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Screening resistance to chestnut blight in young chestnut trees derived from *Castanea sativa* × *C. crenata* hybrids

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Abstract

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In 2010, 2011 and 2012 four trials were carried out to prove a reliability of the new screening method for resistance to chestnut blight caused by *Cryphonectria parasitica.* In the selected trees of hybrid origin (*C. sativa* × *C. crenata)*) 10 cm long cut-branch sections were inoculated with mycelia of three virulent isolates and one hypovirulent isolate (in two last trials) and cultivated for 7 days in the dark at 25 °C with 95% humidity. Then the bark of branch segments was peeled off and the size of necrotic lesions formed on the wood tissue around the inoculation place was measured. The size of necrotic areas varied by sampling date, type of fungus strains and tested trees. In all three summer trials necrotic lesions were larger than lesions in a spring trial of 2011, in which still dormant stem sections were used. Unlike the summer trials in the spring trial higher differences in the size of necrotic lesions among tested trees and among used virulent strains were observed. In the spring trial interactions between tested trees and fungal isolates were not so frequent like in summer trials where more trees exhibited different response to the same virulent strain. Majority of trees showed different susceptibility in particular trials. Observed high variation of reactions of tested trees to both virulent and hypovirulent isolates has pointed at the need to prove other screening methods, and to find such one, that would be highly effective to reveal an inherited resistance and/or a lower degree of susceptibility to chestnut blight.

Key words

branch sections, *Cryphonectria parasitica*, hypovirulent isolate, necrotic lesions, seasonal variation, virulent isolate

Introduction

Since the first occurrence of chestnut blight in Slovakia in 1976 (Juhásová, 1983), this disease caused by *Cryphonectria parasitica* (Murrill) M. E. Barrhas has spread to all main chestnut growing areas and caused mass extinction of chestnut trees (Juhásová et al., 2011). As long as several decades in all Europe there has been an effort to reduce and minimize the dissemination and the devastating effect of chestnut blight disease. In affected areas, the attempts to control the disease by chemical, legislative and phytosanitary measures were reported as insufficient (Jaynes and Van Alfen, 1977; Elliston, 1981). Therefore the effort has been concentrated on biological control based on application of hypovirulent isolates of the fungus (Heiniger and Rigling, 1994).

In Slovakia no natural hypovirulent isolates were found out till now (Adamčíková et al., 2012), so French and Hungarian hypovirulent isolates were used to prepare suitable isolates for biological control in our area (Ondrušková et al., 2010). The success of biological control was very variable and it fluctuated from 5 to 72.7% (Juhásová et al., 1997 and 2005).

Successful managing bio-control of chestnut blight requires a rapid, convenient and reproducible virulence test. Standard tests involve inoculation of the fungus into living trees (Elliston, 1978; Griffin et al., 1978), excised stems (Elliston, 1985; Fulbright et al., 1983), or apple fruit (Fulbright, 1984) with incubation times of 2–3 months, 5 weeks, and 3 weeks, respectively. Field research on living trees can be difficult because several healthy trees of similar size and developmental stage must be found for replicate inoculations with the fungus. Genetic variation among the trees can introduce another variable to this test. In addition, canker development from field inoculations can be poor during late fall and winter. The cut-stem method, in which comparable cut-stem sections from a tree (or trees) are inoculated with fungi in the laboratory, can be used to overcome the seasonal limitations of field tests and to minimize size, developmental-stage, and genetic variations. However, the cut-stem virulence-test incubation period is also long and requires constant, high humidity. Results from apple fruit inoculation test can be obtained more quickly, but because this tissue is substantially different from chestnut, the conclusions drawn from the fruit data may not always be directly applicable to chestnut. McCarroll and Thor (1985) found that inner-bark tissue from American chestnut has a detectable reaction to fungal products in vitro. As a result, this type of tissue has potential for use in fungal virulence tests. Following this finding a rapid *C. parasitica* virulence test was developed using excised bark- and wood-tissue sections with incubation time only of 4 days (Lee et al., 1992). Unlike the standard tests where canker area formed on the bark surface was evaluated, in this test necrotic areas formed on inner-bark- and wood tissue sections were measured.

In our study we have combined method of cut-stem sections with the method of bark- and wood-tissue sections and we evaluated size of necrotic areas formed on inner bark and wood tissue on the stem sections in which bark and wood tissues were not separated prior to mycelium inoculation. The main aim of this study was to assess reliability of the new, rapid method of screening chestnut for resistance and/or lower susceptibility to chestnut blight especially in relation to different date of sampling. At the same time we have attempted to assess: (i) interaction of fungal isolates with host trees in necrosis induction, (ii) interaction of necrotic lesions size with type of fungal isolates, (iii) seasonal effect on the necrotic lesions size.

Material and methods

Fungal isolates and cultural conditions

The following virulent strains of *C. parasitica,* each from a different vegetative-compatibility (vc) group, were used in the study: M1297 (MAT-1, vc group EU1, Pian Ne, Swiss), M1115 (MAT-2, vc group EU2, Gorduno, Swiss, Rigling, 1995), EU12 (SA 16 from European vc tester database, Tonara, Italy, Cortesi et al., 1998). These strains were found to be the most, medium and least virulent, respectively, following previous virulence tests on cut-stem sections. In two last trials (29th June 2011 and 13th July 2012) in addition to virulent strains also a hypovirulent one was used in the tests. This strain named as I22 × IHB2 (MAT-1, vc group EU12, Príbelce, Slovakia) was produced by pairing a virulent strain isolated from the tree I22 (a seedling *C. sativa* from the family TV21o.p./15 grown in Príbelce orchard) with hypovirulent strain IHB2 coming from Hungary (Radócz, 2001). Fungal strains were grown on Malt extract agar (MEA) in Petri dishes for 7 days at 25 °C.

Origin of host trees

Eight seedlings derived from open pollination of eight different *C. sativa* × *C. crenata* hybrids, one seedling derived from open pollination of a *C. sativa* × *C. sativa* tree and one graft *C. sativa* × *C. crenata* were used as host trees in the trials (Table 1).

Parental trees of the tested accessions were derived from both interspecific and intraspecific crosses of a *C. sativa* tree TV21 on locality Tlstý Vrch with *C. crenata* and *C. sativa* trees respectively grown in Spain, Pontevedra. In 1984 two-year-old seedlings of these crosses together with other seedlings derived from other crosses were planted in Arboretum Mlyňany on an experimental plot. In 1997 in village Príbelce a chestnut orchard was established by planting seedlings from interspecific and intraspecific hybrids grown on this experimental plot. In 1999 most of seedlings were grafted by scions coming from female parents of the seedlings. However a great part of grafting was unsuccessful so rootstocks remained as permanent accessions in the orchard. In 2010, first year of the experiment, seedlings were 15 years and the graft 13 years old.

Table 1. Host trees used in susceptibility test, their names, origin and parentage

Tree name	Tree origin	Parent origin / parent No
B4	Seedling	*C. sat.* 21 × *C. sat.* E/4
B10	Seedling	*C. sat.* 21 × *C. cren*. E/7
E4	Graft	*C. sat.* 21 × *C. cren*. E/6
F3	Seedling	*C. sat.* 21 × *C. cren*. E/13
F4	Seedling	*C. sat.* 21 × *C. cren*. E/4
H3	Seedling	*C. sat.* 21 × *C. cren*. E/7
H4	Seedling	*C. sat.* 21 × *C. cren*. E/3
I3	Seedling	*C. sat.* 21 × *C. cren*. E/5
I4	Seedling	*C. sat.* 21 × *C. cren*. E/11

All trees included to the experiment were of good health condition at the time of testing and showed good growth performance. As the experiment was not designed primarily for screening and selection of the resistant chestnut accessions, the eight different genotypes were considered to be a sufficient number for the experiment.

Excised-branch inoculation

One or two branches with no signs of blight infection were excised from the selected trees and then cut to the sections of 10 cm in length. Number of branch sections per tree varied from 9 to 15 depending on the number of used *Cryphonectria* strains. Each strain was applied in 6 repetitions in single tree (three branch sections, each with two inoculations). In each tree, both thinner and thicker branch sections were included to the trial in order to assess the effect of branch thickness on the necrotic lesion size. Two bark plugs, each on the opposite site of the section, were removed from each branch section by using 7-mm diameter cork borer. Agar plugs of the same size from the edge of actively growing colonies of the isolates were inserted into holes with mycelium facing downwards. Sterile agar plugs as controls were inserted to the bored holes in the same number of sections as used for each fungal isolate. The inoculation sites and both cut ends of branch sections were sealed with Parafilm to prevent desiccation and the branch sections were placed to cultivation chamber in the dark at 25 °C with 85% relative humidity. In total, four trials were performed, each of them having started with collecting branch samples on the following dates: 6th July 2010, 19th April 2011, 29th June 2011 and 13th July 2012.

Seven days after inoculation, the branch sections were picked out from cultivation chamber and diameter of each section in its middle position was measured. Subsequently bark and wood tissues of branch sections were separated and on wood tissues the length (a) and width (b) of the necrotic lesions were measured. As area of necrotic lesions was in general of ellipsoid shape, formula for the ellipse area calculation was used (a × b × 0.785).

Data analysis

Data obtained in each of four trials were evaluated separately because the trials included both the same and also different trees. The size of necrotic lesions was evaluated by analysis of variance where as sources of variance were assumed the trees, isolates and interaction trees × isolates. Differences between the mean values were evaluated by Duncan's multiple range test. Statistical package Statgraphics 5.1 was used for data processing.

Results

Variation of necrotic lesions size among trees and dates

Correlations between diameter of branch segments and necrotic lesion size were very low and in individual trials varied from r = –0.2658 to 0.2365 and were not significant at P = 0.05. Following this finding, thickness of branch segments was not assumed as source of variation in analysis of variance of necrotic lesion size.

In particular dates a size of necrotic lesions varied among trees with different results. The trees with the smallest lesions in one date had the largest or medium large lesions in other dates. For instance trees H3 and H4, both seedlings derived from two *C. sativa* × *C. crenata* hybrids, had in 2010 the smallest lesions, next year in the spring of 2011 H3 the smallest and H4 the largest spot but in the summer of 2011 both trees had the largest lesions (Table 2). In the next year 2012 the tree H4 died out because of chestnut blight but H3 was healthy without any symptoms of disease and had medium large necrotic lesions in the test. The tree B10 with symptoms of chestnut blight in 2012 had in this year the smallest necrotic lesions on average among seven tested trees.

Table 2. Mean necrotic area on wood-tissue of cut-branch sections of the selected host trees after infection by all isolates at different dates

Infection date							
29-06-2010		12-04-2011		22-06-2011		13-07-2012	
Tree name	Necrosis [mm^2]	Tree name	Necrosis [mm^2]	Tree name	Necrosis [mm^2]	Tree name	Necrosis [mm^2]
H3	750.55 a	H3	347.30 a	E4	740.72 a	B10	566.87 a
H4	755.14 a	E4	430.38 b	I4	832.28 ab	I4	672.80 b
I3	806.14 a	B10	494.55 bc	I3	877.08 b	E4	703.80 b
B4	835.39 ab	B4	506.08 c	H4	993.80 c	H3	719.19 b
I4	865.39 ab	I4	582.80 d	F4	1,039.09 c	B4	738.97 b
E4	958.18 bc	H4	610.37 d	B10	1,039.57 c	F4	746.72 b
F3	1,008.29 c			H3	1,050.36 c	I3	862.56 c

Means followed by the same letter are not significantly different at P = 0.05. Numbers of observations (n) for the trees in individual dates were as follows: 16, 16, 36, 36.

The mean size of necrotic area on inoculated cut-branch sections differed in individual trials. The smallest necroses were observed in cut-branch sections inoculated on 12th April 2011 (on average 495.2 mm^2). Necrotic area increased during the remaining dates as follows: July 13, 2012 – 717.3 mm^2, June 29, 2010 – 875.6 mm^2 and June 22, 2011 – 939.0 mm^2. In the trial performed in April 2011 the most differences occurred among tested trees compared with other dates.

Variation in necrotic lesions size in relation to fungal isolates

Original grading of the virulent strains by intensity of their virulence was in accord with the observed results only during the last test in 2012 (Table 3). In June 2010 the strain EU12 classified as the least virulent was the most virulent and formed necrotic lesions significantly bigger ($P < 0.05$) than strain M1297 classified as most virulent. In June 2011 all three virulent strains induced necrotic lesions of the same size ($P > 0.05$). In April 2011 the strain EU12 gave rise significantly smallest ($P < 0.05$) necrotic lesions but M1297 classified originally as the most virulent initiated formation of significantly smaller ($P < 0.05$) lesions than M1115. A hypovirulent strain used in two last dates initiated significantly smaller ($P < 0.05$) necrotic lesions than virulent strains especially in the test conducted in June 2011.

Interaction of fungal isolates with host trees in necrosis induction

In each of four trials some host trees responded to the same fungal isolate in another way than remaining host trees. In the first trial where only two fungal isolates were used it was tree I4, which was more susceptible to the strain M1297 than to EU12. All remaining trees were more susceptible to EU12 than to M1297 forming necrotic lesions of bigger size. In the second trial where virulent isolates produced lesions of significantly different size ($P < 0.05$), trees I4, H3, H4 and B10 responded to fungal strains in a different way than was average for all trees (Fig. 1). In the third trial where all three virulent isolates produced necrotic lesions of the same size on average, their effect in each of single trees was different but most of differences were not significant. In all host trees necrotic lesions induced by hypovirulent strains were significantly smaller ($P < 0.05$) than those induced by virulent strains except fourth trial, tree I4. In this trial, the highest effect of M1297 calculated for all trees was proved only in three trees (B4, I4 and E4) and in other trees its effect was on the level of either second or third virulent isolate.

Discussion

Data on area of necrotic lesions varied in this study in relation to several factors. Dates of test performance showed the most significant effect on this variation. Lesions developed on stem sections collected in dormant period (beginning of April) were two times smaller than those developed on stem sections from late June. Similar results were also obtained by Guérin and Robin (2003) after inoculation of 4-year-old *C. sativa* sprouts *in situ* and *in vitro* on stem segments. In both trials, lesion length was lower in April than in June, more markedly in excised chestnut segments. Generally in all trials bark susceptibility was higher during the vegetative period of the chestnut than during the dormant season. The authors recommend using for inoculation tests chestnut segments excised during the vegetative period rather than dormant segments (higher percentage of infected segments when inoculated by spores and higher lesion development when inoculated by mycelium). However, the proposed method might not be reasonable in all cases. For instance, if we wish to distinguish host trees of moderate blight resistance

Table 3. Mean necrotic area on wood-tissue of cut-branch sections developed after infection of virulent (M1297, M1115, EU12), hypovirulent (I22 × IHB2) isolates and pure culture medium (control) at different dates of infection

Infection date							
29-06-2010		12-04-2011		22-06-2011		13-07-2012	
Isolate	Necrosis [mm^2]	Isolate	Necrosis [mm^2]	Isolate	Necrosis [mm^2]	Isolate	Necrosis [mm^2]
Control	184.9 a	Control	114.5 a	Control	271.8 a	Control	89.4 a
M1297	1,117.1 b	EU12	535.3 b	I22 × IHB2	536.5 b	I22 × IHB2	605.1 b
EU12	1,324.7 c	M1297	600.1 c	M1297	1,271.3 c	EU12	890.6 c
		M1115	731.1 d	EU12	1,275.6 c	M1115	956.8 c
				M1115	1,339.7 c	M1297	1,044.9 d

Means followed by the same letter are not significantly different at $P = 0.05$. Numbers of observations (n) for the isolates and control in individual dates were as follows: 48, 35, 42, 48.

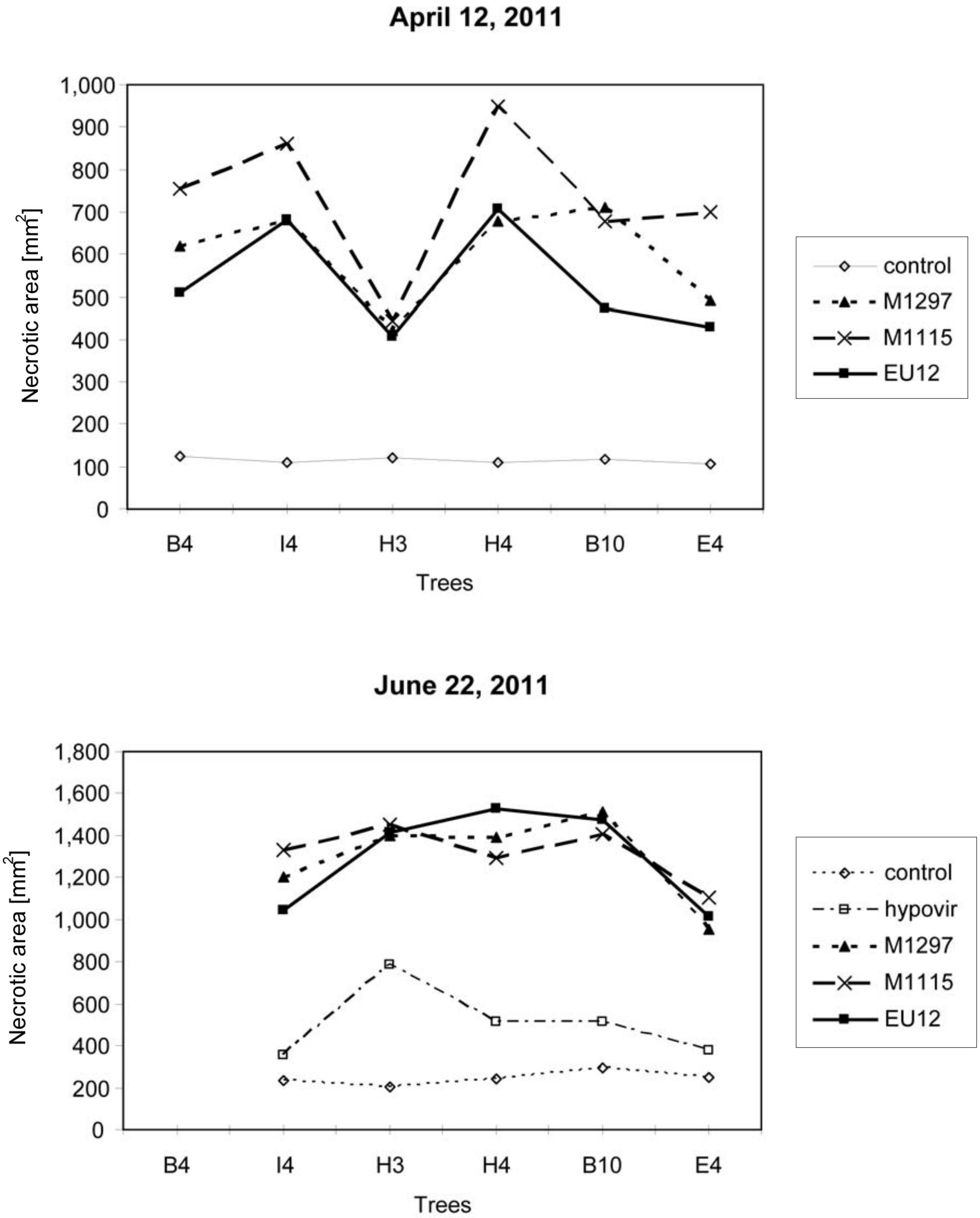

Fig. 1. Interaction of host trees and fungal isolates in forming necrotic area on in vitro cultivated branch segments in two different dates. M1297, M1115 and EU12 are virulent isolates and hypovir is the hypovirulent isolate I22 × IHB2.

from hosts of low or else high resistance, a more rapid growth of inoculated isolates may make even eventual genetic differences between tested trees in susceptibility to chestnut blight. Namely in our study just inoculations performed on branch sections from dormant period (early April 2011) resulted to the most distinct differences among host trees as well as among fungal isolates in the size of necrotic area. At the end of June 2011, when necrotic lesions were larger, differences among host trees were less distinct and among tested virulent strains absent at all. Similarly, relatively uniform response of tested trees to inoculated isolates was also at end of June 2010 and in July 2012, when in five out of seven trees necrotic lesions were of the same size.

Seasonal variation in canker development appeared to result from seasonal variation of chestnut susceptibility particularly of intrinsic susceptibility of chestnut cortical tissues and from the direct effect of meteorological factors on *C. parasitica* development (Guerin and Robin, 2003). Just during summer, susceptibility of chestnut cortical tissues might be higher in all tested trees but in spite of this, individual trees may exhibit different response to different isolates. Although responses of particular host trees to different virulent isolates (interaction host tree × isolate) varied also in dormant period, the variation was higher in summer period. In this context, Elliston's (1978) recommendation for using dormant stems to carry out pathogenicity tests seems to be reasonable.

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Influence of soil moisture on litter invertebrate community structure of pine forests of the steppe zone of Ukraine

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Abstract

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An analysis of litter invertebrate community structure of 141 pine forest ecosystems in the steppe zone of Ukraine in five moisture gradations has been carried out. The absolute number of macrofauna individuals in the pine forests of the steppe zone of Ukraine is on average 5 times lower than in the corresponding moisture conditions of deciduous forests. The average number of species and the Shannon-Weaver diversity index increases from mesoxerophilous to hygromesophilous conditions. The relative number of saprophages increases when the degree of moisture increases from mesoxerophilous through to mesohygrophilous conditions. Zoophages do not change their number with optimisation of moisture conditions; only the number of their species increases. The relative number of polyphages decreases as the soil humidity increases. It is possible to observe significant deviations in the size structure of pine forest macrofauna in comparison with that of steppe zone deciduous forests: the share of 4–7 mm size group increases and the number of individuals with >20 mm body length decreases in most moisture gradations. In pine forest litter, Formicidae occupy the first place among dominants, in hygromesophilous and mesohygrophilous forest types Porcellionidae make up more than a quarter of macrofauna, while Lycosidae and Carabidae dominate in all moisture gradations. The results of the studies allow us to assess the importance of pine forests in the steppe zone as low for preserving biological diversity of litter macrofauna.

