent laboratory experiments with EPH usage against *H. abietis* were inconsistent or not very promising (Williams et al. 2013; Ansari & Butt 2012; Samsinakova & Novák 1967; Waldenfels 1975; Wegensteiner & Führer 1988; Wegensteiner 1989; Mc Namara et al. 2018). In spite of that, fungi are assumed to have potential to be successfully implemented into an integrated system, which deals with the problem of seedling damage by the large pine weevil. Feasibility and sustainability of their field application as control agents of *H. abietis* depends on the selection of fungi strains, formulation of inocula and application techniques (Lalík pers. observ.). One application possibility is to infect a carrier with EPF, on which EPF can grow and produce spores. In the year 2019, we submitted the Slovak patent application PP-79-2019 with such a carrier, and in the year 2020 an international patent application PCT/SK2020/050007 was submitted. This carrier with *B. bassiana* fungus and an appropriate attractant for *H. abietis* seems to be a good tool for targeted population reduction.

3.4.2 Entomopathogenic nematodes

More than 30 families of entomopathogenic nematodes (EPN) parasitizing or differently linked to insects are known (Poinar 1990; Kaya & Stock 1997). Due to the potential of biological control, experiments focusing on 7 families (Mermithidae, Allantonematidae, Neotylenchidae, Sphaerularidae, Rhabditidae, Steinernematidae and Heterorhabditidae) have been performed. Most attention has been paid to the last two families because they can be cultivated, formulated, and used for to control of a wide spectrum of insect pests within a short period (Lacey et al. 2001).

EPN can be found in most ecosystems over the whole world and on all continents except Antarctica. Currently, more than 96 species of Steinernematidae family and 21 species of Heterorhabditidae family are known (Shapiro-Ilan et al. 2017, 2018). Steinernematidae and Heterorhabditidae families of EPN are lethal insect pathogens. In nature, they play a role in the regulation of natural insect population, their main application type is in the form of watering with aqueous solution as a biocontrol reagent. The unique partnership between EPN and symbiotic bacteria pathogens for insects helped to ensure their success as reagent for biological control (Griffin et al. 2005).

Both families, Steinernematidae and Heterorhabditidae, have the only freely living stage of infectious larva (IL) of the third instar, which transmits symbiotic bacteria of *Xenorhabdus* and *Photorhabdus* genera in this order (Boemare et al. 1993). IL can enter insect body through a mouth opening, rectum, or breathing holes, and then move towards the hemolymph. Some species are able to enter through insect cuticle (Bedding & Molyneux 1982; Peters & Ehlers 1994). For example, *Heterorhabditis* is able to do so using the front dorsal tooth (Bedding & Molyneux 1982).

Steinernema carpocapsae excretes proteins suppressing the immune system of a host, which could help the release of their symbiotic bacteria. It is not known if *Heterorhabditis* also excretes similar proteins (Frost & Clarke 2002).

As soon as IL is inside the host hemolymph, EPN release symbiotic bacteria. Insect haemolymph is extremely rich in nutrients, due to which bacteria exponentially multiply and cause insect death within 24 to 48 hours. IL feed on symbiotic bacteria and are decomposing host tissue. After EPN start to eat, they develop to the fourth larval phase and subsequently to the adult stage in order to reproduce. Depending on available nutrients and sources, more than one EPN generation can occur inside the host insect (Dix et al. 1992). Hundreds of thousands of IL can develop in a big host and can get out of the dead insect within several days, and then they begin to search for a new host (Griffin et al. 2005).

If IL leave the host body, they keep the skin of the previous instar as a coat. The coat prevents them, especially *Heterorhabditis*, from drying, freezing, or infection by fungal pathogens (Wharton & Surrey 1994). *Steinernema* lose their coat quite easily when they move in soil, while the case of *Heterorhabditis* is attached to the body more firmly, and larvae do not take it off so easily (Dempsey & Griffin 2003).

3.4.3 Parasitoids

Bracon hylobii (Ratzeburg 1848) is another natural enemy of *H. abietis*. It is a wasp from the Braconidae family, the distribution of which is closely linked to *H. abietis* (Heqvist 1958; von Waldenfels 1975; Gerdin 1977). The wasp is about 5 mm long and is active from May to November. The female reacts to vibrations of actively consuming larvae of *H. abietis* (Faccoli & Henry 2003). It penetrates the bark, under which a larva is located, with its ovipositor, and injects paralysing venom in it (Wharton 1993). Afterwards, it lays eggs directly on the body or in the vicinity of larva. After hatching, wasp larvae feed on the content of the paralysed larva of *H. abietis* until only its cuticle remains. Before pupation they spin their cocoons, and come out after 7 to 10 days, in the case of unfavourable conditions they remain in the cocoon and come out later (Dillon & Griffin 2008). They can cause a 30% mortality of *H. abietis* larvae (Munro 1914; Crooke & Kirkland 1956; Dillon et al. 2008), which is however not sufficient during the weevil outbreak to reduce damage caused by abundant number of new adults.In agriculture, populations of natural enemies of pests have long been replenished with individuals bred in laboratories if the natural elimination of pests is insufficient (e.g. releasing ladybirds to fight against aphids). Scientists from the University of Ulster and the Galway-Mayo Institute of Technology examined the possibilities of breeding this parasitoid wasp for applications in Ireland. However, breeding the wasp in laboratory conditions is demanding, because it requires larvae of *H. abietis* as hosts, and the application results were not positive (Dillon et al. 2008). Mass production and application of the parasitoid wasp *B. hylobii* requires further research.

3.4.4 Ants

Ants are predators that were successfully applied as biological control in agriculture (Way & Khoo 1992). Apart from consuming the prey, they can scare a wide spectrum of species from arthropods to vertebrates (with their aggressive behaviour when defending their area) (Rico-Gray & Oliveira 2007).

The red wood ant (*Formica rufa*) is an aggressive, territorial ant species (Savolainen et al. 1989). They build characteristic mound nests and a system of tracks that remain stable during the whole active season (Buhl et al. 2009). These tracks often lead to tree crowns, where ants collect honeydew produced by aphids (Skinner 1980). Ants have long been considered as potential means for biological protection of forests (Adlung 1966). It was revealed that by placing sugar baits to newly planted spruce seedlings, damage by *H. abietis* was reduced by 30% in comparison with the plots without such baits (Maňák et al. 2013, 2015). This was probably caused by the defensive behaviour of ants that were defending their food source and thus scared *H. abietis* adults, which wanted to feed on seedlings. High ant density can disturb *H. abietis* and other herbivore insects, which reduces their damage, and hence, ants can be useful for integrated forest protection. Nevertheless, mechanisms of interactions between *H. abietis* and ants need further research.

4. Economic damages

The only work that summarised the data on damages of forest stands caused by *H. abietis* is the one written by Långström & Day (2004). We tried to update the data from this work, particularly the damaged area of stands in individual countries. It is quite difficult to obtain these data because some countries consider this weevil as an important pest or are not able to obtain such data from forest owners. Table 2 presents an overview of countries, from which we were able to obtain the data.