Key words

biodiversity, litter invertebrate communities, moisture conditions, pine forests, structure of domination, trophic structure

Introduction

The Samarsky coniferous forest (the left-bank part of Dnipropetrovsk region) forms the southern limit of the natural range of *Pinus sylvestris* (Linnaeus, 1753) within the steppe zone of Ukraine (Belgard, 1971). Only artificial plantations of this tree species can be found further to the south. An annual excess of the amount of precipitation over evapotranspiration by 200–400 mm is typical of natural pine forests in a forest zone. However, the reverse applies to the Samarasky coniferous forest, where the amount of precipitation is actually 150–250 mm lower than the amount of evapotranspiration. This causes certain specific features of the cycling of matter and the composition of animal population of this particular pine forest.

Natural communities of *P. sylvestris* L. and artificial plantations of this species in southern Ukraine are distributed on light-textured types of soil (sand and supersand). The low water-holding capacity of such soils causes rapid evaporation after rainfall, which results in the fact that microbiologically fallen needles do not have time to decompose to the same extent as in forest-steppe or forest zones (Belgard, 1971). The accumulation of a thick layer of fresh litter and, below that, older litter prevents the development of herbaceous vegetation in pine forests, and the low crown closure is prone to overheat the soil surface and the litter during daylight hours (Dougherty et al., 1994; Knight et al., 1994). The compounds released during decomposition of needles (stilbene, various phenolics, lignin and its derivatives), also adversely affect the passage of specific stages of ontogenesis of certain species of litter macrofauna (Hänninen et al., 2011; Pan and Lundgren, 1996; Schanz et al., 1992).

Litter fauna of pine forests has been studied mostly in the north and in the centre of the range of *Pinus sylvestris* L. (Atlegrim and Sjoberg, 1995; Halme and Niemela, 1993; Niemela et al., 1988, 1989, 1992). Some families of litter invertebrates of these types of ecosystems have been studied better than others. For example, for Carabidae, indicative types and systems have been defined, that change their number in the gradient of certain environmental factors (Jukes et al., 2001; Luff et al., 1989; Pollet and Desender, 1987; Rainio and Niemela, 2003; Small et al., 2006), and for Lycosidae – trophic links to prey populations have been analysed (Persons et al., 2001).

The effect of anthropogenic factors on forest ecosystems in arid zones is much more significant in comparison with similar types of forests in the optimum moisture conditions of the forest zone (Apigian et al., 2006; Didham, 1997; Gutierrez et al., 2004; Huhta et al., 1967; Moroz et al., 2011; Schowalter, 1995). Pine forest ecosystems in the steppe zone of Ukraine have only received fragmentary attention from scientists (Brygadyrenko, 2003; Brygadyrenko and Komarov, 2008; Fedorchenko and Brygadyrenko, 2008; Moroz et al., 2011). A general analysis of the litter macrofauna structure of these ecosystems has not been carried out so far.

In this connection, the aim of this paper is to assess the effect of moisture conditions on the main characteristics as well as the trophic, size and taxonomic structure of the litter macrofauna of the pine forests of southern Ukraine.

Material and methods

Invertebrates were collected with the help of soil traps (500 ml volume with 20% *NaCl* solution) during all the vegetation periods of 2001–2013. We examined 141 forest ecosystems with *P. sylvestris* L. as the dominant plant in the following moisture conditions: mesoxerophilous – 30, in xeromesophilous – 51, in mesophilous – 21, in hygromesophilous – 25, in mesohygrophilous – 14. Sample plots differed in the density of vegetation in the tree layer, the percentages of dominant tree and shrub species, the degree of plant litter accumulation, the texture of the soil, the salinity of the soil solution and other characteristics. When selecting sample areas, we tried to cover as fully as possible all the variety of ecological conditions typical for a certain moisture gradation. The ecosystems under examination were natural forests (50–200 years of age) as well as artificial plantations (20–80 years of age). Moisture gradations in the 141 ecosystems examined by a standard scale (Belgard, 1971) were using, first of all, the herbaceous plant community and correcting the results according to the data provided by the visual inspection of the test plot and the soil moisture analysis at the time of the inspection.

Collection was carried out on the territory of Dnipropetrovsk, Zaporizhzhya, Nikolayev, Donetsk and Kharkiv regions. Soil traps were checked every 5–7 days during 20–185 days (from 3 to 24 samples per every test plot). In order to compare the structure of the communities, 20-day time intervals were analyzed (mid-June), the precise timing varying slightly each year but being identical in each particular summer for each test plot.

The degree of diversity of macrofauna communities was assessed with the most widely used diversity indexes (Pilou, 1977; Shannon and Weaver, 1949). The groups that exceed 3% of the total number of the community were considered dominants in the taxonomic structure. Statistical processing of the results was performed in the software package Statistica 8.0. The diagrams show median, 25–75% quartiles and the selected outlier data points can (outliers – º, extremes – *). Only average values of the characteristics under discussion are given in the text due to the limited space of the paper. To compare the samples, the univariate analysis of variance was used, the differences between the values of the various moisture gradation characteristics being considered reliable at $P < 0.05$.

Results and discussion

Main characteristics of the communities

The number of invertebrates in the pine forest litter is on average 5 times lower than in the corresponding moisture conditions of deciduous forests (24.5 and 129.0 samples/100 trap-days accordingly). At the same time, certain test plots differ significantly due to the greatly increased number (up to 100–320 samples/100 trap-days) of Formicidae (*Formica cinerea* (Mayr, 1853), *F. fusca* (Linnaeus, 1758), *F. glauca* (Ruzsky, 1896) dominate). The change of moisture conditions in pine forests does not significantly affect the total number of litter invertebrates (Fig. 1a): the lowest numbers are present in minimal in mesohygrophilous moisture conditions (7.6 samples/100 trap-days), the maximum numbers – in xeromesophilous conditions (40.0 samples/100 trap-days).

The average number of species in the soil traps significantly increases during a 20-day period of their exposition from 10.3 in mesoxerophilous up to 20.4 in hygromesophilous conditions while not significantly decreasing up to 16.7 in mesohygrophilous moisture conditions of the soil (Fig. 1b). It is of interest that in natural deciduous forests of the region studied, the number of species in similar moisture gradations varies within the range of 17.6–22.8, that is, a practically constant number of invertebrate species can be observed in the ecosystem of deciduous forests. Low pine crown closure causes significant daily fluctuations in temperature of pine forest leaf litter, which leads to the decrease of the species number in extreme conditions (in mesoxerophilous and xeromesophilous moisture conditions).

Shannon-Weaver diversity index (Fig. 1c) decreases significantly during the transition from mesoxerophilous (2.15 bits) to mesohygrophilous moisture conditions (2.96 bits). There are no significant changes in the Shannon–Weaver index (its average value does not differ from pine forests and is equal to 2.57 bits) in deciduous forests of southern Ukraine with similar moisture gradations. The evenness of the community structure – Pielou index (Fig. 1d) – does not change significantly: average values change within the range of 0.635–0.747 bits in various moisture gradations in the pine forests.

Trophic structure

The average share of phytophages in the trophic structure of litter macrofauna increases from 5.1% in mesoxerophilous to 10.6% in mesohygrophilous moisture conditions (Fig. 2a). There are far fewer phytophages than other groups of invertebrates in the litter fauna (maximum – 35.6% in one variant of hygromesophilous pine forests), the range of their number fluctuations for different moisture gradations is also much smaller. The number of phytophage species in the soil traps increases with the significant increase of moisture from 1–2 to 3–5.

The relative number of saprophages (Fig. 2b) significantly increases during the transition from mesoxerophilous (19.4%) to mesohygrophilous (29.4%) moisture conditions. A similar tendency can also be observed in natural deciduous forests of the steppe zone of Ukraine. The relative number of saprophages in the litter macrofauna of pine forests (21.6%) is significantly

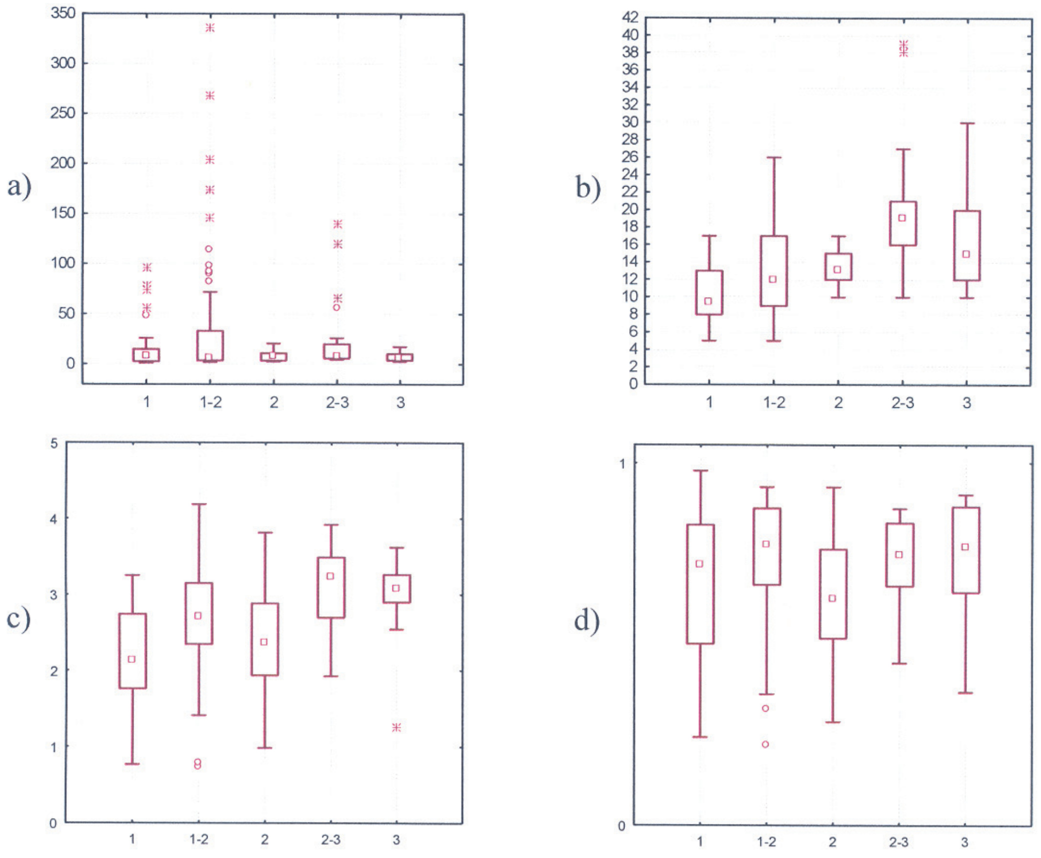

Fig. 1. Main characteristics of litter invertebrate communities of the pine forests of the steppe zone of Ukraine: a) total number (samples/100 trap-days), b) the number of species, c) Shannon index (bits), d) Pilou index (bits); abscissa – moisture conditions (1 – mesoxerophilous, 1–2 – xeromesohpilous, 2 – mesophilous, 2–3 – hygromesophilous, 3 – mesohygrophilous), ordinates – values of characteristics.

lower than for deciduous forests (39.9%). The average number of saprophage species in one test area does not change in various moisture gradations, making up 2–4 species on average.

No significant change was registered in the relative number of zoophages (Fig. 2c) with increasing humidity in a pine forest (minimum share in hygromesophilous – 21.6%, maximum – in xeromesophilous conditions, 36.5%). In deciduous forests, the share of zoophages is significantly lower (16.2% compared with 28.7% for pine forests) ranging from 12.2% to 22.4% for different moisture gradations. The average number of zoophage species in pine forests increases from 3–5 species in mesoxerophilous to 7–10 species in hygromesophilous soils. Thus, the conditions of existence for the majority of predatory invertebrate species in the litter of drier pine forests are not favourable, but the absence of most species of this trophic group is made up for by increased number of the few remaining species of zoophages, keeping their number in this trophic group at a stable level.

The relative number of polyphages decreases significantly (Fig. 2d) from 52.2% in mesoxerophilous and 61.4 in mesophilous to 25.9% in mesohygrophilous moisture conditions making up 43.3% on average of the litter macrofauna of pine forests in numbers. The average number of polyphage species increases from 2–3 to 3–5 with increasing moisture. In deciduous forests of similar gradations a similar tendency is observed: the relative number of polyphages in the litter decreases with increasing moisture, but the average number of species remains constant.

Size structure

In the extreme conditions of pine forests there is a simplification of the size structure of the litter macrofauna (Fig. 3). The dominant group of invertebrates,

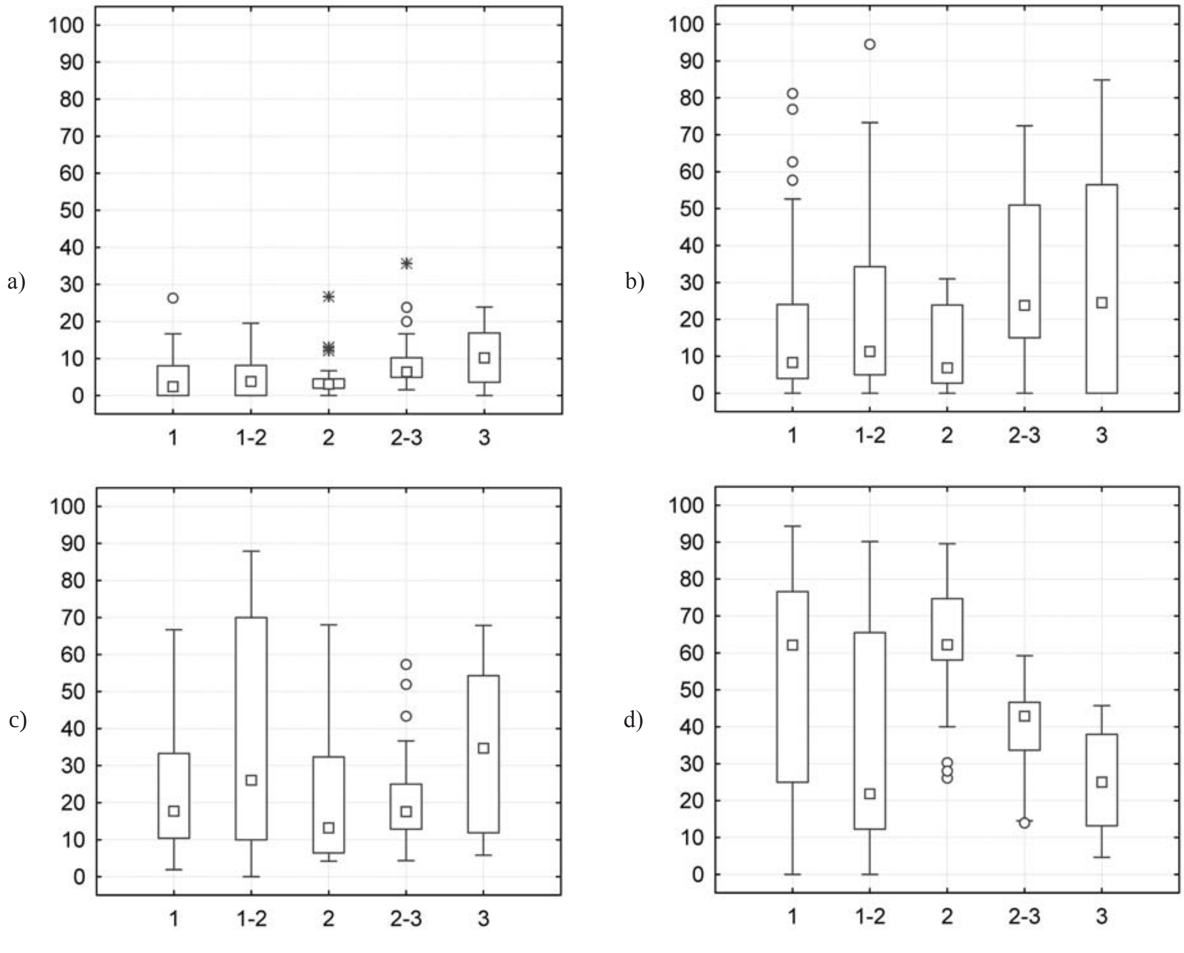

Fig. 2. Trophic structure of litter invertebrate communities of of the pine forests of the steppe zone of Ukraine: a) phytophages, b) saprophages, c) zoophages, d) polyphages; abscissa – moisture conditions (1 – mesoxerophilous, 1–2 – xeromesohpilous, 2 – mesophilous, 2–3 – hygromesophilous, 3 – mesohygrophilous), ordinates – share of the trophic group in numbers (%).

as in other forest types of the steppe zone, consists of invertebrates with the length of 4–7 mm. According to our observations, for different moisture gradations of natural deciduous forests the height of this peak is within the range of 23.4–65.7% and for artificial deciduous plantations – 31.0–52.0%. The height of the peak for pine forests on the chart of the size structure is minimal for xeromesophilous forest variants (50.8%),

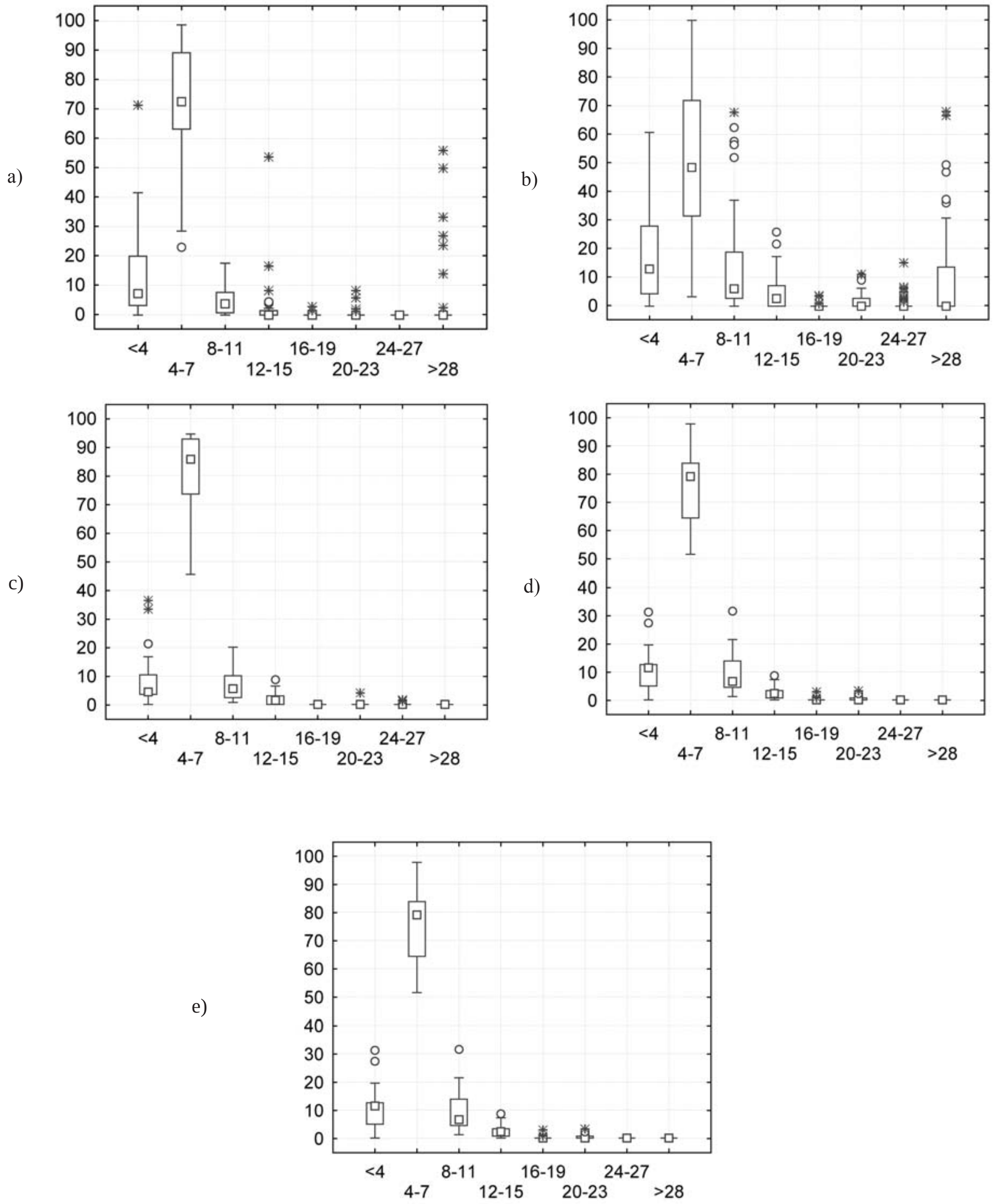

Fig. 3. Size structure of litter invertebrate communities of the pine forests of the steppe zone of Ukraine: a) mesoxerophilous, b) xeromesophilous, c) mesophilous, d) hygromesophilous, e) mesohygrophilous moisture conditions; abscissa – individual body length (mm), ordinates – share of this size group individuals in numbers (%).

and for other surveyed moisture gradations it fluctuates within the range of 71.1–80.4%.

The share in number of the size groups exceeding 20 mm of their body length reaches its maximum (13.0%) in xeromesophilous moisture conditions. It decreases sharply to 0.4–1.0% in mesophilous, hygromesophilous and mesohygrophilous moisture conditions. The low number of species exceeding 20 mm in body length is a characteristic feature of the pine forests of the steppe zone of Ukraine.

The average share of the smallest size group with the body length of less than 4 mm in the community of invertebrates does not exceed 20% of the total number in any of the pine forest humidity gradients (this varies from 9.2% in mesophilous to 17.6% in hygromesophilous moisture conditions). The share of this size group in deciduous forests of the steppe zone is significantly higher. This reduction in the number of invertebrates belonging to the group with the smallest body length is associated with violent fluctuations in hygrothermal conditions (this applies both at the seasonal and twenty-four-hour level) and the unfavourable chemical composition of the diet for many groups of saprophages (the presence in the needles of difficult to assimilate compounds and substances that affect the endocrine system of invertebrates).

Additional peaks for the size groups of 12–15 and 16–19 (as opposed to individual variants of deciduous forests) are absent in the diagram of the size structure.

The qualitative composition of the size structure (the size structure of the community calculated by the number of species) is characterized by the same features as the quantitative composition. Thus, significant deviations in comparison with deciduous forests of the steppe zone can be observed in the size structure of the macrofauna of the pine forests' litter: the relative number of 4–7 mm size group is significantly higher (50.8–80.4%), and the number of individuals with the body length of more than 20 mm decreases sharply in most humidity gradients.

Taxonomic structure

In the litter of mesoxerophilous pine forests of the steppe zone of Ukraine (Fig. 4a), Formicidae (average percentage of dominance in numbers – 49.9%), Lycosidae (13.2%), Julidae (6.9%), Lygaeidae (6.8%) and Carabidae (4.8%) are predominant.

In xeromesophilous variants of pine forests (Fig. 4b), the litter macrofauna is composed principally of Formicidae (34.6%), Lycosidae (13.3%), Carabidae (12.9%), Julidae (11.1%), Isopoda (4.3%) and Lithobiidae (3.0%).

Mesophilous moisture conditions (Fig. 4c) are characterized by the dominance of Formicidae (59.7%), Lycosidae (10.2%), Carabidae (9.0%), Tenebrionidae (5.3%) and Isopoda (4.4%).

In the pine forest communities of hygromesophilous moisture conditions (Fig. 4d), Formicidae (37.3%), Isopoda (25.8%), Lycosidae (9.9%), Carabidae (8.1%) and Lygaeidae (4.0%) predominate.

The complexes of mesohygrophilous variants of pine forests of the steppe zone of Ukraine are significantly different from those characterized above (Fig. 4e): Isopoda (26.9%), Formicidae (23.2%), Carabidae (16.5%), Lycosidae (9.1%), Phalangiidae (6.2%) and Lygaeidae (3.6%).

Thus, in all moisture gradients of the pine forests studied, except for the most humid of the variants considered, the Formicidae family (23.2–59.7%) occupies the first place among the dominants. In hygromesophilous and mesohygrophilous variants of pine forests, the Porcellionidae family of Isopoda order makes up more than a quarter of the number of macrofauna. In all the moisture gradients studied, Lycosidae (9.1–13.3%) and Carabidae (4.8–16.5%) predominate, and the relative number of the latter increases with increasing humidity.

Conclusions

The specific composition of the leaf litter fauna of the *P. sylvestris* L. ecosystems studied, which differs dramatically from the litter fauna of deciduous forests of the steppe zone, may be explained by a combination of the extreme hygrothermal conditions and chemical composition of the litter. Pine forests are characterized by the dominance of invertebrates that are adapted to significant migrations by flight and by ground locomotion, species with hard (sclerotized) external skeleton, species that accumulate or synthesize toxic substances in the hemolymph. With the increase of humidity, the limiting impact of the lack of moisture decreases, and in the most humid of the considered moisture gradients, it is possible to observe a gradual approach of the litter community structure to that of zone types of deciduous forests. On the whole, ecosystems of pine forests are much poorer in species composition than deciduous forests of similar moisture gradients or areas of sand steppe or meadows.

Even optimum hygromesophilous moisture conditions, which allow the formation of a rich litter macrofauna in natural deciduous forests of the steppe zone, do not lead to the formation of a complex and diverse community in the pine forest, whose grass and shrub layer is represented by only a few species of flowering plants. The ecosystems of natural and artificial pine forests do not significantly differ according to the structure and the main characteristics of the litter macrofauna.

The present work is part of a comprehensive monitoring study of the litter macrofauna of forest ecosystems of the steppe zone of Ukraine. The main characteristics and features of the structure considered

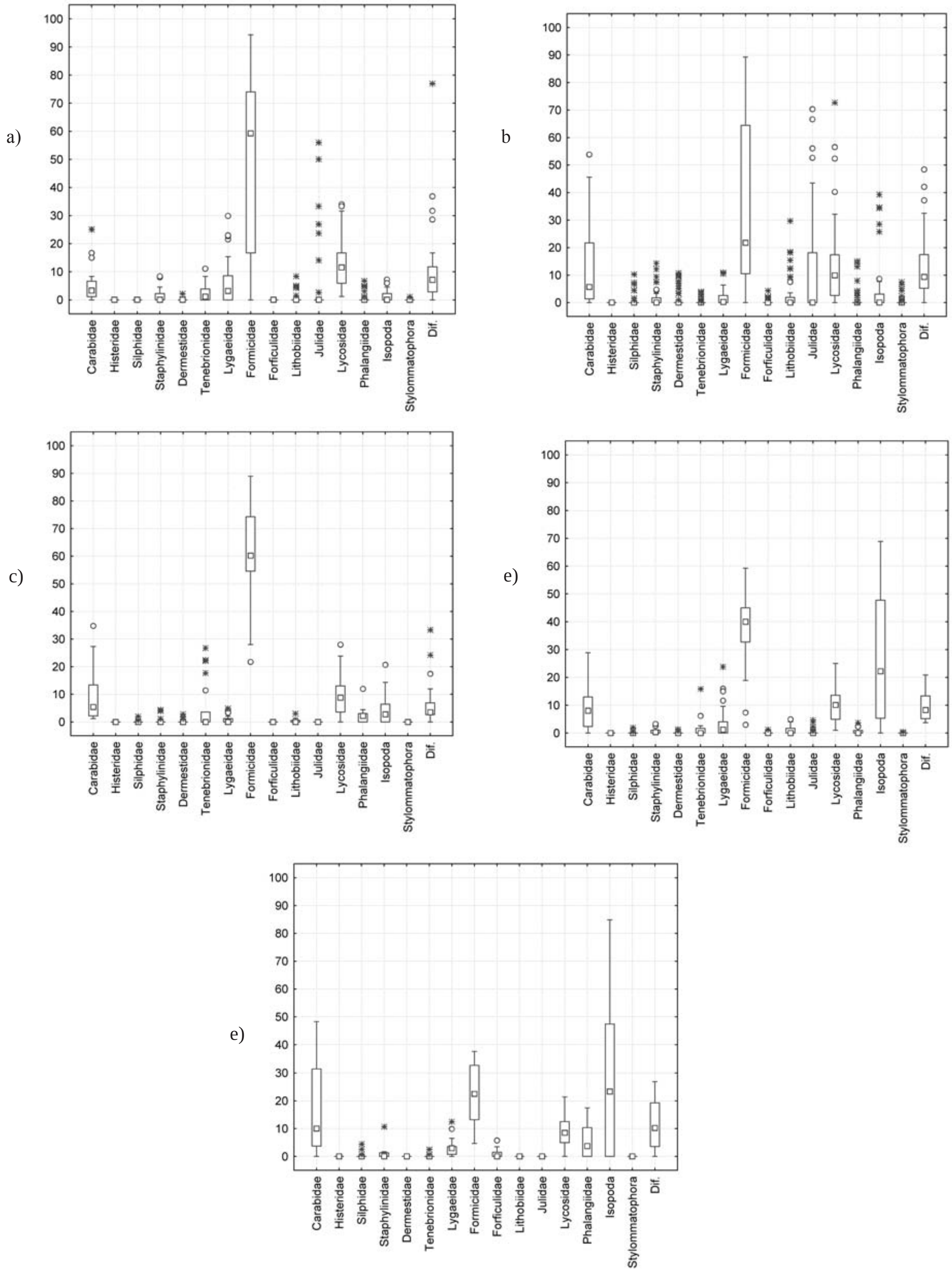

Fig. 4. Taxonomic structure of litter invertebrate communities of the pine forests of the steppe zone of Ukraine:
a) mesoxerophilous, b) xeromesophilous, c) mesophilous, d) hygromesophilous, e) mesohygrophilous moisture conditions;
abscissa – dominant taxonomic groups, ordinate – share of this taxonomic group in the community in numbers (%);
Dif. – species of other taxonomic groups.

here compel us to assess the value of the steppe zone pine forests for the conservation of biological diversity of the litter macrofauna as low in comparison with other types of forest communities in the steppe zone.

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Diversity and ecology of polypores in urban vegetation of northern, central and southern Slovakia

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Abstract

Gáper, J., Sliacka, I., Hvolková, L. 2014. Diversity and ecology of polypores in urban vegetation of northern, central and southern Slovakia. *Folia oecol.*, 41: 17–23.

During the years 2010–2011, we recorded 102 findings of polypores including 20 taxa in three different kinds of urban vegetation of northern, central and southern Slovakia. The most abundant taxa were *Phellinus igniarius* s. l. (16 findings) and *Fomes fomentarius* (L.) J. J. Kickx (11 findings). There was also a broad range of the host plants (15 genera, 22 species). The most abundant species of the host plants were *Acer platanoides* L. (12%), *Cerasus serrulata* (Lindl.) Loudon (11%) and *Salix* × *sepulcralis* 'Tristis' (10%). 53% of polypores colonized stem, fewer of them (21%) colonized branches. We observed the polypores in the town parks, street lines (alleys) and streamside stands. City parks and alleys were the most often habitats of polypore occurrences.

Keywords

diversity, ecology, host plant, polypore, urban vegetation

Introduction

Wood-destroying fungi represent serious problems in urban conditions. They are causing wood rots of infected woody plants and they have an unfavorable influence not only on the health of hosts but also on their aesthetic appeal. Although their harmful activity in the association with trees and shrubs has been studied extensively, relatively few specific studies have been conducted on the urban mycoflora in Europe (Tello et al., 2005). Supuka et al. (1991) mention that parasitic mycoflora of urban areas is very various. The fungi are getting in this environment from forest nursery, surrounding forest stand, orchards and gardens. From there they are transferred by the wind, the insects, by the rain, and very often by human on the woody plants, which are grown for improvement of environment. There are number of factors which influence polypore spreading. Overall, there are biotic and abiotic agents, for example Takemoto et al. (2010) indicate unfavourable weather conditions (drought, heat, low temperatures, overhumidity, ongoing global warming). Sinclair et al. (1987) also stated that the fungus might aggressively colonize trees stressed by heat and drought. Similarly, Schwarze et al. (2000) also consider that weather conditions such as humidity, temperature fluctuations, and UV radiations definitely cause degradation of wood after a certain time. Concerning abiotic agents, there are some examples of fungal spreadings – traffic, industries, incorrect tree maintenance, construction work near trees, and deliberate man-made injuries. Furhermore, Balder (1994) revealed nitrogen enrichment in the subsoil caused by dog urine in urban situation. He considered that high nitrogen contents in the wood can lead to higher rates of degradation by fungi, or cause increased disposition for decay. In

Slovakia, predominantly Gáper with his co-workers (Gáper, 1996; 1998; 2001; 2002; 2003a; 2003b; Gáper and Gáperová, 1999; Gáper and Repáč, 2003) are concerning with diversity and distribution of polypores and wood-decaying fungi in urban areas. Tello et al. (2005) state that polypores such as *Bjerkandera adusta* (Willd.) P. Karst., *Fomes fomentarius* (L.) J. J. Kickx, *Phellinus igniarius* s. l., *Trametes versicolor* (L.) Pilát and some others are always common in urban areas if the appropriate host is present. Furthermore, Tello et al. (2005) state that species with a narrow host range show great differences in abundance when their hosts are distributed unequally.

There are also some differences in the occurrence and distribution of particular fungal species according to the size of a populated area. The greatest species number was recorded in the largest cities. Some wood-destroying fungi such as *Ganoderma australe* (Fr.) Pat. occur almost exclusively in areas of the human habitation or areas influenced by the human activity (known as synantropic species) (Tello et al., 2005).

The goal of this work is to find out polypore diversity and their binding to host trees in selected type of the urban vegetation areas in observed towns.

Materials and methods

The field research was realised in 8 towns of northern, central and southern Slovakia: Dolný Kubín, Tvrdošín, Námestovo, Ružomberok, Zvolen, Detva, Lučenec, and Fiľakovo. In-field research ran for two years. The findings are recorded in 3 categories of urban vegetation: town parks, street lines (alleys), and streamside stands. Findings were repeatedly taken place in all three sampling sites. The harmonogram of sampling was as follows:

1st collection: October–November 2010
2nd collection: May–June 2011
3rd collection: November 2011.

The polypores were collected according to the harmonogram of their fructification growing period. There were the fruiting bodies of the expected higher incidence and greater diversity of occurring polypores in spring and autumn. Abstraction activities were performed repeatedly in the same localities. Occurrence of new findings was recorded on host trees. We recorded essential characteristics of occurring polypores, host trees, and habitat in which host occurred during abstraction in the field.

The patterns of the field record were as follows: sequential sample number, type of fungus, town, street, who collected, who determined, collection date, GPS coordinates of finding, altitude, type of host tree species, type of fruiting bodies, the number of fruiting bodies, place of fruiting bodies on tree species, description of tree damage (presence of cavities, rots, crown-dying and other visible damage; in case of cavity – height, depth and width of the cavity), diameter of the trunk and description of habitat conditions. The sample material obtained in the field research was therefore determined in the laboratory. Individual wood-decaying polypores were determined by using of the designation keys (Hagara et al., 2005; Hansen and Knudsen, 1992; Breitenbach and Kränzlin, 1986; Jülich, 1984). The evaluation of the results was necessary for creating the comprehensive database containing all collected data during the field research.

Results

During the field research in the years 2010 and 2011 we recorded 102 findings, 20 taxa and 13 genera of polypores in the selected towns of northern, central and southern Slovakia (Fig. 1.). As it is obvious from the chart, most commonly occurring taxon was *Phellinus igniarius* s. l., which accounted for 13.73% of all the findings. As relatively frequent taxa, *Fomes fomentarius* (L.) J. J. Kickx (10.78%), *Bjerkandera adusta* (Willd.) P. Karst. (8.82%), and *Trametes versicolor* (L.) Pilát (8.82%) were recorded. Rare taxa were *Ganoderma resinaceum* Boud., *Inonotus hispidus* (Bull.) P. Karst., *Inonotus rheades* (Pers.) Bondartsev & Singer, *Phellinus alni* (Bond.) Parmasto, and *Spongipellis spumeus* (Sowerby) Pat. (Fig. 2).

The individual samples were collected in three selected kinds of the urban vegetation – town parks, street lines (alleys) and streamside stands. The town parks and street lines were the most frequent habitat of polypores. A great number of findings was recorded in the towns of Zvolen and Lučenec. For the town of Zvolen, there were recorded 30.69% of all findings and 20.79% in the town of Lučenec. The fewest findings were recorded in the towns of Fiľakovo and Námestovo, there was approximately equal number of the polypores – 2.97% of all recorded findings (Fig. 3).

The most frequently occurring polypores were in the town parks of Zvolen and Lučenec, no incidence was recorded in this category in Námestovo. The most frequently occurring polypores were recorded in the street lines of Zvolen, Dolný Kubín, and Detva and no occurrence was recorded in Námestovo. For streamside stands, the most frequently occurring polypores were in Zvolen and Ružomberok and no occurrence was in Fiľakovo, Dolný Kubín and Detva (Fig. 4).

The polypores colonized 14 genera of the host trees. The most often were *Acer platanoides* L., *Aesculus hippocastanum* L., *Cerasus serrulata* (Lindl.) Loudon and *Salix* × *sepulcralis* 'Tristis'. The highest number of taxa of the host plants was recorded in Zvolen and Detva. For Zvolen it was 10 taxa and 8 taxa in Lučenec. 5 taxa of polypores were recorded in Detva and Tvrdošín. The least spectrum of the host plants was in Dolný Kubín (2 taxa).

Fig. 1. The map of field research of distribution of polypores in the towns of Námestovo, Tvrdošín, Dolný Kubín, Ružomberok, Zvolen, Detva, Lučenec, and Fiľakovo.

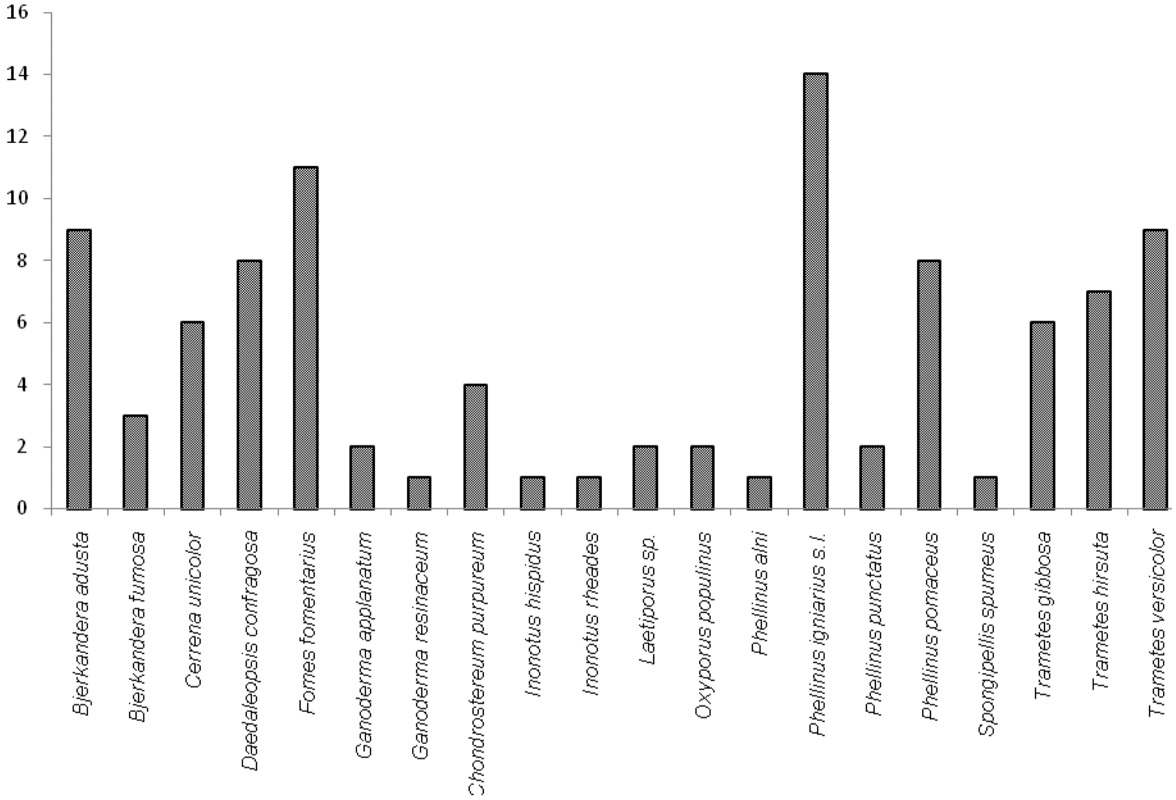

Fig. 2. The number of all polypore species collected from all selected towns (%).

Mentioned taxa were associated with different parts of host plant and they were as it follows: *Bjerkandera adusta* (Willd.) P. Karst., *Cerrena unicolor* (Bull.) Murrill, *Daedaleopsis confragosa* (Bolton) J. Schröt., *Phellinus pomaceus* (Pers.) Maire, *P. igniarius* s. l., *Trametes gibossa* (Pers.) Fr., *T. hirsuta* (Wulfen) Lloyd, and *T. versicolor* (L.) Lloyd. *Bjerkandera fumosa* (Pers.: Fr.) P. Karst. was observed on stem and stump, and *F. fomentarius* (L.) J. J. Kickx on stem and branches. Other taxa were bound to a specific part of host plant:

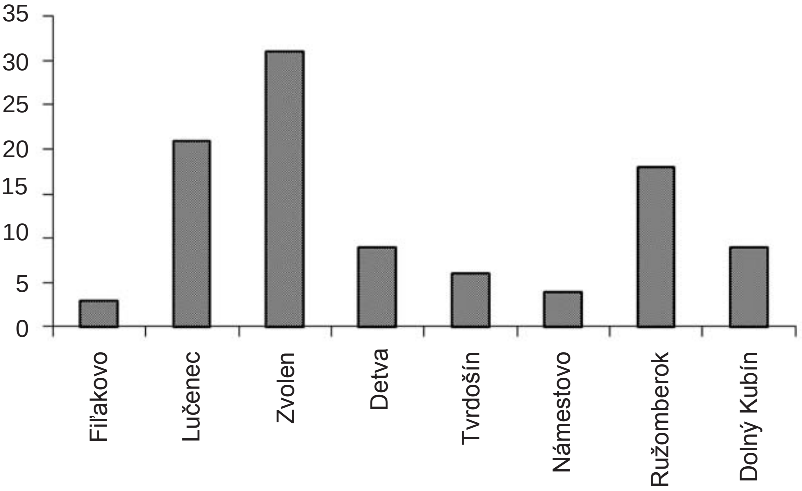

Fig. 3. Comparison of abundance of polypores in each town (%).