We estimated costs of losses by pine weevil damage costs. The price of one damaged seedling and subsequent planting of a new one was set to 1 € (the price of one seedling was 0.27€ and the planting costs were 0.23 €), and 3,000 pieces of spruce seedlings are planted per hectare in Slovakia. In the year 2019, 160 million of coniferous seedlings were planted in Finland at in an area of 114,000 hectares. That means that approximately 1,400 seedlings are planted per hectare on average. Average losses are at a level of 2–4% (pers. com. H. Viiri). According to Hanssen et al. (2017), seedling damage by *H. abietis* causes 10% mortality in Norway. Planting density varies with site index, but generally lies within 1,800–2,500 seedlings ha^{-1}. Annually, they reforest approximately 20,000 hectares (pers. com. I. S. Fløistad.). Hence, around 2,000 hectares are damaged every year. In the analysed countries, more than 18,000 hectares were damaged by *H. abietis* in the year 2019. From Sweden and Estonia, we obtained only the data on estimated damages, that were determined to be more than 26 million €. In the UK, preventing *Hylobius* damage has been estimated to cost the

Table 2. Overview of known, estimated damages and economic losses caused by *H. abietis* in some European countries

Country	Area of forests damaged by *H. abietis* in the year 2019/ mean year 2015–2019	Source	Economic loss calculated by us million €
Austria	2588 / 1988	BFW – G. Hoch 2020	7,764
Croatia	0 / 0	D. Matošević	0
Czech Republic	2294 / 3881	M. Knížek et al. 2020	6,882
Hungary	0 / 40	G. Csóka	0
Estonia	data not available	T. Drenkhan	0,75
Finland	3500	H. Viiri	5,25
France	3000	B. Boutte	9,0
Germany	722	D. Wonsack	2,166
Lithuania	150 / 295	K. Grigaliūnas	0,45
Netherlands	0	L. Moraal	0
Norway	2000	I. S. Fløistad	4.0
Romania	1000 – 2000	M. L. Dudumann	4.5
Poland	2203 / 3777	IBL. Poland 2020	6.61
Slovakia	500 – 1000	personal observation	2,25
Sweden	data not available	Mattsson 2016	25,5
Switzerland	0	B. Wermelinger	0
United Kingdom	data not available	Moore et al. in preparation	45
Total	18 707 ha		120,122

forest industry at least £4 million per year (Leather et al. 1999) (nearly £7 million in 2019, adjusted for inflation), but if indirect impacts such as delays to revenue received are included, total losses are estimated to be ~£40 million per year (Moore, E. Wilson, I.H. Willoughby, T. Connolly, I. Sayyed, K. Leslie, et al., in preparation). In total, it was estimated that damages by *H. abietis* on seedlings exceeded 120 122 000 € in the analysed countries in the year 2019.

No data could be obtained from Spain which are known for their large areas of coniferous forests, and the widespread occurrence of *H. abietis* (Långström and Day 2004; Gregoire & Evans 2004).

5. Summary

Within Europe, adults of the large pine weevil annually destroy several tens of thousands hectares of forests and cause damages of more than 120 million € per year. Biology and a life cycle of *H. abietis* are well studied and described. We can consider this species as the most important pest of young coniferous stands. It occurs from southern parts of Europe up to Northern Europe. It was able to adapt to environmental conditions, which determine its larval development that can last from half a year in warm regions up to five years in cold regions. Adults are also long-lived and can live up to four years. A great number of protection methods to protect seedlings is available. Chemical protection is the most effective, but this approach is being abandoned due to negative impacts on the environment. Individual protection of seedlings based on applying various coatings on stems seems to be appropriate. A disadvantage of this mechanical protection is that it does not reduce the number of weevils in nature. Using natural enemies, such as entomopathogenic fungi and nematodes, is the most suitable method to reduce insect population. These bioagents naturally occur in the environment. The goal of biological protection is to increase their population and to introduce them into the natural environment of *H. abietis*. They can be applied by sprinkling or watering of stumps and seedlings. A disadvantage of such an application is that fungi spores are sensitive to UVC radiation and do not stay active for a long time. A suitable alternative seems to be their application on a carrier, on which fungi can survive for several months. Such a carrier can be placed directly next to a stump or a seedling. Many methods for seedling protection are available, therefore; it is better to use something less effective than to do nothing.

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ORIGINAL PAPER

Overwintering mortality of the oak lace bug (*Corythucha arcuata*) in Hungary – a field survey

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Abstract

The North American oak lace bug (*Corythucha arcuata*) was first discovered in Europe (Norhern Italy) in 2000. It started a rapid area expansion in the last decade and has been reported in 20 countries so far. Almost all European oaks are suitable hosts. On top of the host availability, abiotic factors like weather/climate may also have a decisive impact on its further spread and future outbreaks. We conducted a simple field survey within three years, at five locations to estimate the overwintering mortality of the species. Our results suggest that not even a relatively harsh winter (as 2016/2017) caused severe mortality in the overwintering populations. The average mortality of the nine year/location combinations was 30.6% (range 9.1–58.5%). Based on this, the low winter temperature is unlikely to restrict its further spread, therefore continuing area expansion can be predicted.

Key words: invasive insects; area expansion; climate change; abiotic limitation; overwintering success

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1. Introduction

Alien species are appearing at an accelerated rate worldwide, including European countries (Roques 2010; Csóka et al. 2010; 2012; Tuba et al. 2012; Smith et al. 2018). While some of them do not have any evident impact in the newly colonized areas, others may become invasive, rapidly expanding their area and imposing severe pressure on the invaded area's ecosystems both from an economic and an ecological point of view. The chance of a non-native species becoming invasive strongly depends on the suitability of the environmental conditions including availability of host plants (Csóka et al. 2019; Paulin et al. 2020), effects of native natural enemies (Csóka et al. 2009; Panzavolta et al. 2018; Kos et al. 2020). The weather and climate may also be decisive factors from the point of the establishment and the future of the established populations. Different weather parameters may have different impacts on the different life stages of insects (Neuvonen & Virtanen 2015). Among many others, overwintering success is an extremely important issue (Leather et al. 1993; Marshall et al. 2020; Vétek et al. 2020). Cold winter temperatures have both lethal and sub-lethal impacts on overwintering insects (Turnock & Fields 2005; Scaccini et al. 2020). Any information on the overwintering success of a new non-native insect is vital to make predictions on its further spread and expected importance.

Insects can be classified as either freeze-tolerant or freeze-intolerant (or freeze-avoidant). The extracellular freezing is not lethal for the freeze-tolerant, they regularly freeze between −5 to −10 °C, or at even lower temperature. After this they can be cooled to far lower temperatures (some species even as low as −50 °C). With temperature increase they thaw and recover showing normal functions and development (Bale 1993). The freeze intolerant (freeze-avoidant) insect may avoid freezing by lowering their super cooling point (SCP). SCP is the lower lethal temperature for the freeze-avoidant, since the ice formation is intracellular (Sinclair et al. 2015). On top of the extreme low temperature many other factors may have significant effect on the insects' survival (Sinclair et al. 2003).

The North American oak lace bug (*Corythucha arcuata* (Say 1832) – Heteroptera: Tingidae) is a recent invader in Europe, first discovered in Italy in 2000 (Bernardinelli et al. 2000). In the last decade, it showed an explosive area expansion and has been reported from 20 European countries (Paulin et al. 2020). As almost all native deciduous oaks are suitable hosts for the oak lace bug (OLB), at least 30 million hectares of oak for-

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ests provide acceptable hosts for it in Europe (Csóka et al. 2019). Although there are major gaps in knowledge concerning the further spread and damage of the OLB, it seems potentially very dangerous both from an economic and an ecological point of view (Nikolic et al. 2019; Csóka et al. 2019; Paulin et al. 2020).

OLB adults stop feeding in late autumn (October/November) and migrate to their overwintering microhabitats. These are under raised bark, bark crevices, branch forks covered by leaf litter, etc. Overwintering adults are rarely found on the soil surface under the leaf litter, but the vast majority of the overwintering bugs use tree trunks, branches and dead logs on the ground. No overwintering larvae have been found in Hungary so far. They finish overwintering and climb up on the trees starting from early/mid-April. It is assumed that the mild winters and early spring might help the further area expansion and outbreaks. However this is not yet supported by scientific results and no published information are known on temperature demands of OLB either.