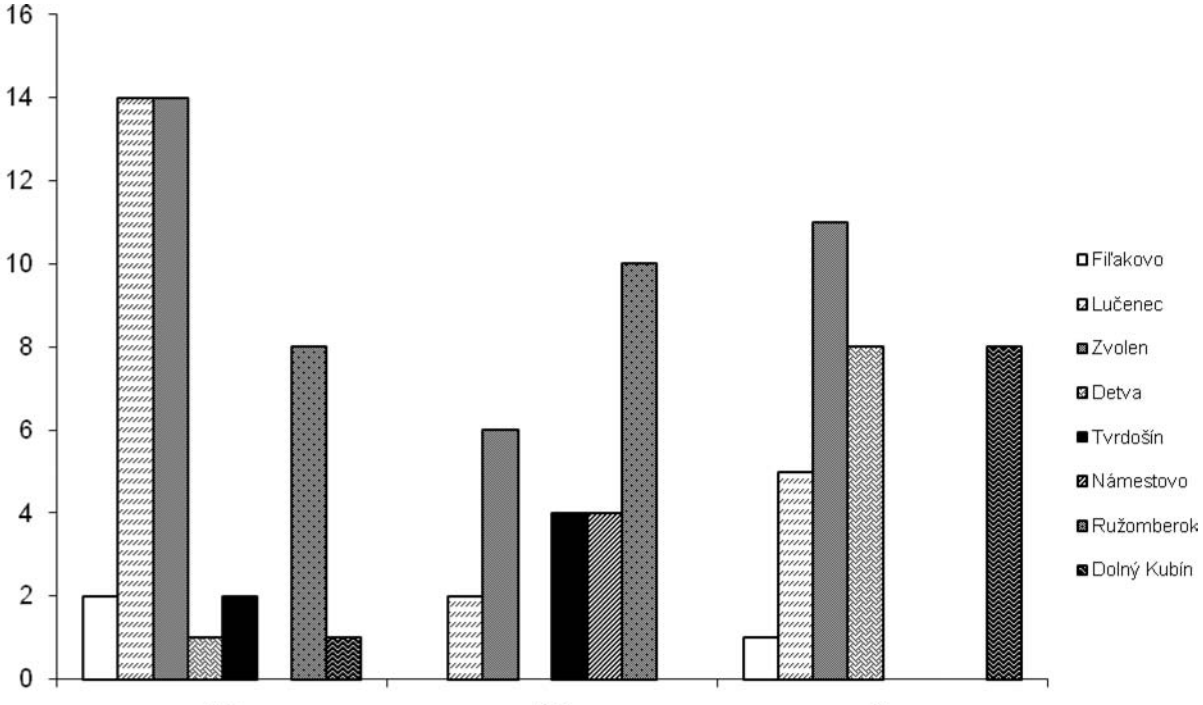

Fig. 4. The comparison of polypore numbers bound to categories of urban vegetation in individual towns. Explanations: SL, street line (alley); SS, streamside stand; TP, town park.

Ganoderma applanatum (Pers.) Pat., *G. resinaceum* Boud., and *Inonotus rheades* (Pers.) Bondartsev & Singer on the base of stem; *Chondrosteum purpureum* (Pers.) Pouzar, *Laetiporus* sp., *Oxyporus populinus* (Schumach.) Donk and *Phellinus alni* (Bondartsev) Parmasto on stem; *Inonotus hispidus* (Bull.) P. Karst. and *Phellinus pomaceus* (Pers.) Maire on branches and *Spongipellis spumeus* (Sowerby) Pat. in cavity (Fig. 5). The greatest number of polypores was observed on the stem. These findings accounted for 53% of the

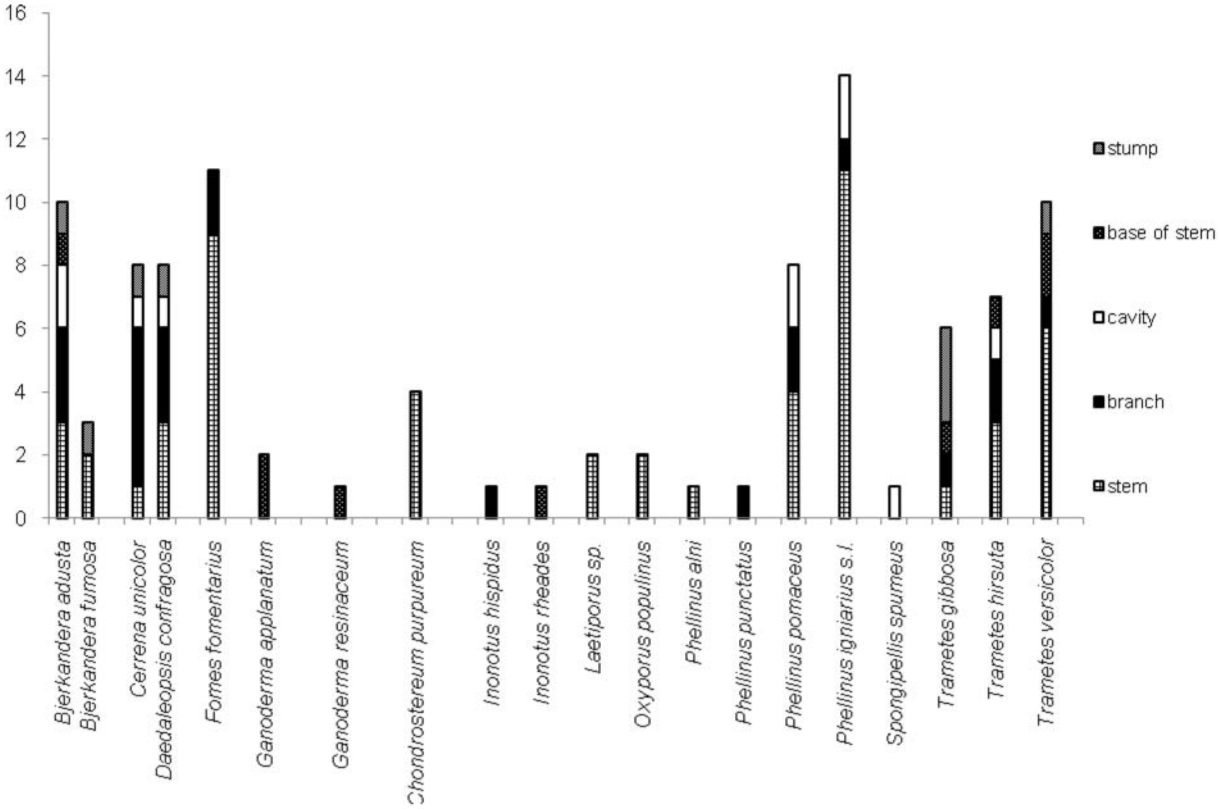

Fig. 5. Binding of polypore taxa to the parts of the host plants.

comprehensive spectrum. On the stumps, we recorded 21% of all polypore findings. In the cavities, there were 10% of all polypores, on the branches 9%, and on the base of stem, only 7% of all findings were recorded (Fig. 6).

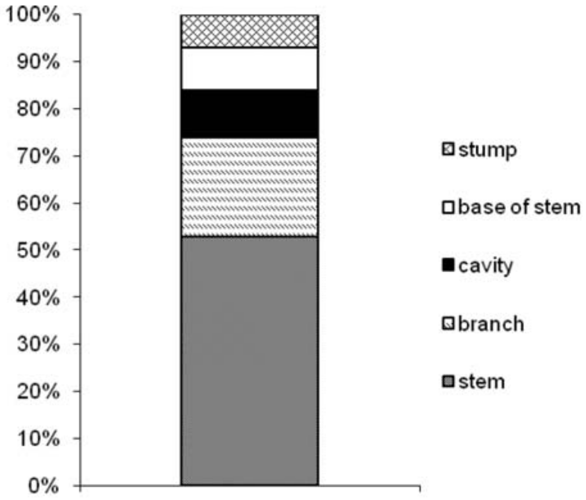

Fig. 6. Proportional representation of all polypores for host plants colonization.

Discussion

The occurrence and the species diversity of the polypores are affected by environmental pollution and nature habitats. Hagara et al. (2005) report that *Fomes fomentarius* (L.) J. J. Kickx is found very abundantly on living and dead wood of different species of the hardwoods. In our case it is occurred more widely and represented until 10.78% of all collected species.

Polypores were bound to 20 taxa of woody host plants. Schwarze et al. (2004) indicate that great number of wood-destroying fungi which cause white rot occur on the hardwoods, what we can confirm. Tomalak et al. (2011) point out the fact that caring of trees should already be started by selecting of the type in urban vegetation. It is appropriate to plant trees originating from the northern coastal areas, exposed to strong winds and salt spray in our conditions. We found that the most invading tree plant species are *Acer pseudoplatanus* L., *Acer platanoides* L., *Aesculus hippocastanum* L., *Salix × scepulcralis* 'Tristis'. The vitality of these plants is reduced mainly by frosts in our northern towns observed and by products of human activity. Hudeková (2002) concludes that in spite of attempt to applicate domestic plants their utilization is difficult in urban polluted environment. There are frequently attacked species of *Acer* spp., as well as trees used for landscaping and fruit production purposes as *Cerasus avium* (L.) Moench, *Malus domestica* Borkh. or *Prunus spinosa* subsp. *dasyphylla* in our research. ASHMORE (2003) reports that substances that pollute the environment can assist in the process of wood-destroying fungi infection of plants by altering the surface of the host plant in terms of surface chemistry, permeability, wettability or changing the character of the leaf exudates in the urban environment. The rate of air pollution often has an impact on the species composition of fungal communities. Up to now, however, these interactions are not well understood. A further factor is the humidity. There is negligible percentage of growing areas relative to the asphalt surface in urban vegetation. Wood-decaying fungi require high temperature and appropriate humidity, mostly at the site of host plant infection for their growth. Polypores were taken from three categories of urban vegetation – town parks, street lines, and streamside stands. The greatest percentage of polypores was taken from town park category. The environment of the park mainly reminds of forest ecosystems by its habitat conditions. There is created enough dense tree crown cover, which prevents rapid evaporation. There is also a high proportion of grassland preventing rapid outflow of the surface water. The second category, the richest for the findings from which we took the polypores, were the street lines. Taking into account the site conditions, it can be argued that in all categories of urban vegetation areas, where the incidence of polypores was noticed, habitat was the most stressful for the host plants. The trees are directly influenced by the smog produced by the traffic. The trees are also directly exposed to the salination. We can confirm that trees were in that second richest category of findings influenced by smog and salination even in five of the eight towns. TELLO et al. (2005) also report that trees are highly influenced by salt spray, when passing cars spray aboveground biomass of trees by melted emulsion of the snow and the salt. Therefore, there is altered wettability of wood surface, and also the technical salt gets into the soil and disturbs the root system.

Conclusions

During 2010–2011 we recorded 102 findings of polypores (20 taxa, 13 genera) in categories town parks, street lines (alleys), and streamside stands in the towns of Námestovo, Tvrdošín, Dolný Kubín, Ružomberok, Zvolen, Detva, Fiľakovo, and Lučenec. The most often taxa were *Phellinus igniarius* s. l. (13.73%) and *Fomes fomentarius* (L.) J. J. Kickx (10.78%). The town parks and street lines were the most frequent habitats of polypores. A great number of findings was recorded in the towns of Zvolen (30.69%) and Lučenec (20.79%). The polypores colonized 14 genera of host trees. The most often were *Acer platanoides* L., *Aesculus hippocastanum* L., *Cerasus serrulata* (Lindl.) Loudon, and *Salix* × *sepulcralis* 'Tristis'. The polypore taxa colonized different parts of host plant. The greatest number of polypores was observed on the stem (53%) and on the stumps (21%).

Acknowledgements

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Influence of some growth retardants on growth, transpiration rate and CO_2 fixation of *Caryopteris incana* 'Heavenly Blue'

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Abstract

Harmath, J., Schmidt, G., Forrai, M., Szabó, V. 2014. Influence of some growth retardants on growth, transpiration rate and CO_2 fixation of *Caryopteris incana* 'Heavenly Blue'. *Folia oecol*., 41: 24–33.

In an experiment between May and October 2011, the effect of growth retardants Alar 85 SP 0.4% and 1%, Bumper 25 EC 0.1% and 1%, Cultar 1% and 2%, Cycocel 0.3% and 1%, Mirage 45 EC 0.2% and 1% was tested on *Caryopteris incana* 'Heavenly Blue' young plants. The young plants were sprayed with the retardants three times during the summer. The influence of these retardants on the transpiration rate, stomatal conductance and net CO_2 fixation of leaves was measured by LCi infrared gas analyzing instrument three times: first on the 1st day, second on the 24th day, and third on the 43rd day after the last spraying. The most effective growth retardant was Cultar used in 2% and 1% concentration (that gave the smallest plants), followed by Cycocel and Alar 85 SP; while the effect of Bumper 25 EC and Mirage 45 EC was minimal. The growth retardants affected not only the growth of the plants. They had also some (weak) effect on the transpiration rate, stomatal conductance and net CO_2 fixation of leaves. In most cases Cultar 1% and/or 2% and in some cases Mirage 1% showed the lowest values in comparison with all the other treatments, but no statistical correlation was found between their dwarfing effect and the results from LCi measurements.

Keywords

Caryopteris incana, growth retardants, net CO_2 fixation, stomatal conductance, transpiration rate

Introduction

Chemical nature and mechanism of action of various growth retardants

According to Dicks (1980) the growth retardants are synthetic compounds that reduce the shoot elongation. This is achieved in the sub-apical meristem and is realized by inhibiting the biosynthesis of the precursors of gibberellins acid. In such a way, they inhibit the operation of gibberellins, which take a key role in cell elongation of plants, Schuh (2012).

According to Quinlan and Richardson (1984), paclobutrazol has antagonistic interaction with endogenous gibberelins, whereas Steffens et al. (1983) stated that paclobutrazol is an inhibitor of gibberelin synthesis.

Cycocel presumably inhibits cell growth but does not reduce the number of cells. The stress caused by it helps in flowering. This retardant is generally recommended for plants with thick foliage (Fodor, 1996). Alar 85 inhibits cell division and delays flowering. It is recommended for plants with thin (weaker) foliage (Biza, 1995).

Besides retarding the growing process, some retardants are also used for other purposes in practice: in plant propagation, for influencing of plant habit, for influencing of flowering and fruit set control, for programming the ripening process, for increasing ecological tolerance and/or resistance (Armitage, 1994; Basra, 2000).

The most important growth retardants and their use in ornamental horticulture

Many growth regulators are used experimentally, but the transition to approved usage is being delayed for several reasons. A number of mergers, buy-outs, and other dispositions of chemical companies has led to a decrease in the number of commercial compounds available (Cutler, 2004).

The most important compounds available on the Hungarian market are shown in Table 1.

Table 1. The most important dwarfing chemicals on Hungarian market (according to the written information on the label of the preparations)

Trade name	Active ingredient
Alar 85 SP	Daminozide 85.0%
Bumper 25 EC	Propiconazole 250 g l^{-1}
Cultar	Paclobutrazol 250 g l^{-1}
Cycocel	Chlormequat (2-chloroethyl) trimethylammonium chloride 11.8%
Mirage 45 EC	Procloraze 450 g l^{-1}

From Hungary, Mohamed (1997) reported that Daminozide, Chlormequat and Paclobutrazol treatments were effective on reducing the plant height and producing dwarf plants, first of all at higher concentrations. Test plants were: *Tagetes, Petunia, Torenia, Rudbeckia, Buddleja, Hibiscus, Fuchsia, Solidago.* Kisvarga et al. (2010) used CCC (chlormequat), Caramba (metconazol), Cultar (paclobutrazol), Regalis (prohexadion-calcium) and Toprex (difenoconazole and paclobutrazol) to see the reaction of these growth retardants on *Scabiosa atropurpurea, Godetia grandiflora, Coreopsis grandiflora.* Regalis, Toprex and Cultar gave dwarf plant and increased the number of shoots. Köbli et al. (2010) tested the dwarfing effect of two fungicides, Bumper 25 EC (propiconazole) and Mirage 45 EC (procloraze) comparing to Alar 85 SP (daminozide). Testplant was *Ismelia carinata.* Most effective was Alar 85 SP.

From the other countries, Sytsema and Glas (1983) reported that combined spray with daminozide and ethephon on *Forsythia intermedia* 'Lynwood Gold' shortened the lateral branches and improved flower bud formation and thus improved the ornamental value.

Goulston and Schearing (1985) reported that paclobutrazol can be applied as a foliar spray or as a soil drench, according to user preference, and compared with currently used standards, it can produce superior quality of plants and quantity of flowers of certain species, often with earlier flowering.

Kristensen and Adriansen (1988) reported that *Hebe* × *franciscana* 'Variegata' plants treated with paclobutrazol gave some remarkable results (measured at the time of flowering) compared with control plants. One spray with 10 mg l^{-1} paclobutrazol caused suitable growth retardation and doubled the number of inflorescences per pot.

Joustra (1989) concluded that regulation of growth and flowering of ornamental shrubs often offers good possibilities to grow them as a pot plant for interior decoration (for example *Cupressus macrocarpa* 'Goldcrest'), but besides the possibilities several problems still exist.

Wilkinson and Richards (1991) used paclobutrazol mainly to reduce the shoot extension of *Rhododendron* 'Sir Robert Peel', but flowering was also more abundant after the treatment. All of the drench rates tested (0.05 to 0.4 g pot^{-1}) resulted in prolonged shoot growth reduction and greatly increased the number of flowers, but flowers produced at the higher drench rates were grossly malformed and unacceptable.

Kessler (1998) used ancymidol, paclobutrazol drench, daminozide, and flurprimidol to decrease the plant height of *Coreopsis verticillata* 'Moonbeam' plant height. Paclobutrazol sprays were not effective. The other treatments decreased the plant height, compared with control plants.

In experiments of Cramer and Bridgen (1998) the most attractive potted plants of *Mussaenda* L. were produced with two spray applications of daminozide at 5,000 mg l^{-1} or two drench applications of ancymidol at 0.5 mg pot^{-1}.

Papageorgiou et al. (2002) reported that paclobutrazol reduced lateral shoot elongation and plant height of *Lavandula stoechas* increased the number of nodes within lateral shoots, but delayed the time to anthesis. In contrast, chlormequat reduced plant height with no effect on flowering.

In experiments of Matysiak (2002) application of daminozide at 6,000 mg l^{-1} and paclobutrazol at 100–400 mg l^{-1} to two-year-old magnolia 'Alexandrina' and 'Susan' significantly increased the number of flower buds in comparison with untreated plants. Single application of chlormequat (1,000, 2,000, or 4,000 mg l^{-1}, ethephon (250, 500, or 1,000 mg l^{-1}), and prohexadione-Ca (125, 250, or 500 mg l^{-1}) failed to affect flower bud production in magnolia.

The aim of the studies

In the present experiments *Caryopteris* was used, which is an autumn flowering woody plant. This shrub is mainly sold as a container-grown garden plant. Smaller and more compact plants would need fewer place in the nursery, and perhaps could be marketed not only as outdoor but also as pot-grown indoor flowering plants. Such plants can be obtained by regular pruning but the use of growth retardants would be probably a more effective and cheaper (labour-sawing) way. There are, however, no literatures yet about of the chemical dwarfing of *Caryopteris*.

The aim of the experiment was to study the effect of growth retardants partially in order to decrease the plant size and the growing time, and also to study their effect on leaf gas exchange.

Material and methods

The experiment was carried out in the Experimental Field of Corvinus University of Budapest, Faculty of Horticultural Science in Soroksár, in young plants of *Caryopteris incana* 'Heavenly Blue' propagated by softwood cuttings in 2011.

The cuttings were taken on 16th May, rooted and planted into 9 × 9 cm pots on 27th June. In growth control treatments, the young plants were sprayed three times (on 28th July, on 18th August, on 6th September) with growth retardants, each in two concentrations as follows:

- Alar 85 SP in 0.4% and 1%
- Bumper 25 EC in 0.1% and 1%
- Cultar in 1% and 2%
- Cycocel 0.3% and 1%
- Mirage 45 EC 0.2% and 1%
- Control plants untreated.

Every treatment contained 64 plants. The treated and untreated plants were placed in a sunny place and irrigated depending on the weather conditions (usually every day).

At the end of the dwarfing experiment (on 22nd September 2011) shoot length, blossom attributes, number of nodes per shoot and branching per shoot were measured.

Instrumental measurements of photosynthetic active radiation (PAR) in µmol m^{-2} s^{-1}, leaf surface temperature in °C, stomatal conductance in mol m^{-2} s^{-1}, transpiration rate in mmol m^{-2} s^{-1} and net CO_2 fixation in µmol m^{-2} s^{-1} were made by infrared gas analyzer (IRGA, called LCi device of ADC Scientific Ltd.) three times: 1. one day after the last spraying (7th September), 2. on the 24th day (30th September) and 3. on the 43rd day (19th October) after the last spraying. The measurements were made in the middle of the day (between 11:00 am and 14:00 pm) in full sun on one leaf per plant, in six repetitions.

All data were statistically analyzed by ANOVA using the statistical package SPSS Statistics program (SPSS 19.0 for Windows). Data were separated by Tukey-test at level $p = 0.05$.

Results

Effect of growth retardants on the growth and development

Shoot length

The most effective chemical was Cultar used in 2% and 1% concentration, because this treatment gave the smallest plants (Table 2). Their shoot length was on average 13.7 cm and 17.17 cm, respectively. This was followed by Cycocel 1% and 0.3%, Bumper 25 EC 1%, Mirage 45 EC 1%, Alar 85 SP 0.4% and 1%, Control and Bumper 25 EC 0.1%.

Table 2. The effect of some growth retardants on *Caryopteris incana* 'Heavenly Blue' growth / developmental parameters on 20–21st September 2011 (5 weeks after the last spraying)

Growth retardants	Shoot length cm	Number of nodes			Total number of nodes on the mainshoot	Number of side shoots	Flowering stage*
		to the first visible flowerbuds	to the first open flowers	to the last open flowers			
Control	47.34	8.66	9.56	13.20	13.20	2.89	3.44
Cultar 2%	13.70	9.55	10.14	10.66	12.30	2.06	1.70
Cultar 1%	17.17	9.41	10.38	11.38	11.41	2.45	2.25
Alar 1%	45.91	8.48	9.00	14.53	13.00	4.52	3.09
Alar 0.4%	45.13	8.53	9.59	13.17	13.08	4.86	3.22
Cycocel 1%	34.02	8.88	10.06	12.84	12.88	3.17	2.91
Cycocel 0.3%	34.08	10.31	9.94	12.75	12.78	2.63	2.89
Mirage 1%	44.47	9.56	12.16	13.41	13.41	5.05	2.64
Mirage 0.2%	47.59	8.91	9.92	13.50	13.50	3.83	3.31
Bumper 1%	41.41	9.92	11.28	13.98	13.64	4.27	2.84
Bumper 0.1%	46.61	8.38	9.45	13.22	13.25	3.77	3.39

*Flowering stage of flowers: 0, no flower; 1, flowerbuds just visible; 2, flowerbuds have elongated; 3, flowerbuds show colour; 4, half of flowers buds are open; 5, all flower buds flowering.

Nodes to the first visible flowerbuds

As regards the development of flower buds, the most effective chemical was Bumper 25 EC used in 0.1% concentration (Table 2). Young plants from this treatment set up buds on 8.38th nodes on average. In the other treatments first flower buds occurred on 8.48th–10.31st nodes, with Alar 85 SP 1% giving the lowest and Cycocel 0.3% the highest value. The differences, however, were not statistically significant.

Nodes to the first open flowers

When Alar 85 SP 1% was used the first open flowers were found on 9th nodes, in case of Mirage 45 EC 1% they appeared after 12.16 nodes (Table 2). In the other treatments flowering started from 9.45th–11.28th nodes on average. These values are about one node more than the number of nodes to the first flowerbuds, because in most cases the flowerbuds in the lowest inflorescence did not open at all.

Nodes to the last open flowers

The best results were given by plants treated by Alar 85 SP 1%, where the last flowers developed on the 14.53rd node, while in the other treatments last open flowers were found between the 10.66th and 13.98th node (Table 2). Thus, plants treated with Alar 85 SP 1% had more flowers per shoot, than plants treated with other retardants.

Total number of nodes per mainshoot

Plants treated by Cultar 1% showed the lowest (11.4), and plants treated by Bumper 1% the highest (13.6) total number of nodes (Table 2). However, it is to note, that in spite of great differences in the minimal and the maximal shoot length (13.7 cm and 47.59 cm respectively), the number of the nodes showed only slight alternations.

Average number of side shoots

The number of side shoots showed the highest value in Mirage 45 EC 1% treatment (5.05), and the lowest value in Cultar 2% treatment (2.06) (Table 2). The other treatments gave intermediate values, their number of side shoots was between 2.45 and 4.86.

Flowering stage

Those growth retardants which most effectively decreased the plant height (for example Cultar 2%), had also delayed approximative one week the flowering stage (Table 2). The less effective retardants (Mirage 45 EC and Bumper 25 EC) flowered at the earliest time, parallel with Control plants.

Effect of growth retardants on the photosynthetic active radiation, leaf surface temperature, stomatal conductance of leaves, transpiration rate and net CO_2 fixation of leaves

Photosynthetic active radiation (PAR) and leaf surface temperature

Table 3 shows that differences between treatments were small and not statistically significant neither in PAR values nor in the leaf surface temperature (they depended more on the weather conditions than on the retardant treatments). The second measurement gave the highest values in all treatments.

Stomatal conductance of leaves

In the first measurement (day 1 after the last spraying) leaves treated by Mirage 45 EC 0.2% had the highest (0.44 mol $m^{-2} s^{-1}$), and leaves treated by Cultar 1% the lowest stomatal conductance (0.28 mol $m^{-2} s^{-1}$ (Fig. 1).

In the second measurement the stomatal conductance decreased in all measurement. Leaves treated by Alar 0.4% showed the highest (0.28 mol $m^{-2} s^{-1}$) and plants treated by Mirage 45 EC 1% the lowest (0.1 mol $m^{-2} s^{-1}$) values.

In the third measurement the leaves treated by Cycocel 0.3% exhibited the highest (0.15 mol $m^{-2} s^{-1}$), and leaves treated by Mirage 45 EC 1% the lowest (0.05 mol $m^{-2} s^{-1}$) stomatal conductance.

In the first and the third measurement, the differences in stomatal conductance were not statistically significant, but in the second measurement significant differences were found between the highest and the lowest values.

Transpiration rate of leaves

In the first measurement, the leaves of plants treated by Cultar 1% showed the lowest transpiration rate (6.13 mmol $m^{-2} s^{-1}$) and, leaves treated by Mirage 45 EC 0.2% showed the highest (7.33 mmol $m^{-2} s^{-1}$) transpiration rate (Fig. 2). The differences were not statistically significant.

In the second measurement, plants treated by Mirage 45 EC 1% and Cultar 2% had resulted the lowest (4.27–4.31 mmol $m^{-2} s^{-1}$) and plants treated by Bumper 1% and Alar 85 SP 0.4% resulted the highest (7.35–7.5 mmol $m^{-2} s^{-1}$) transpiration rate. The differences were statistically significant. Results given by the other treatments (including the untreated control) were close to each other.

In the third measurement the transpiration rate of each treatment was considerably lower than at the earlier measurements. Application of Mirage 45 EC 1% caused the lowest (1.23 mmol $m^{-2} s^{-1}$), and application of Cycocel 0.3% caused the highest (2.82 mmol $m^{-2} s^{-1}$) transpiration rate. These differences were significant.

Table 3. PAR values on leaves (µmol m^{-2} s^{-1}) and leaf surface temperature (°C) of *Caryopteris incana* 'Heavenly Blue' plants in the first, second and third measurement after the last spraying

Growth retardants	PAR values on leaves [µmol m^{-2} s^{-1}]			Leaf surface temperature [°C]		
	First (Day 1)	Second (Day 24)	Third Day 43)	First (Day 1)	Second (Day 24)	Third (Day 43)
Control	1,113.50	1,555.33	1,171.33	34.53	38.05	27.40
Cultar 2%	1,169.17	1,517.67	948.17	34.78	38.85	27.90
Cultar 1%	1,112.17	1,582.67	1,025.50	34.73	37.72	27.87
Alar 1%	1,031.00	1,402.50	949.83	34.47	38.65	27.88
Alar 0.4%	972.67	1,577.17	1,028.67	34.25	38.03	28.05
Cycocel 1%	1,087.00	1,547.83	1,150.33	34.65	37.37	27.83
Cycocel 0.3%	1,074.50	1,429.50	1,044.33	34.48	37.73	27.93
Mirage 1%	1,091.67	1,444.17	1,048.83	34.77	39.67	28.07
Mirage 0.2%	1,094.33	1,434.17	1,208.17	34.53	37.95	28.02
Bumper 1%	1,017.00	1,569.00	1,090.50	34.60	38.18	27.93
Bumper 0.1%	1,029.50	1,570.67	1,066.33	34.47	38.65	27.82
Average	1,072.05	1,511.8	1,066.55	34.57	38.26	27.88

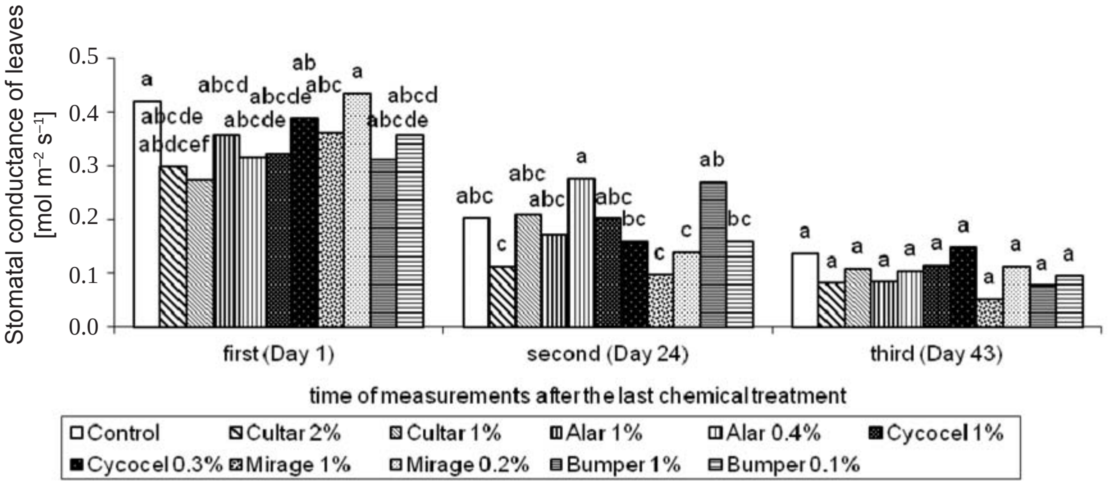

Fig. 1. Stomatal conductance of leaves (mol m^{-2} s^{-1}) of *Caryopteris incana* 'Heavenly Blue' treated by different growth retardants. Columns marked with different letters differ significantly from each other at level p = 0.05, according to the Tukey LSD test.

Net CO_2 fixation of leaves

In the first measurement the net CO_2 fixation of the plants treated with Mirage 45 EC 0.2% and that of the Control plants were somewhat higher from the other treatments, but the differences were not statistically significant (Fig. 3).

In the second measurement plants treated with Cultar 1%, Cycocel 1%, Bumper 25 EC 1%, showed the highest (17.57–18.95 µmol m^{-2} s^{-1}), plants treated with Mirage 45 EC 1% and Cultar 2% showed the lowest (9.15–9.37 µmol m^{-2} s^{-1}) net CO_2 fixation.

In the third measurement plants treated with Cycocel 0.3% had the highest (20.41 µmol m^{-2} s^{-1}) and Mirage 45 EC 1% had the lowest (5.19 µmol m^{-2} s^{-1}) net CO_2 fixation of leaves.

Differences between the highest and lowest values were statistically significant in the second and third measurements.

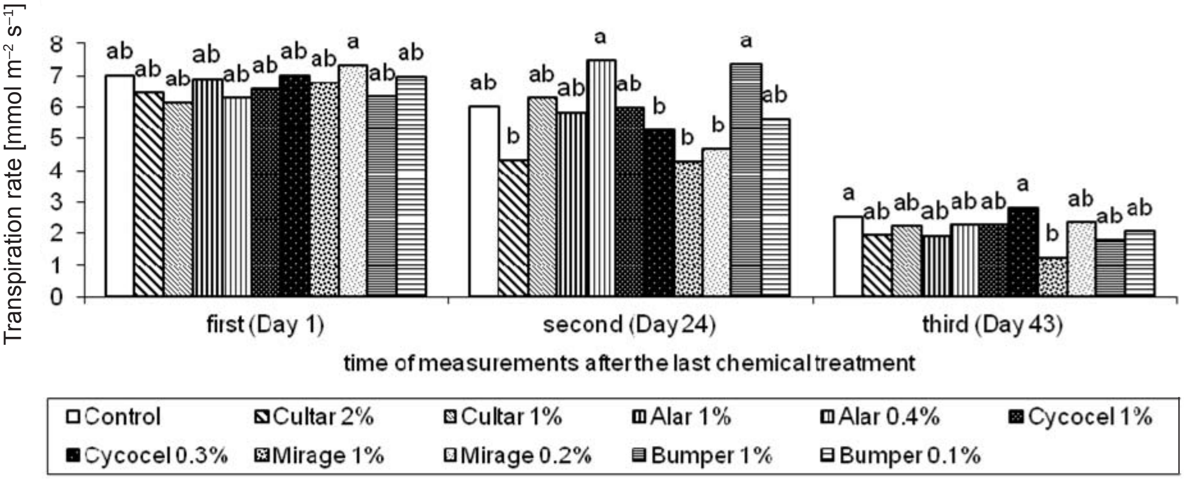

Fig. 2. Transpiration rate (mmol $m^{-2} s^{-1}$) of *Caryopteris incana* 'Heavenly Blue' plants treated by different growth retardants. Columns marked with different letters differ significantly from each other at level p = 0.05, according to the Tukey LSD test.

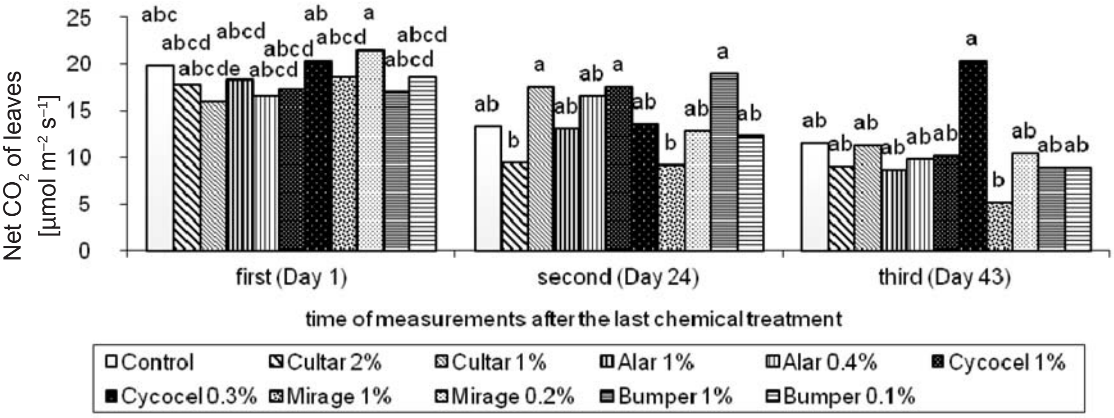

Fig. 3. Net CO_2 fixation of leaves (µmol $m^{-2} s^{-1}$) of *Caryopteris incana* 'Heavenly Blue' plants treated by different growth retardants. Note: Columns marked with different letters differ significantly from each other at level p = 0.05, according to the Tukey LSD test.

Discussion

As seen from the review of literature (see Introduction), numerous experiments with growth retardants were made on glasshouse pot-plants for indoor use: MOHAMED, 1997; KRAUSE, 2003; RAJALEKSHMI, 2009; KISVARGA et al., 2010; KÖBLI et al., 2010. These chemicals were also successfully used on open ground shrubs (JOUSTRA, 1989; WILKINSON and RICHARDS, 1991; MOHAMED, 1997; KRISTENSEN and ADRIANSEN, 1998; PAPAGEORGIOU et al., 2002;) and also on fruit trees (EREZ, 1984; SANSAVINI, 1984; MAX et al., 1986; RADEMACHER et al., 1992; RADEMACHER, 1995).

In our experiments, *Caryopteris* plants treated with some growth retardants became much smaller, but the number of internodes showed only slight changes. It means that the chemicals carry out their dwarfing effect not by decreasing the number but by shortening the internodes. This suggestion is in accordance with the results of CATHEY (1975), JIAO et al. (1986), KOCHANKOV et al. (1989), MOHAMED (1997), MATYSIAK (2002), HANSON et al. (2003), KRAUSE et al. (2003), HARMATH and SCHMIDT (2010), KISVARGA et al. (2010), KÖBLI et al. (2010). In some cases chemicals increased the flower bud initiation on the shoots of plants (Alar 0.4% and 1%), thus improved their ornamental value. Several growth retardant influenced the flowering time (in some cases the flowering time was earlier or delayed) compared with control plants similarly to researches of Goulston and Schearing (1985), Wilkinson and Richards (1991), Kristensen and Adriansen (1998) and Matysiak (2002).

In Hungary, LCi measurements of transpiration rate, stomatal conductance and net CO_2 fixation of leaves have been made mainly in fruit orchards so far (Gyeviki, 2011; Gyeviki et al., 2012) and on street trees on urban environment (Forrai, 2011; Forrai et al., 2011 and 2012).

In other countries, the studies of interaction between dwarfing with growth retardants and the photosynthesis and transpiration started earlier:

Abod and Webster (1991) reported that foliar sprays of tetcyclasis or paclobutrazol (at 50 or 500 mg l^{-1}) reduced shoot extension growth and total leaf area and increased root/shoot dry weight ratios of young transplants of *Malus, Tilia* and *Betula*. Both treatments reduced total water use in these three species, but the effects on rate of transpiration and stomatal conductance were small.

Deyton et al. (1991) reported that paclobutrazol applied on 'Cardinal' strawberry plants (*Fragaria* × *ananassa* Duch.) for reducing the number of runners, decreasing runner length, and increasing lateral crown development has also reduced the leaf area per treated plant. The leaf net photosynthesis and stomatal conductance were higher in the treated than in the control plants.

Hunter and Proctor (1994) reported that paclobutrazol applied as soil drench on grapevines, reduced the photosynthetic CO_2 uptake rate of leaves. The inhibition of vegetative growth may have contributed to this reduction.

According to Kasele et al. (1995) BAS110 W (250 mg kg^{-1}) and an ethephon treatment reduced total plant leaf area of *Zea mays* L. plants and increased the stomatal density, chlorophyll contents, stomatal conductance and CO_2 exchange rate of leaves.

In experiments of Thetford et al. (1995) uniconazole applied as a foliar spray on rooted stem cuttings of 'Spectabilis' forsythia (*Forsythia* × *intermedia* Zab.) increased the chlorophyll concentration, stomatal density and net photosynthesis of the most recently matured leaves.

Gaussoin et al. (1997) reported that plant growth regulators like mefluidide and flurprimidol increasingly used for high turf production, decreased the carbon dioxide exchange rate, increased the chlorophyll content and leaf weight for the tested species.

Seal and Gupta (2001) reported that *Sida acuta* Burm. f. (Malvaceae) treated with Na-dikegulac (100, 200, 500 mg kg l^{-1}) increased the photosynthetic rate, total biomass production, total seed yield and also total alkaloid content in leaves and roots over control.

In experiments of Tari (2003) paclobutrazol inhibited the stem elongation and primary leaf expansion of bean seedlings, and also reduced the relative water content in plants, increased the stomatal density on both leaf sides. The transpiration rate on a unit area basis did not change significantly or increased in the treated leaves. Paclobutrazol not only amplified the stomatal differentiation but increased the differences between the adaxial and abaxial stomatal conductances of the primary leaves.

According to Sheena and Sheela (2010) triadimefon (a triazole compound) increased survival per cent of micropropagated *Gladiolus grandiflorus* L. plantlets, caused a retarding effect on height (14.86 cm) and increased plant root number. Plants treated by triazole exhibited a lower stomatal conductance which indicated the reduced water loss from the leaves.

Xu et al. (2010) reported that chlorocholine chloride used in concentrations of 0.5, 1.0 and 2.0 g l^{-1} significantly increased net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, and the contents of chlorophyll in ginkgo leaves.

According to Choi et al. (2012) a single application of bitertanol (125 mg l^{-1}) retarded leaf and stem growth of watermelon plant, decreased photosynthesis rate, stomatal conductance and transpiration rate, and increased significantly the water use efficiency of the leaves, compared to those of control plants.

Roseli et al. (2012) reported that paclobutrazol significantly reduced plant height and leaf area of *Syzygium myrtifolium* plants, and also reduced the photosynthetic rate (transpiration rate) of the treated plants as compared to the control. However, stomatal conductance was not affected significantly.

In experiments of Wu et al. (2012) leaf spraying and root drench of potted rose ('Shijizhichun') with paclobutrazol, chlorcholinchloride or mepiquat chloride, caused plant height decrease by shortening of the internodes. The plants became compact and blossomed normally, with big ornamental value. The chemical treatments also increased the chlorophyll content and, improved the photosynthetic efficiency in leaves.

In our research some growth retardants (and/or in some concentrations) increased the stomatal conductance, transpiration rate and net CO_2 fixation of leaves of the treated plants, but not all of the three measuring times. Mirage 0.2% increased the photosynthetic parameters at the first, Alar 0.4% and Bumper 1% at the second, and Cycocel 0.3% at the third measurement. These results are comparable with the researches of Deyton et al. (1991), Thetford et al. (1995), Seal and Gupta (2001) and Xu et al. (2010), who reported that plant growth retardants increased the net photosynthesis rate and stomatal conductance of leaves of the treated plants.

However, in our research paclobutrazol (Cultar 2%) did not increase but decreased the stomatal conductance, transpiration rate and net CO_2 fixation of leaves (at all the three measurement times) similarly to researches of Roseli et al. (2012). Mirage 1% decreased the transpiration rate and net CO_2 fixation of leaves in the second and third measurement.

It means that the different growth retardants have several effects on stomatal conductance, transpiration rate and net CO_2 fixation of leaves in diverse trees, shrubs and perennials.

In the case of *Caryopteris* this question has not been studied by other researchers yet. The results of the present paper are, therefore new in respect of this genus.

Conclusion

It can be concluded that by growth retardants, compact flowering bushes of *Caryopteris* can be obtained. From the practical size it is increasing their market value and also reduces the production cost. Plants treated with growth retardants became smaller, and in some cases chemicals increased the flower bud initiation on the shoots of plants. From the scientific side, it is proved that growth retardants decreased the size of the plants mainly by shortening the internodes.

In our case, Cultar 2% gave the best results (the smallest plants), but the flowering time was strongly delayed by this retardant (approximately one week). This suggests that besides the shortening of internodes, it also held back the plant growth and development (Table 2).

The growth retardant treatments had some effect on the transpiration rate, stomatal conductance and net CO_2 fixation of leaves too. These effects were the strongest on the 24th day after the retardant treatments and the weakest on the 43rd day. The reason was probably partially the degradation of chemicals and also the decreasing of their concentration (a sort of dilution) in the increased volume of the constantly growing (larger sized) plants.

At all the three measurements Cultar 1% and/or 2% and in some cases Mirage 1% showed the lowest transpiration rate, stomatal conductance and net CO_2 fixation of leaves in comparison with the other treatments, but (due to the limited number of data) no statistical correlation was found between the growth rate (dwarfing) and the mentioned parameters.