In order to obtain information on its overwintering success, we conducted a simple field survey within three years at five Hungarian locations already invaded by the OLB. The finding of the first year's survey was already published (Csepelényi et al. 2017), but its results have also been incorporated in this study.

2. Methods

Adult oak lace bugs were collected from their overwintering microhabitats in the second half of March prior to their emergence (early/mid-April). In all three years, at all locations samples were taken from at least ten trees, normally providing different overwintering microhabitats (raised bark, bark crevices, branch forks covered by leaf litter, etc.). Sampling dates differed between years and locations mainly due to different spring weather conditions. The main aspect was to sample before the bugs leave the overwintering microhabitats, since the late sampling would have overestimated the mortality. Locations, dates of samplings and sample sizes are provided in Table 1. After keeping the bugs at room temperature (20–22 °C) for 24–36 hours, living and dead bugs were counted. Temperature dates were obtained either from local meteorological stations or from the daily reports of the Hungarian Meteorological Service. Based on these data, the following temperature variables were calculated/considered:

- Average temperature of the period (average of the daily average temperatures).
- Average of the daily minimum temperature of the period.
- The lowest temperature measured in period.
- Lowest 10-day running average of the daily minimum temperatures in the period.

The "period" always means the time window from December 1st until the last day prior the sampling date. For example, December 1st 2016 – March 15th 2017 at Gyula. Measured and calculated temperature variables are provided in Table 2.

Mortality rates were correlated with the meteorological variables listed above.

3. Results

Mortality rates of the 9 year/location combinations (presented in Table 3) ranging from 9.1% to 58.5% show considerable variation. The average of all combinations merged is 30.6%.

None of the four meteorological variables gave any significant correlation with the mortality rates at 95% significance level.

Table 1. Sample sizes (number of adult *C. arcuata*) and sampling dates (in bracket) in three years at five locations.

Winter/Location	Békéscsaba 46.6728°N 21.1431°E 85 m a.s.l.	Gyula 46.6940°N 21.3350°E 86 m a.s.l.	Mátrafüred 47.8308°N 19.9658°E 355 m a.s.l.	Szarvas 46.8759°N 20.5314°E 87 m a.s.l.	Szolnok 47.2042°N 20.1814°E 86 m a.s.l.
2016/2017	3,187 (03.20–24)	201 (03.16)	—	683 (03.30)	
2018/2019	—	1,329 (03.20)	386 (03.29)	—	4,929 (03.21)
2019/2020	—	2,582 (03.19)	1,816 (03.19)	—	2,519 (03.20)

Table 2. Temperature variables within three years at five locations.

Temperature variables	Winter/Location	Békéscsaba	Gyula	Mátrafüred	Szarvas	Szolnok
Average of daily average temperatures	2016/2017	0.7	0.1	—	0.9	—
	2018/2019	—	2.4	2.0	—	2.8
	2019/2020	—	3.1	2.4	—	4.3
Average of the daily minimum temperatures	2016/2017	−2.8	−3.3		−2.8	
	2018/2019	—	−1.2	−1.3	—	−0.9
	2019/2020	—	−0.8	−0.7	—	1.1
The lowest temperature measured	2016/2017	−19.0	−18.0	—	−19.0	
	2018/2019	—	−7.7	−9.3	—	−13.0
	2019/2020	—	−9.5	−10.1	—	−8.0
Lowest 10 days running average of the daily minimum temperatures	2016/2017	−8.1	−7.5		−7.5	
	2018/2019	—	−3.1	−6.6	—	−3.9
	2019/2020	—	−3.1	−2.5	—	−1.2

Table 3. Percentage of dead *C. arcuata* in samples collected in three years at five locations.

Winter/Location	Békéscsaba	Gyula	Mátrafüred	Szarvas	Szolnok	Average
2016/2017	21.4%	51.7%	—	44.1%	—	39.1%
2018/2019	—	58.5%	18.7%	—	44.6%	40.6%
2019/2020	—	13.0%	9.1%	—	14.4%	12.2%
Average	—	41.7%	13.9%	—	29.5%	30.6%

4. Discussion

Bernardinelli (2006) compared the climatic conditions of the OLB's native range (Eastern USA and Canada) and Europe and concluded that most of Europe's climate may allow the further spread of the species. Zielinska & Lis (2020) concluded that the climate in Southern Poland is suitable for the OLB, and the presence/abundance of oak forests may also increase the chance of its establishment. Our results seem to support their conclusions.

The mortality rates did not show significant correlation with our four meteorological variables. The 9 data points is likely not enough to reveal correlations if they are any. But it is already evident that the mortality rates are rather low, even during a relatively harsh winter (2016/2017).

It is clear that the overwintering mortality rates are influenced by many factors other than the extremely low winter air temperature. The state of the overwintering microhabitats (tree exposure, thickness of bark, etc.), number of thaw-freeze transitions, rate of temperature change, cumulative chill injury might be very different, resulting in different chances of survival (Sinclair et al. 2003).

The availability and quality of the pre-overwintering food quality also have important roles, both in the success of overwintering and the post-overwintering performance, as demonstrated by Zvereva (2002) for the leaf beetle, *Chrysomela lapponica* and by Trudeau et al. (2010) for *Malacosoma disstria*.

In warm periods during overwintering, insects may use their energy sources and this can have negative impacts on them on the longer term (Hahn & Denlinger 2011; Sinclair 2015). It can be particularly important for insects starting overwintering with low energy reserve. In other words, insects starving before overwintering will have a lower chance to survive and even if they survive, they will have less resource to use for post-wintering activities (dispersal, mating, etc.). The relative importance of this aspect is likely becoming more important as warmer periods during winters are becoming more frequent.

In case of the OLB, the pre-overwintering starvation seems likely for location/year combinations we studied (except Mátrafüred 2018/2019). The abundance of the OLB was always high enough for overexploitation of food sources by late summer or early autumn, resulting in a uniform discoloration and desiccation of foliage on large contiguous territories. In this situation, the majority of the population either starved or had to feed on other secondary hosts (*Rubus*, *Acer*, etc.). But even so, the mortality rates remained relatively low. This may mean that winter low winter temperature itself is unlikely to restrict the further spread to the East, North and West.

Jun et al. (2010) studied the supercooling point (SCP) and the cold hardiness of the closely related *Corythuca ciliata* in China, where this species is an invasive pest of *Platanus* trees. The average SCP was −11.49 °C for males and −9.54 °C for females. They found *C. ciliata* freeze-intolerant, but chill-tolerant, tolerating subzero temperatures by supercooling. It worth mentioning that supercooling points of a given species' individuals may show considerable variation geographically, monthly and inter annually as it was demonstrated by Vétek el al. (2020) for the invasive *Aproceros leucopoda* (Hymenoptera: Argidae). No similar information is known yet for the OLB, although our related cooperative experiments are already in progress.

5. Conclusions

Based on the results, the low winter temperatures themselves do not seem to restrict the further area expansion of the oak lace bug towards North, West or East. However, many factors other than winter temperature can influence the overwintering mortality of the oak lace bug. Additional studies (both in field and laboratory) are needed to clarify the potential climatic limitation of the further spread.

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ORIGINAL PAPER

Ash dieback and contributing factors of forest weakening in provenance tests in the Sumy region

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Abstract
The aim of this study was to evaluate the health condition of *Fraxinus excelsior* L. in provenance tests, with special focus on ash dieback (ADB), but taking into account also other causes of its decline. The research was carried out in the provenance tests of *F. excelsior* in the forest-steppe part of the Sumy region. ADB symptoms were revealed in all provenance tests. For 2012–2019 the health condition index, ADB incidence and severity increased for all provenances except the Steppe. Collar rot was present in all ash provenances. Fungi species were isolated from the stem parts of ash at all provenances. *Hymenoscyphus fraxineus* induced longest necrotic lesions following wound inoculation of stems of 7–10-years-old plants of European ash in the forest while inoculation with both *Cytospora* sp. and *Diplodia* sp. resulted in smaller necroses. The conclusion from other regions about the coincidence the damage of European ash by ADB and collar rots as well as the coincidence the damage of European ash by collar rot and tree colonization by *Hylesinus crenatus* (Fabricius, 1787) is supported.

Key words: *Fraxinus excelsior*; collar rot; health condition; incidence; severity

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1. Introduction

Recently, in many European countries, deterioration of health condition of *Fraxinus excelsior* L. stands has been registered (Matsiakh & Kramarets 2014; Goberville et al. 2016; Enderle et al. 2017; Meshkova & Borysova 2017), which is largely due to the spread of a new invasive pathogen *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya (Queloz et al. 2011; Baral et al. 2014; Gross et al. 2014). The presence of this fungus in the symptomatic shoots of European ash (*Fraxinus excelsior*) from the Sumy region of Ukraine was confirmed by molecular methods (Davydenko et al. 2013b). The occurrence of this pathogen in ash shoots with pronounced symptoms of the disease (necrotic spots on the bark, discoloration) was statistically proven. For trees affected, the gradual death of crowns, the presence of necrotic spots on the bark of shoots, leaves and stems, discoloration of wood and shoots, uneven flowering, and premature fall were registered (Skovsgaard et al. 2010; Metzler et al. 2012; Davydenko & Meshkova 2017).

Provenance tests with long-term history is a very suitable approach to study many issues, among which is the susceptibility of different provenances to some pathogens (Metzler et al. 2012; Stener 2013; McKinney et al. 2014; Enderle et al. 2015). Such provenance tests of *Fraxinus excelsior* were created in 1930 under the leadership of prof. V. M. Andreiev in the Sumy region (Davydenko et al. 2013a).

In 2012, inspection of these provenance tests was carried out with assessment of ash dieback (ADB) incidence and severity as well as the health condition for each provenance (Davydenko et al. 2013a). Over the past years, the progressive development of ash dieback has been registered in many sample plots in different regions of Ukraine (Davydenko et al. 2019). Therefore, it was the basis for repeated assessment of ash dieback parameters in the provenance tests in the Sumy region.

The aim of this study was to evaluate the health condition parameters of *Fraxinus excelsior* in provenance tests, with special focus on ash dieback, but taking into account also other causes of its decline. One aspect of our research on ash dieback was aimed at determining fungi associated with ash trees and the ability of certain fungi to cause necrosis on the shoots of European ash saplings.

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2. Material and methods

The research was carried out in the provenance tests of *Fraxinus excelsior* in the State Enterprise "Trostyanetske Forest Economy" in the forest-steppe part of the Sumy region (50°29'05" N; 34°57'56" E).

Within these stands, 5 provenance tests of *F. excelsior* were tested: Poliskyi, Western Forest-Steppe, Right-Bank Forest-Steppe, Left-Bank Forest-Steppe and Steppe.

From each provenance, 50 living trees of European ash were inspected. Besides diameter at breast height (DBH) several parameters of ash health condition were additionally assessed in each provenance test. Social position within the stand was assessed according to Kraft classification (class 1 – predominant trees, class 2 – dominant trees, class 3 – subdominant trees, class 4 – suppressed trees and class 5 – dying trees) (Lech et al. 2020).

Category of tree health condition was evaluated on a range of visual characteristics (crown density and color, the presence and proportion of dead branches in the crown, etc.) according to National scale (Davydenko et al. 2019). Each tree was referred to one of four categories of health condition (1st – healthy; 2nd – weakened; 3rd – severely weakened; 4th – drying up). Health condition index (HCI) for each plot was calculated as mean of the health condition categories of studied trees.

The incidence of ash dieback, collar rots, and *Hylesinus crenatus* (Fabricius, 1787) (Coleoptera: Curculionidae: Scolytinae) galleries was estimated as a proportion of living ash trees with characteristic symptoms within the sample from each provenance.

Severity of ash dieback for each ash tree was estimated by score: 0 points – no symptoms, 1 point – the presence of several indirect symptoms (uneven leafing, single drying of shoots, individual necroses on healthy shoots, drying up to 10% of branches; 2 points – the presence of these symptoms, drying of more than 10% and up to 50% of shoots and branches, formation necrosis, 3 – drying of more than 50% of the crown, discoloration of wood in the area of necroses, the presence of necroses and drying of leaves, shoots, branches, 4 – damage to 100% of the crown, the presence of necroses even on green shoots and branches, leaves, stems (Metzler et al. 2012). Severity of ash dieback for each provenance was evaluated as an arithmetic average of all trees scores.

By epicormic shoots, the trees were rated as follow: 0 – absence of epicormic shoots; 1 – sporadic epicormic shoots; 2 – multiple epicormic shoots; 3 – total stem coverage with epicormic shoots (Meshkova & Borysova 2017). Each stem collar was examined for the presence or absence of stem necroses and signs of wood decay fungi. The score of wood decay severity (collar rots) considered the part of damaged stem circumference: 0 points – absence; 1 point – up to 25% of circumference; 2 points – 26–50%; 3 points – 51–75%; 4 points – over 75% of circumference (Davydenko et al. 2019).

Fungal isolation. The wood samples were taken by Haglöf increment borer 10 cm or over in length from *F. excelsior* trees randomly. Five samples per each provenance (25 samples in total) were packed individually in Falcon tubes, marked and placed into freezer (−20 °C). After surface sterilization in flame, wood samples were placed in Petri dishes containing ca. 30 ml of 3% malt extract agar medium (MEA) and incubated at room temperature (ca. 21 °C) in the dark. Petri dishes were checked daily and outgrowing fungal mycelia were subcultured to new Petri dishes with MEA. Fungal cultures were divided into groups based on their morphology and for species identification representative cultures from each group were subjected to sequencing of the internal transcribed spacer of the fungal ribosomal RNA (ITS rRNA). Isolation of DNA, amplification and sequencing followed methods described by Davydenko & Meshkova (2017). The thermal cycling was carried out using an Applied Biosystems GeneAmp PCR System 2700 thermal cycler (Foster City, CA, USA): 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 and final extension step at 72 °C for 7 min. Raw sequence data were analyzed using the SeqMan Pro version 10.0 software from DNASTAR package (DNASTAR, Madison, WI, USA). The criteria used for identification were: sequence coverage > 80%; similarity to taxon level 98–100%, similarity to genus level 94–97%. Some fungal species were detected by morphological symptoms (fruit bodies, spores, etc.).

Pathogenicity test. In the inoculation tests carried out in May 2020, the following fungi, considered as putative pathogens, were used: *Alternaria alternata* (Fr.) Keissl., *Didymella macrostoma* (Mont.) Qian Chen & L. Cai, *Hymenoscyphus fraxineus, Epicoccum nigrum* Link, and *Fusarium avenaceum* (Fr.) Sacc., *Cytospora sp. 15* and *Diplodia sp.* (for the last two fungi, only genera were identified). The pathogenicity of these fungi to the *F. excelsior* plants was determined in the field experiment. The stems of 7–10-year-old plants growing in the forest were each superficially wounded by removal of bark (0.5 cm diameter) with a razor blade in 8 cm from the shoot tip. A disc cut from a fungal culture was placed on each wound and then covered by Parafilm strip. In the field experiment, five trees per isolate were used (35 trees per all fungal species), whereas 10 control plants were inoculated with sterile MEA. Observations of both necrosis and tree condition (crown dieback) were made at weekly intervals for 4 months. The data were expressed as mean lesion length. Re-isolations of the test fungi from all the inoculated plants were attempted by plating material from lesions (if present) or from points of inoculation. The lesion material was taken from sections ca. 5 mm both above and below each inoculation point. Fungi growing from the material were re-isolated as described in the above procedures for isolation.