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Influence of individual variation in the trophic spectra of *Pterostichus melanarius* (Coleoptera, Carabidae) on the adaptation possibilities of its population

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Abstract

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Data on the selection of food items by *Pterostichus melanarius* (Illiger, 1798) obtained under laboratory conditions are discussed. An average of 40 prey species was offered to each individual during each separate experiment. Individual *P. melanarius* in forest ecosystems in the steppe zone of Ukraine eat zoophages of the I, II, and III orders, phytosaprophages and phytophages. Most ground beetle individuals prefer phytophages (up to 85%) in laboratory experiments, but certain individuals eat mostly zoophages of the II order, which make up more than 90% in their ration. The trophic preferences of *P. melanarius* can be classified into 5 clusters. In each of the clusters, there are representatives of 3 or 4 orders that belong to different trophic groups. One third of *P. melanarius* individuals feed on the representatives of the Lumbricomorpha order, one quarter on prey items from the Coleoptera and Isopoda orders. In 30% of the individuals studied it is difficult to single out a dominant taxonomic group in the feeding spectrum. During our laboratory experiments half the *P. melanarius* individuals selected prey items with a body mass of less than their own weight, others selected prey items that exceeded their own size. No distinct dependence exists between the individual variation in the trophic spectra of *P. melanarius* and their morphometric characteristics. Thus, *P. melanarius* is able to influence differentially various trophic, size-and-weight and taxonomic groups of invertebrate animals, producing a significant effect on the structure of invertebrate animal communities of the leaf litter.

Key words

Carabidae, feeding spectrum, *Pterostichus melanarius*, trophic preference

Introduction

The trophic pressure of a population on its forage base is defined not only by the number of its individuals, but also by the variety of its trophic preferences, the potential breadth of the trophic niche, its ontogenetic changeability, the influence exerted upon it by anthropogenic factors, the seasonal changeability of the number of prey items, etc. Some authors have studied variations in the trophic spectra of vertebrate species. Trophic variation in invertebrates has been studied to a much lesser degree. There are no data even on the breadth of the feeding spectra for the majority of ground beetle species (Thiele, 1977).

The question of the influence of the variety of individual trophic preferences on the resilience of a population as a whole has yet to be analyzed in the modern scientific literature. The increase in the changeabilty of the forage base (seasonal, spatial, multi-year, anthropogenic, etc.) has caused the disappearance of many species of litter zoophages. This process, first and foremost, concerns the family of ground beetles, among which polyphages are the most widespread.

In the conditions of urban agglomerations, the trophic base becomes extremely atrophied, and the size-and-weight structure of the leaf litter fauna becomes simplified. Therefore, adaptation possibilities for most ground beetle species disappear, which leads to the decrease in the number of species of the latter in the litter complexes of city ecosystems; in a typical urban ecosystem only 3–5 species remain, among which one of the most widespread and, at the same time, most ecologically adaptable species in the majority of European city ecosystems is the dominant – *Pterostichus melanarius* (Illiger, 1798). Many studies have been devoted to the ecological peculiarities of the genus *Pterostichus*: feeding ecology (Pollet and Desender, 1985, 1987; Mauremootoa et al., 1995), egg production (Mols, 1987), effects of landscape and ecosystem structure on diversity of beetles (Burel, 1992), dispersal activity (Frampton et al., 1995).

The incidence and activity of *P. melanarius* in arable land have been analyzed by Fadl et al. (1996), Thomas et al. (1998), Fournier and Loreau (2002), Irmler (2003). This species of beetle plays an important role in forest ecosystems (Magura, 2002). The territorial distribution of *P. melanarius* individuals is connected with the numbers of their potential prey (Guillemain et al., 1997). Behavioral and physiological aspects (Plotkin, 1981; Symondson, 1997; Symondson et al., 1999a, 1999b; Lindqvist and Block, 2001; Paill et al., 2002; Raworth et al., 2004; Thomas et al., 2008) and trophic connections (Skuhravý, 1959; Currie and Digweed, 1996; Currie et al., 1996; Johansen, 1997; Lang et al., 1999; Snyder and Wise, 1999; Collins et al., 2002; Prasad and Snyder, 2004) of *P. melanarius* and other species within this genus have been studied fairly thoroughly.

This species of ground beetle is situated at the top of a trophic pyramid. According to the results of our previous research, its individuals are able to eat not only phytophages and saprophages that are constantly or temporarily present in the litter horizon, but also zoophages of different orders (Brygadyrenko and Korolev, 2006; Korolev, 2010; Korolev and Brygadyrenko, 2012a, 2012b). A complex trophic system is formed, which is capable of self-regulating and is, at the same time, extremely changeable in response to the influence of factors of natural and anthropogenic origin. In our opinion, it is this changeability in the functional role of the *P. melanarius* population that allows the species to survive in the extremely unfavorable conditions under which the majority of other ground beetle species disappear.

The mechanisms and methods of avoiding extreme intra species competition in *P. melanarius* rather than the width of the trophic niche demand thorough study. There are certainly insufficient examples of similar research on polyphages with a rather wide feeding spectrum.

The analysis of morphological variation within populations (which has partially been studied in our previous papers) and its connections with the possibility of the existence of individual trophic preferences for this species of polyphage requires special attention. Specific features of the internal and external structure of Carabidae representatives have been widely used for defining trophic connections of ground beetles (Davies, 1953; Skuhravý, 1959; Zhavoronkova, 1969; Forbes, 1983).

In the work by Simchuk and Ivashov (2012) fermentational polymorphism was studied. This secures maximum adaptation of *Tortrix viridana* L. (Lepidoptera, Tortricidae) individuals to a forage plant of a certain genotype with a certain ratio of phenols in the cell juice. Similar studies for phytophages are few, and, as for polyphages, are not known to us at all. Recently molecular-genetic analysis of the gut content of predator invertebrates has been popular (Kitaev et al., 2011). An enzyme-linked immunosorbent assay was applied by Hagler and Naranjo (1997) in predator gut content research. Bacher et al. (1999) have described the development of a monoclonal antibody (MAb) to the hemolymph of fifth-instar larvae of *Cassida rubiginosa* Muell. (Coleoptera, Chrysomelidae) and concluded that MAb can be used as a tool to identify predator-prey interactions and identify the trophic complex of *C. rubiginosa* Muell.

The assessment of trophic specialization by *P. melanarius* individuals is important for making connections between specific features of one and another species of a polyphage population feeding and their adaptation characteristics.

The aim of this paper is to evaluate the degree of influence of variations in the trophic spectra of individual *P. melanarius* on the adaptation abilities of its population.

Material and methods

The study of trophic spectra individual changeability of *P. melanarius*

The series of experiments on the study of the potential of polyphages to eat various species of invertebrates is described in our previous papers (Brygadyrenko and Korolev, 2006; Korolev, 2010; Korolev and Pokchylenko, 2009; Korolev and Brygadyrenko, 2012a, 2012b), but a potential trophic niche has not always been realized for every *P. melanarius* individual. Like vertebrate-panthophages, *P. melanarius* individuals combine different species of trophic objects in a daily ration in a certain way. The literature is completely lacking in data on research techniques for assessing individual trophic spectra of invertebrate-polyphages. For this reason we have had no option but to develop and suggest our own techniques.

The considerable migration capability of this species prevents us assessing the variety of its trophic spectrum under conditions close to reality (an imago migrates for 10–50 m during 24 hours). It is impossible for us to make an experimental plot of this size, that is why insectariums with the size of 30 × 20 cm were used. They were filled with screen soil of 4–5 cm thick from the imago collection sites, and the natural composition of the litter was imitated. The total number of invertebrates offered to all *P. melanarius* individuals was 190 species, which corresponded to the number and types of litter macrofauna species present in *P. melanarius*'s natural habitat in the region under study.

The invertebrates were collected in the Samarskyi pine forest (Novomoskovsk distr., Dnipropetrovsk region). The study of individual trophic preferences of *P. melanarius* was carried out at the Prysamarskyi International Biological Station named after A.L. Belgard. Of course, this species is common in the urban environment of Dnipropetrovsk. However, due to the likelihood of human disturbance to the collection process, we avoided collection in the city and took our specimens from a relatively natural ecosystem.

A total of 16 specimens of *P. melanarius* imago were taken, 3 male (No 6, 15, 16) and 13 females, the experiments being conducted between approximately 12 and 28 July 2010, just before the reproductive season for this species. Each specimen was placed on its own in a separate insectarium and offered live invertebrate prey items over a period of 24 hours. All 16 specimens underwent experiment simultaneously. A total of 10 to 15 of these experiments were conducted for each individual without interruption. Before each experiment each specimen had been fully fed with larvae of *Calliphora vicina* R.-D. Each *P. melanarius* individual was offered on average 40 species of invertebrates during 24 hours, a single specimen of each potential prey item being provided. On average around 35 samples of those 40 species were eaten. The maximum number of invertebrate species offered to any single *P. melanarius* individual in the course of all the 24 hour experiments was 75 (out of the 190 species offered to the 16 *P. melanarius* specimens as a whole). The species composition of prey was determined according to the real species composition of the litter macrofauna in the modeled population of *P. melanarius*. Consumption of a prey specimen was determined by the presence, absence or partially devoured remains of each potential trophic item through carefully examining the contents in the insectarium at the end of every experiment.

A "points" system of assessment was introduced in order to compare the trophic spectra of *P. melanarius* individuals. 100% consumption of a prey item by an individual *P. melanarius* was assessed at 2 points. 1 point was given for the partial consumption by a *P. melanarius* specimen of a prey item (1–99% consumption of the specimen). Minus 1 (–1) point was given when a *P. melanarius* specimen completely ignored a prey item offered. When a potential prey species was absent in an experiment the score of zero was given. The sum total of points obtained in the course of 10–15 experiments was calculated. Individual feeding spectra of 16 *P. melanarius* individuals were analyzed and 6,840 offerings of potential prey species (sub-experiments) were made in the process of the research.

The data obtained was compared with the use of the cluster analysis methods (Statistica 8.0). The summary table enables us to assess the trophic preferences of *P. melanarius* individuals and illustrates divergences of the feeding spectra of separate individuals of the species under research.

The study of the morphometric characteristics of *P. melanarius* individuals

In order to find out the connections between the morphometric characteristics and the specific features of variations in the trophic spectra of *P. melanarius* individuals, measurements were obtained of the morphological parameters of the imagoes whose feeding spectra were studied in laboratory conditions. The size of the beetles was determined with the help a binocular micrometer eyepiece MBS-10. Fourteen (14) measurements were used which characterize linear dimensions of the body sizes of the individuals under research.

The length of the body regions and their components (head, labrum, clypeus, pronotum, elytra) was determined according to the average line of the body.The length of the head was measured from the front edge of the clypeus to the back edge of the head (excluding the mandibles and labrum). Measuring the width of the head and elytra was performed at the widest point. The pronotum was measured at three points (top, bottom, and the widest point). The length of the body was determined from the labrum to the top of elytra, and the height – according to the average line of the metathorax.

Results

The *P. melanarius* individuals studied show a wide divergence in trophic preferences (Fig. 1). The prey items of *P. melanarius* form 5 clusters, each of which comprises the representatives of different groups of soil-and-litter macrofauna. An interesting specific feature of forage feeding by *P. melanarius* is the frequent combination of quite large prey items (*Aporrectodea caliginosa* (Savigni, 1826), *Rossiulus kessleri* (Lohmander, 1927), *Trochosa terricola* Thorell, 1856, *Porcellio scaber* (Latreille, 1804)) with small prey items

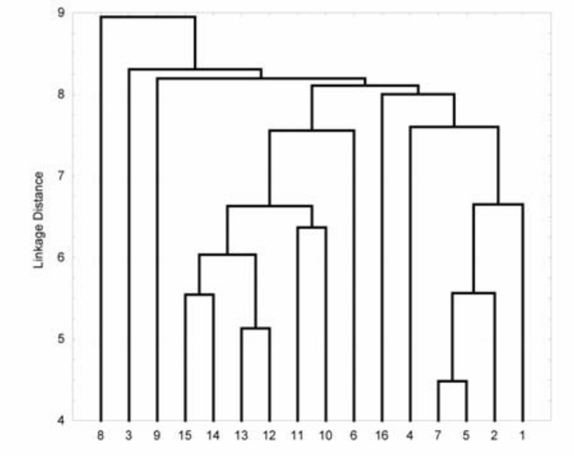

Fig. 1. Grouping of *P. melanarius* individuals according to trophic preferences.

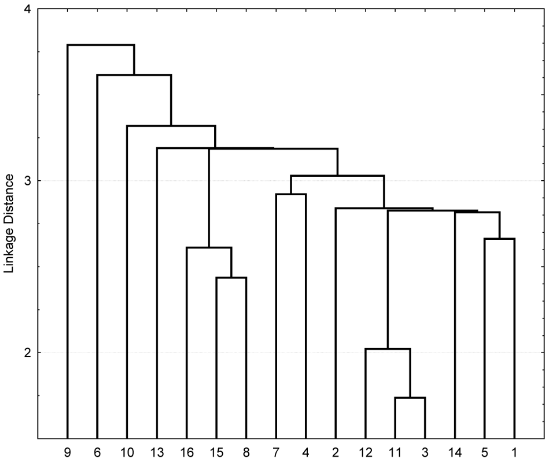

Fig. 3. Groupings of *P. melanarius* individuals based on morphological characteristics.

(*Drusilla canaliculata* (Fabricius, 1787), *Coccinella septempunctata* Linnaeus, 1758, *Bembidion properans* (Stephens, 1829)). It is possible for the representatives of taxons which have rather hard exteriors to be in the same cluster as weakly chitinous objects (Fig. 2). The prey consumption of *P. melanarius* has an almost random character, which is also confirmed by the indiscriminate consumption by the individuals studied of species which belong to different biogeohorizons (inhabitants of litter, soil and herbage).

It has proved impossible to identify any definite clusters of correlation between the trophic specialization of *P. melanarius* individuals and their specific morphological features (Fig. 3). Interestingly, it is impossible to observe even minimum similarity between groups of individuals sharing trophic preferences and the complexes of individuals singled out according to morphological features (see Figs 1 and 3).

For instance, in one cluster of ground beetles, specimen No 1 selected mostly average-sized zoophages,

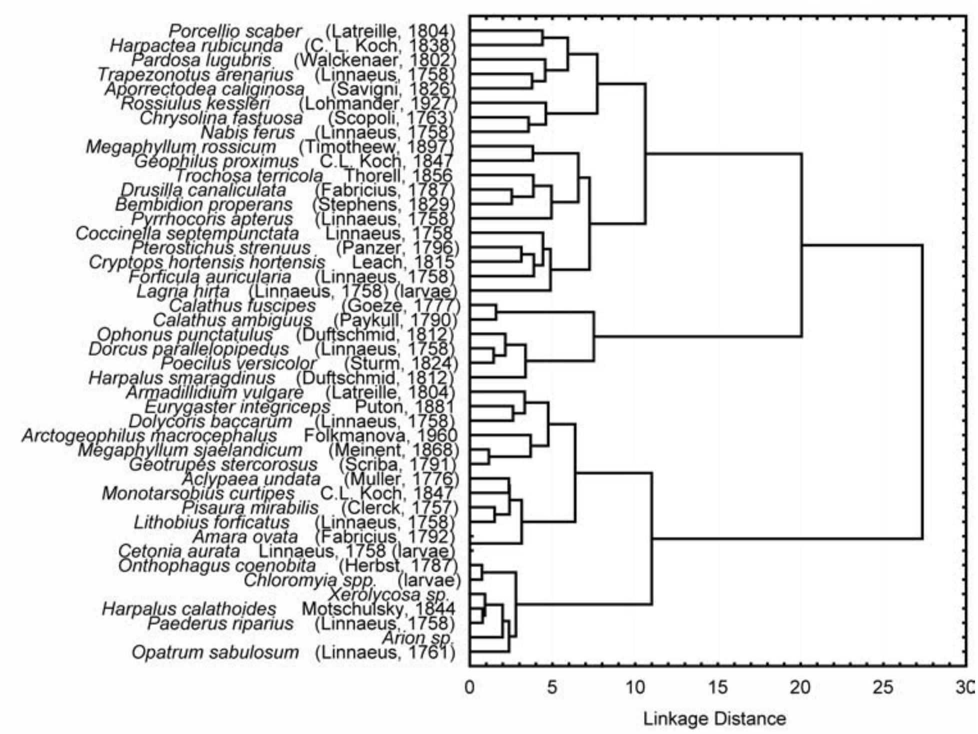

Fig. 2. Cluster analysis of consumption of invertebrate species by *P. melanarius* individuals.

among which the beetles of the Carabidae family, inhabitants of open steppe areas covered with xerophytic vegetation, dominate. At the same time, it is possible to see in the ration of specimen No 14 chiefly large-sized individuals of the Julidae family, which are widespread in a variety of ecosystems. The latter play a primary role in breaking down vegetable organic substances. In another cluster of *P. melanarius*, specimen No 8 consumed large soil-dwelling herbivorous Scarabaeidae larvae, while specimen No 16 preferred small panthophages of the Formicidae family, which are widespread in forest habitats.

The feeding spectra of *P. melanarius* individuals which are similar in morphotype have certain differences. Among invertebrates that were eaten by specimen No 4, phytosaprophages of the Porcellionidae family greatly predominated. They play an active role in breaking down litter and dead wood (Soma and Saito, 1983; Walton, 1987), while *P. melanarius* individual No 7 is characterized by active predation of endogenous Lumbricidae species. Julidae representatives formed an insignificant part of the ration of the both specimens of ground beetles.

In the ration of different *P. melanarius* individuals (average body mass – 214.5 mg) combined by morphological characteristics in one cluster, it is possible to find invertebrates whose weight and size vary widely (Fig. 4). Some *P. melanarius* individuals ate small or average-sized victims (8–511.9 mg), others preferred prey weighing more than 512 mg. Small objects weighing 2–7.9 mg made up an insignificant part (up to 4%) of the ration of specimens No 1–15, while specimen No 16 ate such objects in 34% of cases.

The analysis of the taxonomic structure of invertebrates eaten by *P. melanarius* shows that most of the 16 specimens studied preferred representatives of the Coleoptera, Julida, Isopoda and Lumbricomorpha orders (Fig. 5), to which dominant elements of litter and soil fauna belong. It was observed that some of the *P. melanarius* specimens actively consumed soil pupae of various Lepidoptera species as well as pupae of abundant Formicidae species. This is one of the most abundant groups of the soil-and-litter complex, and its representatives have a very important functional meaning for ecosystems because they take an active part in various environmental processes. A significant percentage of consumption (more than 20%) in the rations of *P. melanarius* specimens No 1, 3 and 5 was made up by fast-moving litter Aranei species (see Fig. 5).

Analysis of the potential prey of *P. melanarius* according to trophic specialization indicates that phytophages, phytosaprophages and zoophages dominate in the rations of individuals of this species (Fig. 6).

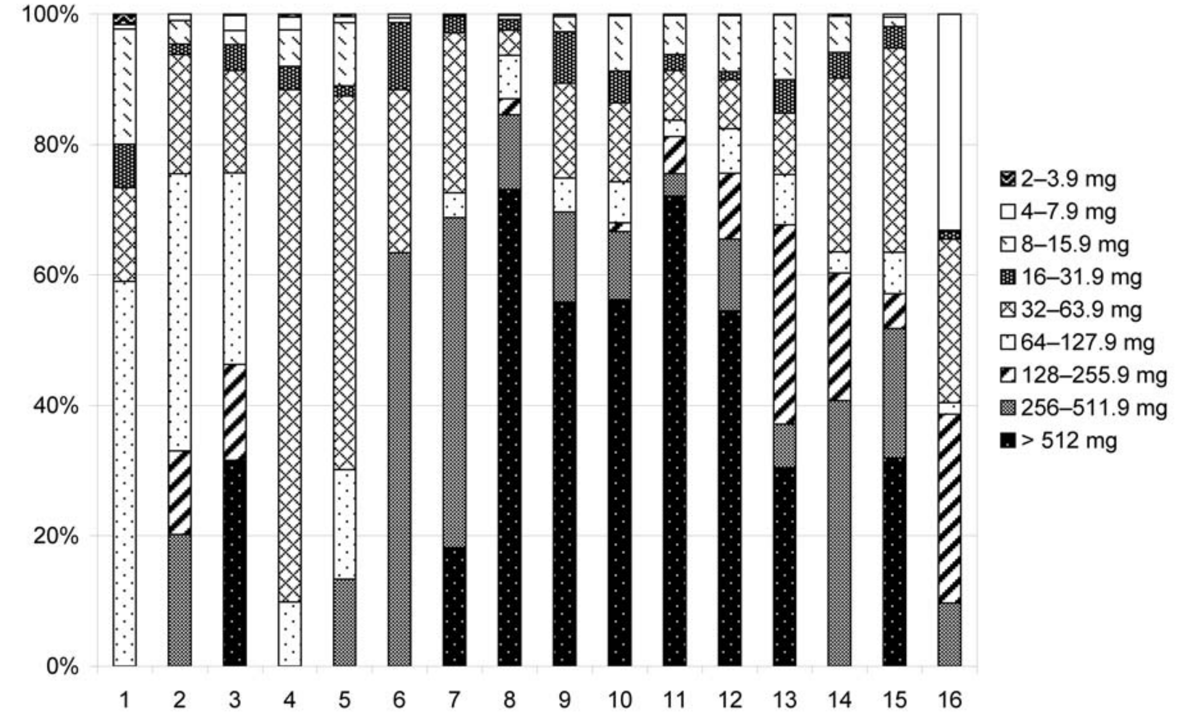

Fig. 4. Size-and-weight structure of the rations eaten by *P. melanarius* individuals under conditions of free choice of trophic objects.