Normality tests, summary statistics, one-way analysis of variance (ANOVA), Tukey HSD test with a significance level of $p < 0.05$ were performed. Shapiro-Wilk test has proved the normality of analyzed parameters (ADB, collar rots and *H. crenatus* incidence and severity, health condition index) when comparing the provenances between each other in 2012 and 2019, and each provenance in 2012 and 2019. 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

The results of the 2012 assessment showed that the diameter (30.3–32.1 cm), Kraft class (2.3–2.6) and health condition index (1.8–2.4) of individual provenances did not have statistically significant differences (Table 1).

In 2019 assessment, the diameter (31.1–33.2 cm), Kraft class (2.2–2.6) and health condition index (2.5–2.8) of individual provenances also did not have statistically significant differences (Table 2).

Statistically significant differences were registered between the provenances in terms of branch dieback and the presence of epicormic shoots (Table 2). The branch dieback was the lowest in the Steppe provenance (5.5%), which is significantly less than in the other provenances, except for the Western forest-steppe. The highest values of branch dieback were assessed in the Poliskyi and Right-bank forest-steppe provenances (15.3%), with the maximum values reaching 50% (Poliskyi).

The prevalence of epicormic shoots ranged from 0.8 points in the Steppe and Left-Bank Forest-Steppe provenances to 1.7 points in the Right-Bank Forest-Steppe. However, Tukey's test showed a significant difference only between Right-Bank Forest-Steppe and two above mentioned provenances ($P < 0.05$).

In 2019 compared with 2012, the DBH increased in all provenances, and the Kraft class a bit increased only in the Left-bank Forest Steppe and Steppe provenances. The health condition index significantly increased in 2019 compared with 2012 for all provenances except the Steppe, for average sample from 2.1 to 2.6.

In 2012, ADB incidence was the lowest in the Western Forest-Steppe provenance (25%), almost twice as high in Poliskyi and Right-bank Forest-Steppe provenance and the highest in the rest provenances (83.3 and 91.7% in the Left-bank Forest Steppe and Steppe respectively) (Table 3). The differences between these three groups of provenances are significant.

Table 3. Comparison of ADB parameters in *Fraxinus excelsior* provenance tests in 2012 and 2019.

Provenance	ADB incidence, [%]±SE		ADB severity, [points] ±SE	
	2012	2019	2012	2019
Poliskyi	58.3±6.97 a	90.0±6.71 d	0.7±0.09 b	1.8±0.12 d
Western Forest-Steppe	25.0±6.12 b	75.0±4.78 e	0.3±0.09 a	1.3±0.09 c
Right-bank Forest Steppe	58.3±6.97 a	70.0±5.12 ae	0.7±0.09 b	1.2±0.12 c
Left-bank Forest Steppe	83.3±5.27 c	100.0±0.00 d	0.8±0.05 b	1.8±0.11 d
Steppe	91.7±3.91 c	75.0±4.78 ce	1.4±0.09 c	1.3±0.09 c
Total	63.3±3.05 a	82.0±3.84 b	0.8±0.04 b	1.5±0.10 d

Note: *Means followed by different letters in each column are significantly different at the 95% confidence level.

In 2019 compared to 2012, ADB incidence increased 1.3 times in total, 3 times in the West Forest-Steppe, 1.5 times in Poliskyi and 1.2 times in the Right-bank and Left-bank Forest-Steppe provenances, however, the increase was significant only for three provenances (Table 3). Significance of increase the ADB incidence for Right-bank Forest- Steppe and of its decrease for Steppe provenance was not proved.

In 2012, ADB severity score was the lowest in the Western Forest steppe (0.3 points) and the highest in the Steppe provenance (1.4 points) (Table 3). The rest three provenances with ADB severity score 0.7–0.8 points did not differ significantly among themselves, but were significantly above the minimum and below the maximum value of this parameter.

In 2019, ADB severity score was the lowest in the Right-bank Forest steppe (1.2 points) and didn't differ statistically from Western Forest-Steppe and Steppe (1.3

Table 1. General parameters of *Fraxinus excelsior* health condition in different provenance tests (2012).

Provenance	DBH, [cm] ±SE	Kraft class, [points] ±SE	Health condition index, [points] ±SE
Poliskyi	30.3±1.21 a	2.5±0.08 a	1.8±0.09 a
Western Forest-Steppe	31.1±1.21 a	2.5±0.06 a	1.8±0.08 a
Right-bank Forest Steppe	31.3±1.30 a	2.6±0.05 a	2.0±0.10 a
Left-bank Forest Steppe	32.1±1.39 a	2.3±0.06 a	2.4±0.09 a
Steppe	31.6±1.23 a	2.5±0.07 a	2.4±0.09 a
Total sample	31.3±0.84 a	2.5±0.06 a	2.1±0.04 a

Note: *Means in each column are not significantly different at the 95% confidence level.

Table 2. General parameters of *Fraxinus excelsior* health condition in different provenance tests (2019).

Provenance	DBH, [cm] ±SE	Kraft class, [points] ±SE	Health condition index, [points] ±SE	Branch dieback, [%] ±SE	Epicormic shoots, [points] ± SE
Poliskyi	31.1±1.0 a	2.5±0.1 a	2.5±0.1 a	15.3±2.3 a	0.9±0.2 ab
Western Forest-Steppe	31.9±1.6 a	2.5±0.1 a	2.6±0.1 a	11.0±1.8 ab	1.1±0.2 ab
Right-bank Forest Steppe	32.2±1.6 a	2.6±0.1 a	2.7±0.1 a	15.3±1.5 a	1.7±0.3 b
Left-bank Forest Steppe	33.2±1.3 a	2.2±0.1 a	2.8±0.1 a	14.5±2.3 a	0.8±0.2 a
Steppe	32.2±1.6 a	2.4±0.1 a	2.6±0.1 a	5.5±1.4 b	0.8±0.2 a
Total sample	32.1±0.63	2.4±0.06	2.6±0.05	12.3±0.91	1.0±0.1

Note: *Means followed by different letters in each column are significantly different at the 95% confidence level.

points each) provenances. The ADB score in Poliskyi and Left-bank Forest Steppe provenances was significantly higher (1.7 points each) comparing to rest provenances (Table 3).

ADB severity score significantly increased over the period 2012–2019 for all provenances except Steppe one.

Collar rot incidence was the lowest (20–25%) in the Western Forest-Steppe and Right-bank Forest Steppe provenances, and significantly higher (40–45%) in other provenances. Collar rot severity score varied from 1.2 to 1.8 in different provenances but the difference between them was not significant. Trees colonization by *H. crenatus* was absent in Western Forest-Steppe and Right-bank Forest Steppe provenances. It varied from 5% (Poliskyi provenance) to 30% (Steppe provenance) but the difference between them was not significant. Severity score of trees colonization by *H. crenatus* was 0.1–0.3 points with also insignificant difference (Table 4).

The results obtained allowed pooling the data for all provenances to test the hypothesis about the possible coincidence ash dieback and collar rots, ash dieback and tree colonization by *H. crenatus*, as well as collar rots and tree colonization by *H. crenatus* (Table 5).

The χ^2-test proved the coincidence the damage of European ash by ash dieback and collar rots as well as the coincidence the damage of European ash by collar rots and tree colonization by *H. crenatus* and doesn't support the hypothesis about the coincidence the damage of European ash by ash dieback and tree colonization by *H. crenatus* (Table 5).

Table 4. Incidence and severity of collar rots and *Hylesinus crenatus* as the most common damages of European ash in provenance tests (2019).

Provenance	Collar rots (CR) incidence, [%] ±SE	severity [points] ±SE	*Hylesinus crenatus* (HC) incidence, [%] ±SE	severity [points] ±SE
Poliskyi	45±11.1 a	1.8±0.21 a	5±4.9 a	0.1±0.05 a
Western Forest-Steppe	20±8.9 b	1.3±0.23 a	0	0
Right-bank Forest Steppe	25±9.7 ab	1.2±0.22 a	0	0
Left-bank Forest Steppe	45±11.1 a	1.8±0.16 a	15±8.0 a	0.2±0.08 a
Steppe	40±11.0 a	1.3±0.22 a	30±10.2 a	0.3±0.11 a
Total sample	35±4.8 a	1.5±0.10 a	10±3.0 a	0.1±0.03 a

Note: *Means followed by different letters in each column are significantly different at the 95% confidence level.

Table 5. Proportion of European ash trees with mixed damage and statistical analysis of correlation between mixed forms of damage in European ash trees (2019).

Damage type A	Damage type B	Proportion of ash trees with symptoms of mixed damage, [%]	Number of inspected trees with the damage: A and B	A without B	B without A	neither A nor B	r	χ^2_{fact}	Conclusion about correlation
ADB	CR	35±4.8	35	47	0	18	0.34	11.8	proven
ADB	HC	10±3.0	10	72	0	18	0.16	2.4	not proven
CR	HC	8±2.7	8	27	2	67	0.32	10.6	proven

Notes: $\chi^2_{0.05} = 3.84$; N = 100; ADB – ash dieback; CR – collar rot; HC – *Hylesinus crenatus*

Table 6. Fungi identified on *Fraxinus excelsior* stem for different provenances.

Fungi	Frequency of isolation, [%]: Poliskyi	Western Forest-Steppe	Right-bank Forest Steppe	Left-bank Forest Steppe	Steppe	All provenances
Alternaria alternata (Fr.) Keissl.	13.04	5.56	0.00	4.08	7.41	6.35
Alternaria sp.	8.70	0.00	18.18	2.04	7.41	4.76
Armillaria sp.	4.35	0.00	9.09	6.12	3.70	3.97
Aureobasidium pullulans (de Bary & Löwenthal) G. Arnaud	0.00	0.00	0.00	0.00	3.70	1.59
Cladosporium sp.	4.35	0.00	9.09	4.08	11.11	6.35
Cytospora sp. 15	0.00	0.00	9.09	8.16	0.00	3.97
Didymella macrostoma (Mont.) Qian Chen & L. Cai	8.70	16.67	0.00	4.08	3.70	5.56
Diplodia sp.	8.70	0.00	0.00	2.04	7.41	3.97
Epicoccum nigrum Link	8.70	5.56	9.09	6.12	3.70	6.35
Fusarium sp.	8.70	5.56	0.00	6.12	0.00	4.76
Fusarium avenaceum (Fr.) Sacc.	0.00	16.67	0.00	0.00	7.41	4.76
Gliocladium sp.	13.04	0.00	0.00	0.00	14.81	5.56
Hymenoscyphus fraxineus (T. Kowalski) Baral, Queloz & Hosoya	0.00	5.56	0.00	8.16	0.00	3.97
Hysterographium fraxini (Pers.) De Not.	0.00	0.00	9.09	0.00	14.81	3.97
Phialophora sp.	0.00	5.56	0.00	4.08	0.00	2.38
Mortierella sp.	0.00	0.00	9.09	8.16	0.00	0.79
Mollisia cinerea (Batsch) P. Karst	0.00	0.00	0.00	6.12	0.00	2.38
Penicillium sp. A	0.00	11.11	0.00	8.16	7.41	7.94
Penicillium sp. B	0.00	0.00	9.09	0.00	3.70	0.79
Pezicula cinnamomea (DC.) Sacc.	4.35	0.00	0.00	4.08	0.00	2.38
Phialocephala sp.	0.00	0.00	0.00	*2.04*	0.00	0.79
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	8.70	11.11	0.00	2.04	3.70	4.76
Pseudocamarosporium brabeji (Marinc., M.J. Wingf. & Crous) Crous	0.00	0.00	9.09	2.04	0.00	1.59
Sordaria sp.	0.00	11.11	0.00	4.08	0.00	3.17
Trichoderma viride Pers.	4.35	0.00	0.00	0.00	0.00	1.59
Unidentified sp. 51	0.00	5.56	0.00	4.08	0.00	3.17
Unidentified sp. 58	4.35	0.00	9.09	4.08	0.00	2.38
Unidentified sp. 74	4.35	0.00	9.09	2.04	2.38	2.38

The 25 wood samples from the ash stem yielded a total of 126 fungal isolates (23 from provenance Poliskyi (Pol), 18 from provenance Western Forest-Steppe (WF), 11 from provenance Right-bank Forest Steppe (RBFS), 47 from provenance Left-bank Forest Steppe (LBFS) and 27 from provenance Steppe (S) respectively), representing 28 taxons, although only 12 of them could be identified to species level (Table 6).

Only *Epicoccum nigrum* was isolated from all five provenances. From among all fungi, *Penicillium* sp. A, *Epicoccum nigrum, Cladosporium* sp. and *A. alternata* occurred most frequently.

Four of the fungal species were isolated only from a single provenance: *Aureobasidium pullulans* (Steppe), *Mollisia cinerea, Phialocephala* sp. (Left-bank Forest Steppe), *Trichoderma viride* (Poliskyi). Each provenance has a large number of unique species but also a number of shared species. The highest diversity in terms of species richness and Shannon's index was found for the Left-Bank Forest Steppe (Fig. 1).

Relatively high species richness was also found for the Poliskyi and Steppe provenances. Lowest species richness was found in the Right-bank Forest Steppe. However, all groups had relatively higher Shannon index values (comparable to samples from Right-bank Forest Steppe). For species richness values, all pairwise comparisons are significantly different ($p < 0.05$) with Tukey tests except Western Forest-Steppe and Right-bank Forest Steppe; and for Wilcoxon test all are significantly different ($p < 0.05$) except Western Forest-Steppe and Right-bank Forest Steppe. For Shannon index values no pairwise comparisons are significantly different with either the Tukey or Wilcoxon test.

Beta diversity statistics/comparisons among the samples were performed with non-metric multi-dimensional scaling (NMDS) analysis (Fig. 2). Figure 2 shows a map for beta diversity with unweighted unifrac distances among groups.

The highest beta diversity levels are found between Western Forest-Steppe and Right-bank Forest Steppe groups as well as Poliskyi and Right-bank Forest Steppe groups. NMDS picture groups samples into rough clusters corresponding to either their geography (Poliskyi, Western Forest-Steppe, Right-bank Forest Steppe, etc.). A similar pattern can be seen in the plot (Fig. 2). The Right-bank Forest Steppe sample is a diverse set of fungal species in comparison to the Poliskyi and Western Forest-Steppe samples that are only a few close associated fungi with *F. excelsior*. The fungi set from Poliskyi samples groups more closely with Steppe samples than the Western Forest-Steppe, Right-bank Forest Steppe and Left-bank Forest Steppe samples (despite the close geographical proximity of Right-bank Forest Steppe and Left-bank Forest Steppe), thus there seems to be strong influence of provenance on fungal community composition. Left-bank Forest Steppe is the sample with much higher species richness than the others.