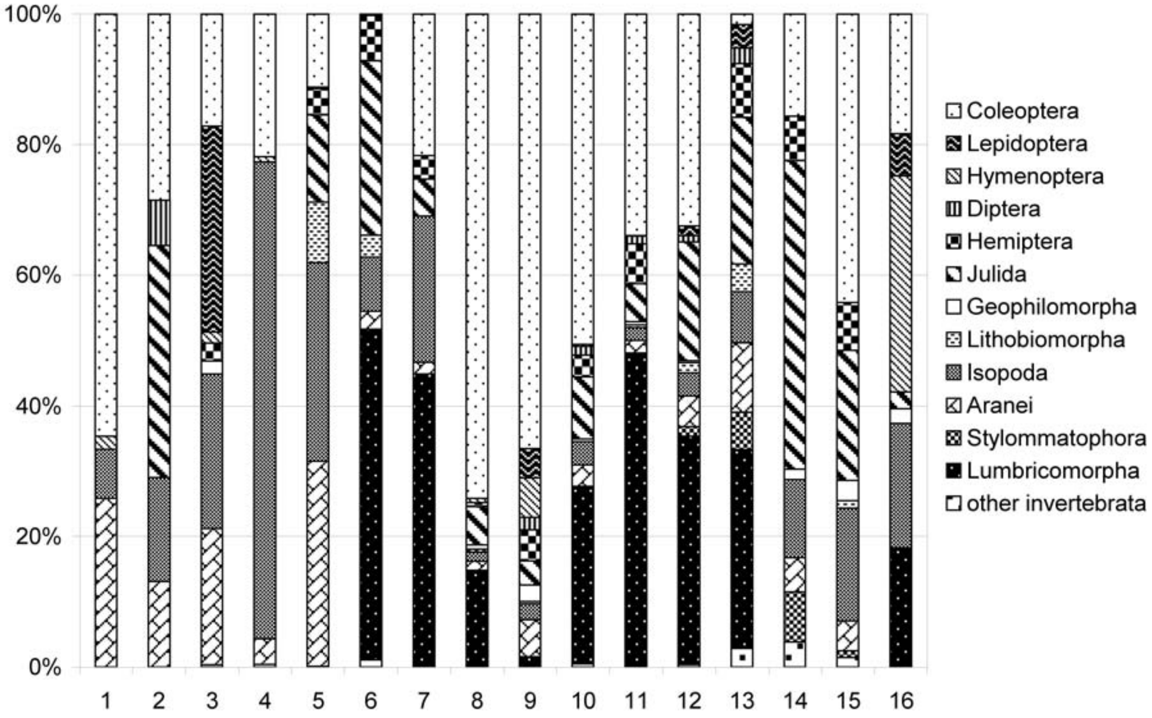

Fig. 5. Taxonomic structure of the rations eaten by *P. melanarius* individuals under conditions of free choice of trophic objects.

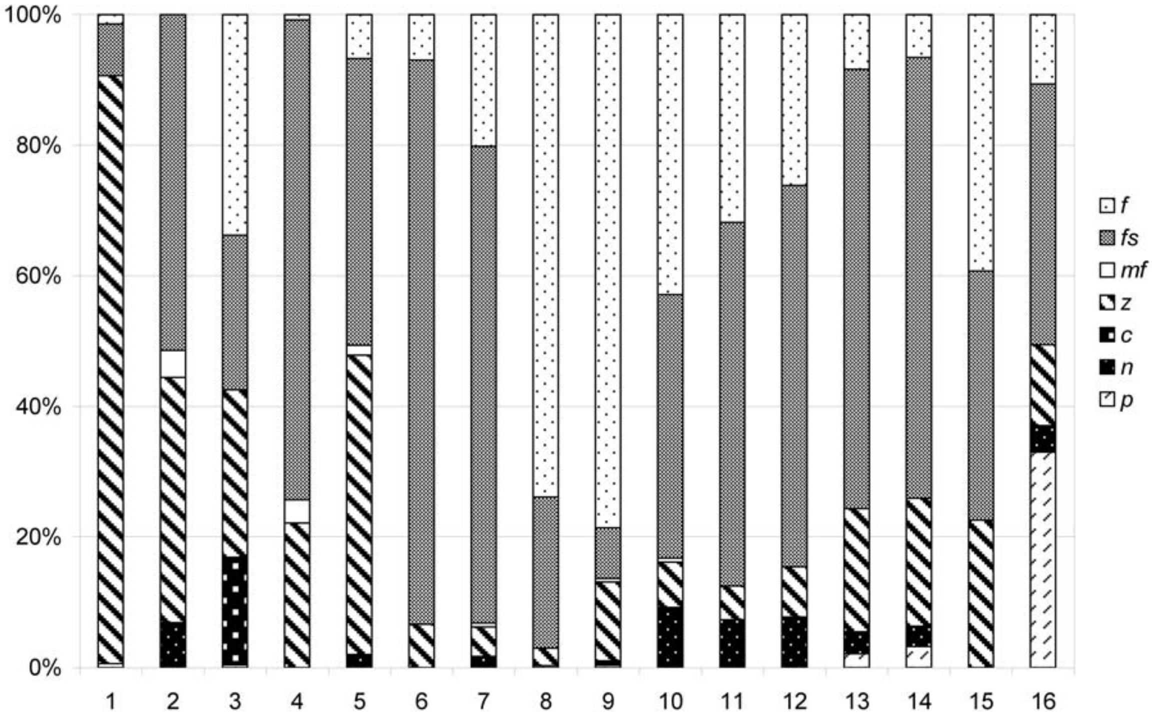

Fig. 6. Taxonomic structure of the rations eaten by *P. melanarius* individuals under conditions of free choice of trophic objects: *f*, phytophages; *fs*, phytosaprophages; *mf*, mixophytophages; *z*, zoophages; *c*, coprophages; *n*, necrophages; *p*, panthophages.

This reveals a strong possibility that the *P. melanarius* population has a strong influence on the formation of the trophic connections of invertebrates at the bottom of the trophic pyramid. The significant percentage of coprophages in the ration of specimen No 3 *P. melanarius* is connected with the occasions of consumption by this individual of fresh *Geotrupes stercorosus* (Scriba, 1791) (Geotrupidae family) corpses. Necrophages in the diet of *P. melanarius* individuals are represented mainly by larvae and puparia of the Calliphoridae and Sarcophagidae families (Diptera) as well as by the Silphidae and Dermestidae families (Coleoptera).

Among invertebrates that make up the base of the feeding spectra of *P. melanarius*, the majority consist of forest forms and habitat generalists (Fig. 7) which are widespread in a variety of biotopes. This testifies to the ecological plasticity and high adaptation possibilities of the population of the ground beetle species under study, which is an integral element of the litter invertebrate communities of most forest ecosystems in Ukraine's steppe zone.

Discussion

Trophic connections of *P. melanarius* have been studied at the level of discrete populations (in a particular field, wood, wetland, etc.). Skuhravý (1959) researched the protein content in the stomachs of this species and found that this beetle has great potential for destruction of agricultural pests. Researchers in the past 20 years (Currie and Digweed, 1996; Currie et al., 1996; Johansen, 1997; Lang et al., 1999; Snyder and Wise, 1999; Collins et al., 2002; Prasad and Snyder, 2004) have developed more modern methods of field investigation, involving such technology as radio-isotope and radio-telemetry. However, field research on the diet of *P. melanarius* has been held back by the limited variety of prey objects at any particular study site (typically about 10–30 species at any site in Ukraine). By studying the trophic spectrum of *P. melanarius* in laboratory conditions we are able to evaluate a much fuller range of potential trophic items.

Our study indicates that in the case of *P. melanarius* there are no groups of individuals clearly differentiated from each other as to trophic specialization. It is astonishing how different individual trophic spectra of *P. melanarius* are with regard to the size groups of their food objects and their taxonomic, trophic, ecosystemic affiliation. However, only 140 species out of 190 studied species of invertebrates were consumed, which is, nevertheless, considerably more than for the other species of ground beetles whose feeding spectra we have studied in laboratory conditions (Brygadyrenko and Korolev, 2006). On the one hand, this is connected with the greater size of *P. melanarius* compared to *P. oblongopunctatus* (Fabricius, 1787), *Harpalus rufipes*

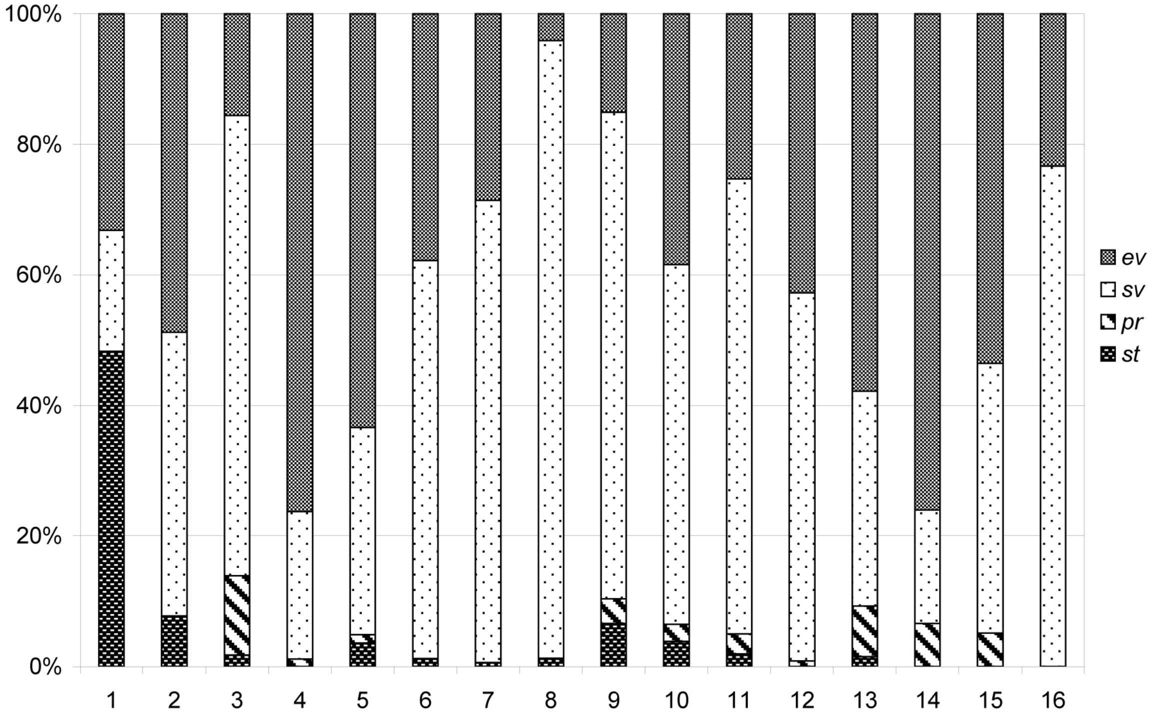

Fig. 7. Ecosystemic structure of the rations eaten by *P. melanarius* individuals under conditions of free choice of trophic objects: *ev*, habitat generalists; *sv*, forest species; *pr*, meadow species; *st*, steppe species.

(De Geer, 1774), *Poecilus versicolor* (Sturm, 1824), *Calathus melanocephalus* (Linnaeus 1758), on the other hand – with the hardness of shells, mobility and the fact that this species belongs to the greatest size-and-weight class in the litter and soil fauna.

Before starting the present research we did not expect to obtain such different trophic spectra for different individuals. The data obtained in the course of experiments provide evidence of the extremely wide trophic preferences of *P. melanarius* compared to other species of ground beetles.

As a result of the ration correlation analysis at the level of orders among 16 *P. melanarius* specimens, only 42 correlation coefficients were found reliable ($P < 0.05$) while 214 correlation coefficients were found to be unreliable. That is, similarity of feeding spectra was registered only in 16.4% of cases. The extremely high percentage of divergence in feeding spectra permits us to state that the variable ration at the individual level is one of the mechanisms by which populations of *P. melanarius* adapt to the conditions of an anthropogenically transformed environment.

Thus, an individual-based approach to tropho-ecological research on ground beetles needs to be extended in order to find the reasons for the extinction of rare species and survival of hardy taxa under the influence of factors of natural and anthropogenic origin (Korolev and Brygadyrenko, 2012a). The ecosystems in which the species with variable individual feeding spectra dominate can probably be considered to be more anthropogenically transformed than the communities in which species with a stable ration dominate.

Conclusions

The *P. melanarius* population that inhabits the forest of Ukraine's steppe zone is situated at trophic level IV in the ecosystem. *P. melanarius* individuals eat zoophages of orders I, II, and III, phyto-saprophages and phytophages as well as panthophages, necrophages and coprophages. Most of the individuals studied preferred phytophages (up to 85%) in laboratory experiments, some individuals consumed predominantly zoophages of order II (zoophages made up over 90% of their ration).

Analysis of the trophic preferences within the *P. melanarius* population reveals 5 clusters, each of which contains representatives of 3 or 4 orders that belong to different trophic groups. A clear association was not found between variation in the trophic spectra of *P. melanarius* individuals and their morphometric characteristics.

In laboratory experiments, half of *P. melanarius* individuals fed on victims with a body mass of less than their own weight while others fed on prey items larger than their own size.

The range of food shows a great variety in taxonomic preferences. One third of the *P. melanarius* specimens studied fed on representatives of the Lumbricomorpha order, one quarter fed on Coleoptera and Isopoda species. It is difficult to single out the dominant taxonomic group in the feeding spectrum in 30% of cases.

Thus, *P. melanarius* is capable of having a differential impact on different trophic, size-and-weight and taxonomic groups of invertebrates. In this manner it influences the structure of soil and litter macrofauna communities.

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Evaluation of the greenery in historic park in Beladice-Beladice, Slovakia

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Abstract

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The work is a part of broader research of historic parks in the South-western region of Slovakia. Beladice is a small village 20 km from Nitra; composed of 4 former villages; the largest one carries the name Beladice. In each of them there is a manor surrounded by a park. Late baroque styled manor rebuilt at the end of 19th century has had an added functionalistic wing since 20th century. Park laid out on 6.6 ha and 2.4 ha was last time treated by Michal Strnad in 1906-07. In 2012 a plant inventory of the whole park was made using an author's method. 800 trees, 35 shrubs and 65 groups of natural seedlings have been evaluated. Altogether 67 taxa were identified; from which 36 are broad-leaved trees, 23 conifers and 8 shrub species. The result of the stock taking is felling proposal for the ill, dangerous and compositionally unsuitable trees. The average health state of the trees is 3.55; their average compositional value is 3.25 and 50% of the plants should be removed due to the overgrown outplantings and lack of maintenance for over last 20 years.

Keywords

historic park, Beladice-Beladice, plant inventory, Slovakia

Introduction

Historic parks represent a specific art of cultural heritage of mankind; their particularity is based on their vividness, they still grow and without a proper long lasting and never ending maintenance they would decay. In fact decaying of a park is in confrontation with the return of the nature. It would overrule the human made composition, sometimes artificial, sometimes demanding, but usually culturally valuable and always intentional. So keeping up of a historic park can be considered as a fight against but still with the nature itself (Supuka et al., 2008).

Tree inventory methods are being developed for many purposes which affect their characteristics, parameters or even profundity and accuracy. As it is very human demanding and time consuming work which can only be done by professionals equipped by botanic and other scientific skills, it is not very common and widely used tool. Its importance on the other hand is broadly recognized, still not sufficiently well financially covered. There is significant difference between American and European approach. As Americans (DiSalvo, 2011) are oriented more on species, genus and families identification; size (defined by diameter in breast height), condition (fair, good, poor and dead), stocking level (potential planting spaces), replacement values (full cost of replacing trees in their current condition) and aesthetic and environmental benefits (Energy savings, Carbon sequestration, Air quality improvement, Storm water processing); European methods differ in separate countries, but are more or less more scientifically oriented (all parameters are measured or qualified by points). English tree stock taking method resembles more American ones (Ciesielski, 2011) as they are oriented similarly just on tree size (diameter in breast height), species composition, tree diversity and vulnerability, tree mortality (wood condition, leaves condition, percentage of deadwood), new outplantings withstanding and pests infestation. Similar to continental approach is presence of maintenance recommendation and remark on sidewalk damages or overhead wires.

We would like to present our approach to tree stock taking methods and their application in praxis. Tree inventory methods used in our region are based more on scientific assessment. Result of the work would be valuable information about dendrological composition of a hundred-year-old park.

Material

As the material of this work can be considered the complete 6.6 ha area of the historic park in village Beladice, local part Beladice (48°20′21″N, 18°17′55″E; 48.339167°, 18.298611°) together with the area of former agricultural school situated in the manor house with 2.4 ha park; finally the area of a cooperative farm neighbouring with the area of the school was not included although in the past it was a part of the manor just with economic use (stables, vegetable garden and so on). According to *Atlas Slovenskej republiky. Landscape atlas of the Slovak Republic*, Miklós, 2002, the village Beladice-Beladice is in warm climatic region, moderately dry with mild winter. Mean annual precipitation is 550–600 mm; mean annual air temperature is 9–10 °C. There are brown soils, phytogeographically area belongs to Žitavská pahorkatina and potential vegetation is *Carici pilosae-Carpinetum* and partially *Ulmenion*; in shorter words – hardwood alluvial forest.

The material itself were just woody plants growing in these two parts which are separated by a fence, owner and also by use. Historic park serves as a free public village area for short term recreation; also it has a transitional character as it is situated between two parts of the village (Beladice and Pustý Chotár). Second part of the park belonging to the school is completely fenced with no access to the public; it is little bit decayed as it has already been abandoned for several years with pure maintenance; it has a private owner.

Originally a renaissance manor house built in 1620, was in 18th century replaced by a late baroque styled manor house; this was at the end of 19th century reconstructed to mostly today's appearance. Also a Szentivanyi family mausoleum situated in the school park was built at this time but in romantic neo-renaissance style. Manor is also equipped with yard with an agricultural function, interesting terrace with pergolas and a glasshouse. During socialistic times a functionalistic wing was added to the manor house needed for school purposes (Kubišta, 2004).

Park itself was probably founded like formal garden with a parterre, but at the end of 19th century was re-stylized and enlarged into romantic landscape park. It still keeps the composition of its gardener Michal Strnad from Topolčany, but after last 20 years with no or very low village maintenance it is already overgrown, with plenty of naturally seeded trees (natural seedlings) aged exactly 10 or 20 years. These trees are already endangering the old ones planted in a composition of an English park. As it was fashionable in 19th century the park was enriched by exotic trees as *Ginkgo biloba* L., *Phellodendron amurense* Rupr., golden pine (*Pinus sylvestris* L. just with yellowish needles), *Liriodendron tulipifera* L. or *Gymnocladus dioicus* (L.) K. Koch. But the most interesting tree was till recent years over 300-year-old *Tilia platyphyllos* Scop.; unfortunately surviving now only like a torso of formerly the hugest tree in the park, this title is now carried by a *Platanus hispanica* Mill. A massive trunk of dead *Fraxinus excelsior* L. overlies a small lake creating a natural bridge. Since 1982 the park has been proclaimed as a Protected Area (Kubišta, 2006).

Methods

The stock taking was done in February 2012, in a dry and cold weather; it was following the geodetic survey using digital gages which needed the deciduous plant without foliage because of high density of plants in inventoried area. As a main method for woody plants inventory, author's method developed for historic greenery was chosen – Kubišta (2008), which is developed out of Machovec (1982) plants inventory methods. Stock taking is conditioned with exact geodetic survey of items position. To each item a set of information expressing their parameters is collected. According to used inventory method can be collected information divided to four types: identification data, characterisation data, evaluation data and operation data.

Identification data is especially the Item Code (IC) consisting of a letter expressing a kind of inventoried plant (T, tree; B, bush, shrub; S, natural seedling…) and a number expressing order of inventoried item (T01, B02, T03…). These Item Codes are marked not just in inventory tables but also in inventory maps to present a real position of the inventoried item. The most important part of identification data is the Name of inventoried item consisting of Latin genus, species and possibly also cultivar name of the plant (for example: *Picea pungens* Glauca).

Characterisation data concerns two types of data; first type is a professional guess of the age of inventoried plants in years set in categories (0–10, 10–20, 20–40, 40–60, 60–80, 80–100, 100–150, 150–200,…). Age guess results from an assumption that average tree ring gain is 0.5 cm per year, so in diameter 1 cm on average stand of medium growing tree (Table 1). According to this assumption also other stand and tree growth groups can be guessed. Second type are dendrometric data as the height of the plants in meters set in categories each 2 and later 5 m high (0–2, 2–4, 4–6, 6–8, 8–10, 10–12, 12–14, 14–16, 16–18, 18–20, 20–25, 25–30, 30–35, 35–40,…), the trunk diameter in centimeters set in categories each 20 cm wide (0–10, 10–20, 20–40, 40–60, 60–80, 80–100, 100–120, …) and finally the crown diameter in meters set in categories each 2 wide (0–2, 2–4, 4–6, 6–8, 8–10, 12–14, …). All dendrometric data can be measured precisely. The height can be guessed or measured with hypsometer or digital gage. Trunk diameter can be guessed or precisely measured with forestry calliper usually in 1.3 m trunk height (so called breast height). Crown diameter is measured in two orthogonal measurements with a measuring tape; the result is always an average value of these two measurements. The accuracy of measurements is sufficient for the historic park renewal projects, as the number of inventoried items is very high and necessary time always short.

Evaluation data is the most scientific part of the plant inventory; it needs long lasting experiences and is not easily describable. There are just two parameters, first is Compositional Value and second is Health State. Although their name seems to be easily recognizable, they are not. Compositional Value logically presents a value of the plant in particular composition, this concerns characteristics like originality of the tree in the composition, location, suitability, proportionality, usability and in some cases also connectivity and inclusiveness (Table 2). All these parameters have 6 degree values (0, 1, 2, 3, 4, 5) representing the percentage of monitored characteristics at each inventoried item. Finally the compositional value is an average value of all these monitored parameters rounded to the closest whole number (0–5).