Pathogenicity tests. Inoculation with *H. fraxineus*, *Cytospora* sp. and *Diplodia* sp. isolates was followed by the development of brownish lesions on all stems of 7–10-year-old plants. The lesions were already obvious after 4 months and significantly longer than control and other isolates. The inoculation trials showed that there were significant differences in the length of necroses ($F = 5.60$, $p = 0.018$) due to the *H. fraxineus* between the three inoculated species. The mean wood necrosis length on the branches of *H. fraxineus* was 25.6 ± 1.5 mm (mean ± standard error) while *Cytospora* sp. and *Diplodia* sp. 11.7 ±1.1 mm and 6.7 ± 1.3 mm, respectively. Moreover, among both the upper and lower lesions, some showed a superficial splitting of the bark. None of the inoculated plants died within 4 months. In re-isolation, *Cytospora* sp. and *Diplodia* sp. were obtained from necrotic sections of all inoculated stems of *H. fraxineus*.

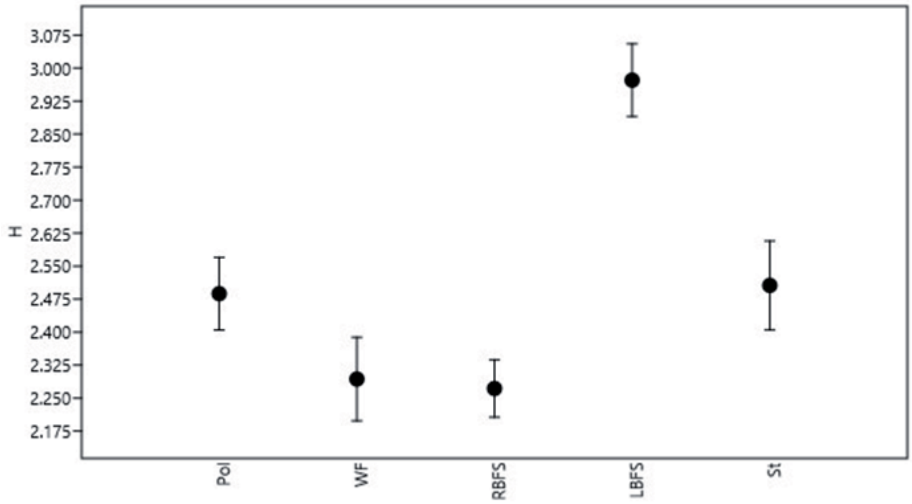

Fig. 1. Box plots of Shannon indices in the five different groups (Pol – Poliskyi, WF – Western Forest Steppe, RBFS – Right-bank Forest Steppe, LBFS – Left-bank Forest Steppe, St – Steppe).

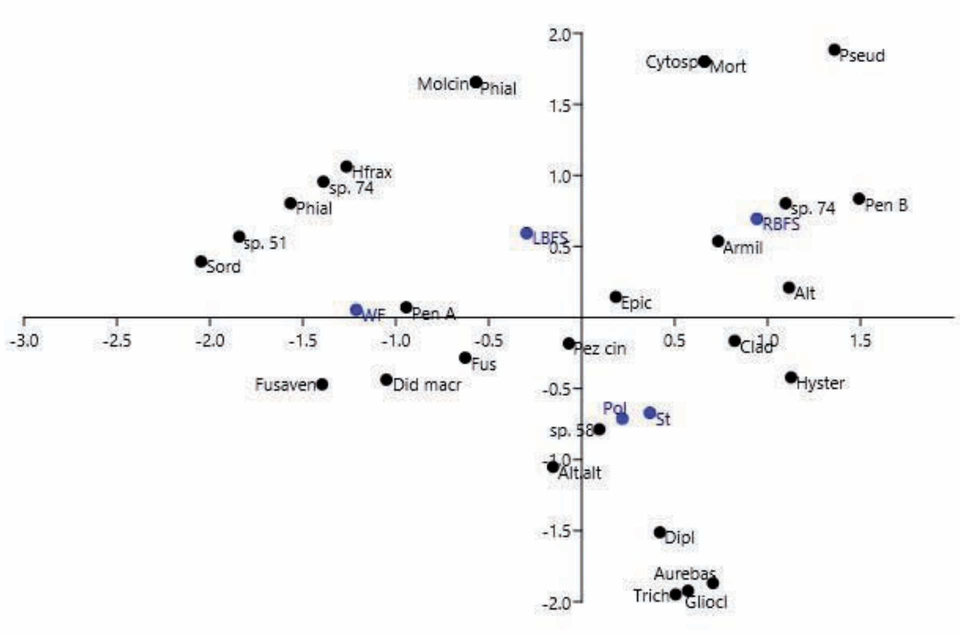

Fig. 2. NMDS plot of fungal species from Pol – Poliskyi, WF – Western Forest Steppe, RBFS – Right-bank Forest Steppe, LBFS – Left-bank Forest Steppe, St – Steppe.

Legends on the plot corresponds to Alt.alt – *Alternaria alternate*; Alt – *Alternaria* sp.; Armil – *Armillaria* sp; Aurebas – *Aureobasidium pullulans*; Clad – *Cladosporium* sp; Cytosp – *Cytospora* sp. 15; Did macr – *Didymella macrostoma*; Dipl – *Diplodia* sp.; Epic – *Epicoccum nigrum*; Fus – *Fusarium* sp.; Fusaven – *Fusarium avenaceum*; Gliocl – *Gliocladium* sp.; Hfrax – *Hymenoscyphus fraxineus*; Hyster – *Hysterographium fraxini*; Phial – *Phialophora* sp; Mort – *Mortierella* sp.; Molsin – *Mollisia cinerea*; Pen A – *Penicillium* sp. A; Pen B – *Penicillium* sp. B; Pez cin – *Pezicula cinnamomea*; Phialc – *Phialocephala* sp.; Sord – *Sordaria fimicola*; Pseud – *Pseudocamarosporium brabeji*; Trich – *Trichoderma viride*; sp 51 – Unidentified sp. 51; sp 58 – Unidentified sp. 58; sp 74 – Unidentified sp. 74.

In other cases, the lesion extension was not observed; however, the slight yellowing of leaves was noted after 4 months from inoculation. *A. alternata, Didymella macrostoma* and *Fusarium avenaceum* produced no symptoms on stems of plants in experiments although all these species induced brown discoloration (ca. 1 mm) around the inoculation points on 7–10-year-old plants. These fungi were re-isolated from the points of inoculation. No lesions developed on any of the control plants and the wounds healed within 4 months after inoculation.

4. Discussion

Invasive pathogen *Hymenoscyphus fraxineus* spread in Europe over 20 years ago (Baral et al. 2014; Gross et al. 2014; Enderle et al. 2015). Its presence in the east of Ukraine, in particular, in the Sumy region, has been confirmed by molecular methods (Davydenko et al. 2013b). In contrast to the western regions (Matsiakh & Kramarets 2014), the disease in the east develops more slowly and does not often lead to the death of trees (Davydenko & Meshkova 2017), which may be due to the features of the climate, in particular, with low precipitation (Hlásny et al. 2014; Krakovska et al. 2017). According to another hypothesis, the origin of ash plays a role in the susceptibility of trees to fungal infection (Metzler et al. 2012; Stener 2013; McKinney et al. 2014). Our research attempted to test this hypothesis in the plots of provenance tests of *Fraxinus excelsior* which were created in 1930 under the leadership of prof. V.M. Andreiev in Sumy region (Davydenko et al. 2013a).

Provenance tests as objects of tree breeding were created primarily to determine the most adapted and productive provenances for specific growing conditions in order to regionalize the supply of seeds for reforestation. The provenances originate from seeds collected from identified stands or regions. However, nowadays they are the only natural models for predicting the impact of climate change consequences (Matyas 1996) as well as to study the tree resistance to new pathogens (Pliura et al. 2011; Metzler et al. 2012). Sometimes, the data from clonal plantations are also considered in the study of resistance (Enderle et al. 2013, 2015). To study the resistance to ash dieback, a provenance trial was established in 2005 in southwest Germany with plots at four sites and eight provenances of *Fraxinus excelsior* (Enderle et al. 2013, 2015). The intensity of the disease in single trees within the provenances varied constantly between completely unaffected trees and their mortality. The study highlights a high genetic variation in susceptibility and considerable genetic potential for resistance breeding in provenances from southwestern Germany.

In a Danish clonal trial comprising 39 *F. excelsior* clones established in 2007 with grafts at two sites, mortality was about 40% in 2013. The average percentage of crown damage increased from 30% in 2007 to over 60% in 2013, with substantial variation among clones (McKinney et al. 2014). In France, only 8% healthy trees and 88% of the trees with more than 5% crown decline were observed. Collar lesions were observed on 33% of the trees and were present in almost all plots (Husson et al. 2011).

Genetic studies of *F. excelsior* clones in Denmark and Sweden did not reveal any differences among populations in resistance to ADB (McKinney et al. 2014). However, in Lithuania progeny trials established in 2005 at three different sites showed significant differences in resistance to ADB among populations (Pliura et al. 2011). This provides good possibilities for the selection of resistant populations. The health condition of trees was influenced also by site conditions and infection pressure from the surrounding stands.

We compared the health scores of five provenances in 2012 and 2019 by different parameters of growth and health condition. Both in 2012 and in 2019, statistically significant differences in DBH, Kraft class, and health condition index between provenances were not found (Table 1, 2). However, in 2019 compared to 2012, the health condition index significantly increased for all provenances except the Steppe. According to National scale (Davydenko et al. 2019), all provenances in 2012 could be considered as weakened ($1.5 < HCI < 2.5$), and in 2019 they all were severely weakened ($2.5 < HCI < 3.5$).

In 2019, a provenance from Steppe had significantly the lowest branch dieback (average proportion of dry branches in the crowns) (Table 2).

At the same time, ADB incidence was the lowest in the Western Forest-Steppe provenance in 2012 and this parameter did not differ from Steppe in 2019 (Table 3). ADB severity was the highest in Steppe in 2012 but was among the lowest values in 2019.

In 2019 compared to 2012, ADB incidence and severity increased for all provenances except Steppe, however maximal values (1.8 points) were assessed for Poliskyi and Left-bank Forest Steppe provenances (Table 3). So the data obtained do not allow us to draw a conclusion about the different susceptibility of individual ash provenances to the ADB. It may be connected with rather high age of trees (about 90 years old).

In different regions, it was previously found out that collar rots (Langer 2017; Davydenko et al. 2019) and stem colonization with bark beetles (Meshkova & Borysova 2017) often accompany infestations of ash with ADB. Therefore, in a survey of provenance tests in 2019, the incidence and severity of collar rots and *H. crenatus* was assessed (Table 4). Collar rot presence was revealed in all ash provenances with the lowest values in Western Forest-Steppe and Right-bank Forest Steppe. Differences between provenances in disease severity according to visual assessment were not significant. Trees colonization by *H. crenatus* was absent in Western Forest-Steppe and Right-bank Forest Steppe provenances, and the differences between other provenances were not significant both by the incidence and severity (Table 4).

Statistical analysis supported the conclusion from other regions about the coincidence the damage of European ash by ash dieback and collar rots (Langer 2017; Davydenko et al. 2019) as well as the coincidence the damage of European ash by collar rots and tree colonization by *H. crenatus* (Meshkova & Borysova 2017) (Table 5). However, the absence of coincidence of ADB and tree colonization by *H. crenatus* may be explained by relatively low incidence of the trees inhabited by *H. crenatus* and the features of this insect to colonize the lowest part of stem with thick bark (Okolow 1970) while ADB reveals first in the crowns (Skovsgaard et al. 2010). We can suppose that the incidence of other ash bark beetles inhabiting upper stem and branches, especially *H. fraxini* (Panzer 1779) (Pedrosa-Macedo 1979) and *H. toranio* (Danthoine, 1788) (Graf 1977), can be more closely associated with ADB. However, the assessment of these insects' galleries is possible only after tree felling.

In the present study, the detected fungal communities were largely composed by the generalist saprotrophic and pathogenic fungal taxa (Table 6). Despite that several pathogenic taxa including *H. fraxineus*, *Cytospora* sp., *H. fraxini*, etc., the fungal community of all provenances showed preference towards saprotrophic species. One to three pathogenic taxa (including *A. alternata*, *E. nigrum*, *Cytospora* sp., *H. fraxineus* and *H. fraxini*) were found in all provenances. Among these, *A. alternata* and *E. nigrum* are known as opportunistic weak pathogens or even saprophytes with a worldwide distribution. *Cytophora* sp. is known as the causative agent of *Cytospora* canker. Other pathogenic fungi were *H. fraxineus* and *H. fraxini* which both are known as a widespread ash dieback-fungi causing massive ash decline. So, the pathogenic fungi were likely the primary cause of disease symptoms. Therefore, we suggest that the fungus *H. fraxineus* is a primary pathogen killing healthy ash tissues. However, later the fungus is losing the competition with other decomposing and saprotrophic fungi being more successful and taking over their domination in dead tissues. So, it explains why necrotic ash shoots usually are colonized by other plant pathogens, endophytes and saprophytes.

Our results for the study of fungal community structure in declining shoots of ash trees in the different geographic provenances as well as inoculation experiment revealed 28 fungal taxa associated with ash trees. Among them, at least three fungal species showed the ability to be pathogens that is the valuable point of this research. Next to *Hymenoscyphus fraxineus* and *Hysterographium fraxini*, *Cytospora* sp. and *Diplodia* sp. have been considered to be involved in serious decline, in combination with abiotic factors. Our study confirmed previous results published. Przybył (2002), Kowalski & Łukomska (2005), Lygis et al. (2006) presented data of endophytic fungi on ash branches with the most characteristic fungi as we did like *Alternaria alternata*, *Diplodia mutila*, *Phomopsis* sp., *Cladosporium cladosporioides*, *Cytospora ambiens* and *Phomopsis controversa*. Fungi belonging to *Phomopsis* and *Cytospora* genera are pathogens of weak trees and are often present in dying shoots (Przybył 2002; Kowalski & Łukomska 2005).

From this point of view, the knowledge of the distribution of pathogenic fungal species associated with *F. excelsior* suffering from ash-dieback phenomenon can be very useful in forest management and diversity conservation programs.

5. Conclusion

Provenance tests of European ash of about 90 years old in Sumy region (Eastern Ukraine) are affected by ash dieback (ADB) caused by pathogen *Hymenoscyphus fraxineus* for at least 10 years.

For 2012 and 2019 the health condition index, ADB incidence and severity increased for all provenances except the Steppe. However, the data obtained do not allow us to draw a conclusion about the different susceptibility of individual ash provenances to the ADB.

Collar rot was present in all ash provenances with the lowest incidence in provenances from Western Forest-Steppe and Right-bank Forest Steppe and the absence of significant differences between provenances in disease severity.

Hylesinus crenatus was not revealed in Western Forest-Steppe and Right-bank Forest Steppe provenances, and the differences between other provenances were not significant both by the incidence and severity.

Fungi isolated from stem of ash trees with varying degrees of decline were identified and the pathogenicity of some of them was determined. Among them, *Hymenoscyphus fraxineus, Cytospora* sp. and *Diplodia* sp. induced different necrotic lesions.

The conclusion from other regions about the coincidence the damage of European ash by ash dieback and collar rots (Matsiakh & Kramarets 2014; Langer 2017) as well as the coincidence the damage of European ash by collar rots and tree colonization by *H. crenatus* (Davydenko et al. 2019) is supported.

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