Compositional value depends on following characteristics of inventoried items:

- *Originality* traces whether the item belongs to the original composition, when it became a part of the composition, or how it fits with its style.
- *Location* checks whether the items position was changed, whether it was moved or removed.
- *Suitability* checks whether the item is suitable in existing composition, whether it fits in there, whether it disturbs or whether it is suitable for the specific use.
- *Proportionality* checks whether the elements have its composed dimensions, whether they are not overgrown or whether their size was set correctly.
- *Utizability* traces whether the particular item is legitimate in the composition, whether there is a reason for its preservation.
- *Connectivity* observes in relevant cases overlap of tree crowns or bushes.
- *Inclusiveness* checks missing items in alee, in bosquets, or in hedges etc.

Health state values are set similarly like Compositional value (0, 1, 2, 3, 4, 5); they show the real condition of the plant item. The parameters of the Health state are the size, typicalness, viability, damage, safety and sustainability. There can also be set other ones according the needs of stock taking (Table 3). Health state is then an average value of all these monitored parameters rounded to the closest whole number (0–5). They are usually the same degree like Compositional value; just in several cases they can be completely opposite. For example very old tree connected with a legend or standing solitarily in formal parterre can have very high Compositional value but the Health state could be very low because of high age.

Health state monitors condition characteristics of inventoried items:

- *Size* shows items of environmental conditions; whether it is flourishing there or declining.
- *Typicalness* is also an expression of environmental conditions during items ontogenesis or human impacts. In bad conditions plants do not achieves typical habitus or growth.
- *Viability* checks health problems like diseases, dry branches, illness changes, pest occurrence. It also monitors the vitality of growth, leaves size, plant growth increase and so on.
- *Damage* checks wounds, inclination, bad human interventions and environment exposition causing the lowering of health state.
- *Safety* evaluates safety risks of items presence in particular composition for visitors.
- *Sustainability* checks the perspective of items endurance in particular composition, its endangerment and resistance.

Operational data set the future of inventoried items following the evaluation data. They are the most important data in stock taking; divided to data influencing the restoration budget (arrangement) and explanation data (comments). Arrangement is an act proposal; it sets the best suitable solution for each inventoried item. There are several acts like felling in 1st phase, felling in 2nd phase, chemical treatment, trimming or other. All of them have a specific price in the budget; according to inventory the preparatory costs can be completely set. Comments do not influence the budget, they just explain the reasons of felling (compositional, health, safety), or display specific growth characteristics of a plant (inclination, trunk disposal and so on), or specify disease, pest or damage (fracture, cavity, decay, fungus and other). Also the compositional position can be displayed closer (solitaire, alee, hedge, vegetation border, vegetation); or mutual influence between plants (incline because of another tree, common crown of more plants); also importance of the plant in composition (dominant) or just interesting undergrowth vegetation which can be used in composition (perennials, bulbs).

Table 1. Age guess according to plant and trunk diameter

Stand / Plant growth	Fast growing plants	Medium growing	Slow growing plants	Dwarf growing plants
Nourishing stand	X – 2	X – 1	X	X + 1
Average stand	X – 1	X	X + 1	X + 2
Poor stand	X	X + 1	X + 2	X + 3

X, measured trunk diameter category (0–10, 10–20, 20–40, 40–60, 60–80, 80–100, 100–120, …); +/–, lowering or increasing of age categories about 1, 2 or 3 categories, just when it is possible, in higher age categories (100–120 and over) is age guess disputable.

Table 2. Compositional value

Parameter / Degree	0	1 (0–20%)	2 (20–40%)	3 (40–60%)	4 (60–80%)	5 (80–100%)
Originality	Naturally grown plants	Unoriginal composition, planted	Close to original composition	Original composition	Identical species in original composition	Original plant from original plantings
Location	Missing item, stump…	Unoriginal location	Expected unoriginal location	Approximately original location	Assumed original location	Original location
Suitability	Completely unsuitable item	Unsuitable item	Rather unsuitable item	Mostly suitable item	Suitable item	Ideally suitable item
Proportionality	Unpreserved	Rather unpreserved	Suppressed	Almost preserved	Preserved	Ideally preserved
Utizability	Illegitimate	Unnecessary	Almost unnecessary	Rather necessary	Necessary	Legitimate
Connectivity	Unconnected	Almost connected, 0–20% overlap	Mostly connected, 20–40% overlap	Connected, 40–60% overlap	Connected, 60-80% overlap	Connected, 80-100% overlap
Inclusiveness	Completely missing item	0–20% rate	20–40% rate	40–60% rate	60–80% rate	Complete, 80–100% rate
…	Resultant Compositional value is an average of all parameters values rounded to closest whole number (0, 1, 2, 3, 4, 5)					

Table 3. Health state

Parameter / Degree	0	1 (0–20%)	2 (20–40%)	3 (40–60%)	4 (60–80%)	5 (80–100%)
Size	None, extremely unsuitable conditions	Minimal, very unsuitable conditions	Substandard, unsuitable conditions	Standard, suitable conditions	Extraordinary, very suitable conditions	Exceptional, extremely suitable conditions
Typicalness	None	Very low	Low	Average	High	Very high
Viability	None	Very low	Low	Average	High	Very high
Damage	Dead	Dying	Strongly damaged	Damaged	Slightly damaged	Undamaged
Safety	Emergency state	Very high risk	High risk	Average risk	Low risk	Very low risk
Sustainability	None	Very short	Short	Average	Long	Very long
…	Resultant Health state is an average of all parameters values rounded to closest whole number (0, 1, 2, 3, 4, 5)					

Results and discussion

Plants inventory in altogether 9.0 ha historic park in village Beladice in its local part Beladice has shown existence of 800 trees, 35 shrubs and 75 natural seedlings (naturally grown groups of young trees or shrubs). Using author's method of plant inventory these parameters of plants have been shown: Number, Name, Age, Height, Crown diameter, Trunk diameter, Compositional value, Health state. For practical purposes Operational and Comment data were set too.

Species analysis has shown existence of 36 broad-leaved trees, 23 conifers, 8 shrubs species or cultivars. For compositional value and health state formulas for arithmetic average were used (Tables 4, 5 and 6). As there is no known plant analysis of this park, result can not be compared with older situation.

Table 4. Plant evaluation analysis

	Age [years]	Height [m]	Crown diameter [m]	Trunk diameter [cm]	Compositional value (0–5)	Health state (0–5)
Trees (800 items)						
Average	62.88	16.31	7.80	47.25	3.25	3.55
Shrubs (35 items)						
Average	36.86	4.69	4.06	–	3.40	3.86
Natural seedling (75 items / 240 subitems)						
Average	13.29	5.71	2.84	–	1.19	4.86

Table 5. Tree species analysis (800 trees**)**

No.	Species, cultivars	Number [specimen]	Percentage [%]
	Broad-leaved trees		
1	*Acer campestre* L.	118	14.8
2	*Acer platanoides* L.	43	5.4
3	*Acer pseudoplatanus* L.	11	1.4
4	*Aesculus hippocastanum* L.	16	2
5	*Alnus glutinosa* (L.) Gaertn.	14	1.8
6	*Betula pendula* Roth	9	1.1
7	*Carpinus betulus* L.	1	0.1
8	*Castanea sativa* Mill.	1	0.1
9	*Crataegus monogyna* Jacq.	1	0.1
10	*Fraxinus angustifolia* Vahl.	1	0.1
11	*Fraxinus excelsior* L.	183	22.9
12	*Fraxinus excelsior* ´Pendula´	3	0.4
13	*Gymnocladus dioicus* (L.) K. Koch	10	1.3
14	*Juglans nigra* L.	3	0.4
15	*Juglans regia* L.	16	2
16	*Liriodendron tulipifera* L.	1	0.1
17	*Magnolia* × *soulangeana* Soul.-Bod. ex Thunb.	4	0.5
18	*Malus pumila* Mill.	2	0.3
19	*Negundo aceroides* Moench	28	3.5
20	*Ostrya carpinifolia* Scop.	2	0.3
21	*Padus avium* Mill.	4	0.5

Table 5. Tree species analysis (800 trees**)** **–** continued

No.	Species, cultivars	Number [specimen]	Percentage [%]
	Broad-leaved trees		
22	*Phelodendron amurense* Rupr.	1	0.1
23	*Platanus hispanica* Mill.	8	1
24	*Populus alba* L.	2	0.3
25	*Populus nigra* ´Italica´	11	1.4
26	*Prunus domestica* L.	2	0.3
27	*Prunus spinosa* L.	19	2.4
28	*Quercus robur* L.	5	0.6
29	*Quercus robur* ´Fastigiata´	1	0.1
30	*Rhus typhina* L.	1	0.1
31	*Robinia pseudoacacia* L.	26	3.3
32	*Salix alba* L.	2	0.3
33	*Salix alba* ´Tristis´	2	0.3
34	*Sophora japonica* L.	3	0.4
35	*Sophora japonica* ´Pendula´	1	0.1
36	*Tilia platyphyllos* Scop.	56	7
	Conifers		
37	*Abies alba* Mill.	2	0.3
38	*Abies concolor* (Gordon & Glend.) Hildebr.	2	0.3
39	*Abies procera* Rehder	1	0.1
40	*Ginkgo biloba* L. (femina)	1	0.1
40	*Ginkgo biloba* L. (mas)	1	0.1
41	*Chamaecyparis lawsoniana* (A. Murray bis) Parl.	4	0.5
42	*Chamaecyparis pisifera* (Siebold & Zucc.) Endl.	2	0.3
43	*Juniperus × media*	8	1
44	*Juniperus chinensis* ´Pfitzeriana Glauca´	3	0.4
45	*Picea abies* (L.) H. Karst.	13	1.6
46	*Picea pungens* ´Glauca´	5	0.6
47	*Pinus nigra* J. F. Arnold	48	6
48	*Pinus sylvestris* L.	2	0.3
49	*Pinus sylvestris* Aurea	1	0.1
50	*Platycladus orientalis* (L.) Franco	4	0.5
51	*Pseudotsuga menziesii* (Mirb.) Franco	13	1.6
52	*Pseudotsuga menziesii* ´Glauca´	1	0.1
53	*Taxus baccata* L. (tree growth)	9	1.1
54	*Thuja occidentalis* L.	1	0.1
55	*Thuja occidentalis* ´Globosa´	6	0.8
56	*Thuja occidentalis* ´Malonyana´	56	7
57	*Thuja plicata* Donn ex D. Don	2	0.3
58	*Thuja plicata* ´Zebrina´	1	0.1
59	*Tsuga canadensis* (L.) Carriere	3	0.4
	Total	800	100%
	Broadleaf trees – 36 species	611 specimen	76.4%
	Needle trees – 23 species	189 specimen	23.6%

Table 6. Shrub species analysis (35 shrubs)

No.	Species, cultivars	Number [specimen]	Percentage [%]
	Broad-leaved shrubs		
60	*Buxus sempervirens* L.	13	37.1
61	*Buxus sempervirens* Bullata	3	8.6
62	*Sambucus nigra* L.	1	2.9
63	*Berberis thunbergii* DC.	5	14.3
64	*Philadelphus coronarius* L.	4	11.4
65	*Rhamnus cathartica* L.	1	2.9
	Coniferous shrubs		
66	*Taxus baccata* L. (bushy growth)	5	14.3
67	*Taxus baccata* Repandens	3	8.6
	Total	35	100%
	Broad-leaved trees – 6 species	27 specimen	77.1%
	Conifers – 2 species	8 specimen	22.9%

Tree evaluation has shown average age of trees which is 62.88 years which is caused by new plantation during socialistic times, natural sowing of trees and decay of the park. In ideal conditions much more plants would survive from the times when the park was founded (19th century) or from the times of the last treatment (the beginning of the 20th century). However the average age of the trees is still impressive. The average age of shrubs is much lower as they live much shorter time, which is just natural. The average age of natural seedlings shows exactly the time when the park lost continual maintenance which happened after the revolution, so approximately 20 years ago.

Average height of the trees 16.31 m shows average to good environmental conditions of the park. Some of the trees in the lowest part of the park reach the height up to 25 m because of stream flat in this part of the park. Upper part of the park is occupied by black pines (*Pinus nigra* J. F. Arnold) which also reach the same height as they like drier conditions. Average height of shrubs is 4.69 m, which is still sufficient. Average height of natural seedlings is 5.71 m which is influenced by the species, ashes (*Fraxinus excelsior* L.) reach 10 m and bushes are much lower.

Crown diameter shows that the overlap of tree crowns is very high, average diameter reaches 7.8 m. As we have 4 ha of areas with trees, bushes and natural seedlings and approximately 4 ha of open spaces and built area, then 800 trees grow on 3 ha of areas together with natural seedlings. Their overlap is then 27% as the average cover of one tree is 47.8 m so together 3.8 ha on area of 3 ha makes a 1.27 multiple overlap. Shrubs have average 4.06 m crown diameter, but they usually grow under the trees and natural seedlings have average 2.84 m crown diameter as they are young. Natural seedlings were not counted in pieces; just the areas they are growing in were digitally measured with result of approximately 4 ha.

Trunk diameter was categorized just on trees and was 47.25 cm which shows again good environmental conditions.

The most important parameter is compositional value which shows the quality of plants in whole composition. Final compositional value of all trees in the park is 3.25; for shrubs it is 3.4 and of natural seedlings only 1.19. Weighted average of compositional value for trees is 3. These values offer a possibility for expressive improvement after making compositional felling in the park.

Health state is little bit better at trees 3.55 and shrubs 3.86 and much better at natural seedlings 4.86 which refer to low age of natural seedlings. Felling of ill trees, damaged and dangerous ones will certainly improve the health state of trees and shrubs. Certain amount of naturally sown trees (natural seedlings) could remain in a composition after a positive selection.

To reach historical look of the park the reconstruction proposal counts with removing of all other natural seedlings (not selected individual trees and shrubs) and felling of 400 trees in two phases (60% in the first step, 40% in the second one). Comparison of a present state and proposed one is visible in Figs 1 and 2. Also approximately 240 new trees are planned to be planted out, 3,000 new shrubs and approximately 6 ha of new lawns.

Krásny et al. (2008) have made similar evaluation of woody plants in historic park in Piestany (Slovakia) where they have come to similar conclusions. Although their work was made in spa park, it has similar use of woody plants as it was grounded mainly in 19th century

Fig. 1. Present State of the Park.

Fig. 2. Felling Plan.

like Beladice historic park. Total number of trees was 1,704 in 82 taxa, 56 broad-leaved trees and 26 conifers; average age was mostly in categories 10–20, 20–40 and 60–80; average compositional value using Machovec method (1982) it was 2.9 and average health state (tree health index) was 3.75; both average values similarly like in Beladice historic park.

Also Feriancová and Štepánková (2006) have evaluated trees in spa park Brusno using Machovec (1982) method combined with other ones oriented on pests and damages. There were 892 trees evaluated; 2/3 of autochthonous and 1/3 of allochthonous taxa. Their average health condition was 3.3 and their compositional (landscaping) value was 3.34, similarly like in Beladice historic park.

Comparison with a historic park tree evaluation can be made with Oravcová (2005) research where she used the same Machovec (1982) method. The results of 684 trees inventory have shown average compositional value 3 and most trees' age in category 40–60 years.

Conclusion

Evaluation of greenery in historic park Beladice, part Beladice, has shown average health state and compositional value of trees and shrubs. There were recognized 67 plant species altogether in 900 specimens (800 trees, 35 shrubs and 65 groups of natural seedlings). This situation can be considered as not suitable for the park condition. That is why compositional outcuttings are necessary. Especially in case of already too overgrown natural seedlings, just several trees can be selected for further use in composition of the park. Also health state of half of trees (400 specimens) causes the need for their outcuttings. As this is not possible in one step two ones are needed, second one following the first one after 20 years (60% in the first step, 40% in the second one). Just after these cleanings it is possible to grow new plants and laws in the park.

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Physiological reaction and energy accumulation of dominant plant species in fir-beech ecosystems affected by air pollution

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Abstract

Kuklová, M., Hniličková, H., Hnilička, F., Kukla, J. 2014. Physiological reaction and energy accumulation of dominant plant species in fir-beech ecosystems affected by air pollution. *Folia oecol.,* 41: 53–61.

Physiological reaction and energy accumulation of assimilatory organs of 4 dominant plant species were studied in fir-beech ecosystems (80-100-year-old stand) and parallel clear-cut area (10-year-old stand) in locality Hliníky situated in the buffer zone of the Slovenský raj National Park. Studied ecosystems are located on the area affected by human activities. The achieved results showed that the influence of stand climate and habitat conditions differentiated the measured characteristics. Significantly higher photosynthesis was observed in assimilatory organs of *F. sylvatica, R. idaeus, D. filix-mas* and *V. myrtillus* species in the fir-beech stand (range 5.79–16.10 µmol CO_2 m^{-2} s^{-1}) compared to the clear-cut area (range 4.54–12.89 µmol CO_2 m^{-2} s^{-1}). *V. myrtillus* and *F. sylvatica* species reacted sensitively with significantly lower values of stomatal conductance (0.24, respectively 0.26 mol m^{-2} s^{-1}) in clear-cut area; *R. idaeus* and *D. filix-mas* showed significantly lower values (0.57, respectively 0.29 mol m^{-2} s^{-1}) in the fir-beech stand. Reduction of Fv/Fm under physiological limit was found for *F. sylvatica* species growing in clear-cut area, where the file of habitat conditions was not probably optimal. Energy content in phytomass of studied plant species varied from 18,511 J g^{-1} (*R. idaeus*) to 20,551 J g^{-1} (*V. myrtillus*). Significantly higher was only the mean value found for *D. filix-mas* species growing in the fir-beech stand (19,049 J g^{-1}) compared to the clear-cut area (18,561 J g^{-1}).

Keywords

ash, chlorophyll fluorescence, energy, fir-beech ecosystems, photosynthesis, stomatal conductance

Introduction

Vegetation reacts sensitively to environmental pollution by growth slowing, production reducing, morphological leaf changes, mortality of sensitive species and reduction of species diversity. Pollutants after release from the source do not remain in the air without changes, physical changes are in progress (movement and distribution in space, turbulent diffusion, changes in the concentration by dilution and other) as well as chemical changes. Industrial pollution is the most serious threat because industry uses highly toxic contaminants and many of them are released to the atmosphere (Atkinson and Winner, 1990; Fiala et al., 1989; Vacek et al., 1999, etc).

On the basis of literature can be assumed that forest stands with its structure and canopy of trees are influencing and changing the ground vegetation and its biomass. Natural forests have strong effects on the composition and structure of plant species mainly because they are the result of changes in light availability (Ódor and Standovár, 2001). Significant disruption of phytocoenoses composition only occurs after the removal of their woody components (Kukla et al., 2003; Kellerová and Janík, 2011). In natural conditions there are often more stressors simultaneously (strong radiation, high temperature, deficiency of water). If the file of habitat conditions is not optimal, the photosynthetic efficiency of plants is reduced (Ammann et al., 1999; Shparyk and Parpan, 2004).

Studied fir-beech ecosystems are located on the area affected by human activities. The Central Spiš has been subjected to a long-term negative influence of mining, metallurgy and engineering industry. The air in forest ecosystems is polluted mostly by harmful substances produced by ore-working and wood-working industry (Kukla et al., 2011). The main components of the pollutants are sulphur (SO_2), nitrogen (NO_x), CO emission and also heavy metals.

With this background, the present study was undertaken with the objective to compare physiological characteristics (photosynthesis, transpiration, stomatal conductance, chlorophyll fluorescence) and energy accumulation of assimilatory organs of *Fagus sylvatica* L., *Dryopteris filix-mas* (L.) Schott, *Vaccinium myrtillus* L. and *Rubus idaeus* L. species in fir-beech stand and parallel clear-cut area, both located on the area affected by air pollution in the buffer zone of Slovenský raj National Park (Western Carpathians).

Materials and methods

Study plots

The research was performed in the first half of July 2012 on 2 monitoring plots situated in the buffer zone of Slovenský raj National Park (NP), Table 1. G1 – clear-cut area is represented by 10-year-old fir-beech stand; G2 is represented by 80-100-year-old fir-beech stand. Soil reaction of Dystric Cambisol on the G1 (pH_{H_2O} 5.1) is strongly acidic and G2 (pH_{H_2O} 4.2) is very extremely acidic. Values of C/N ratio in the upper layers of soils (0–5 cm) on both plots reach the value 10.3. The studied plots are situated in the cool climatic region, with the mean temperature in July, 12–16 °C. The mean annual temperature is 4–5 °C, and average annual precipitation reaches 700–800 mm (Miklós and Hrnčiarová, 2002).

Methods

The soils were classified according to World Reference Base for Soil Resources 1994 (Bedrna et al., 2000). Values of soil reaction were determined potentiometrically – using a digital pH meter Inolab pH 720. Total content of N and C was determined by NCS analyzer type FLASH 1112.

The forest ecosystems were classified according to Zlatník (1976) and the names of plant taxa were given according to Marhold and Hindák (1998). On monitoring plots (area about the size of 400 m^2), leaves of *Fagus sylvatica* (from the bottom third of the tree crown), *Dryopteris filix-mas* (leaves), *Vaccinium myrtillus* (green twigs) and *Rubus idaeus* (shoots growing from creeping root) were sampled. All plant samples were dried at 80 °C for 48 hours and homogenised with a Fritsch planetary micro mill (<0.001 mm).

The gas exchange – rate of photosynthesis (P_N), rate of transpiration (E), stomatal conductance (g_S) and

Table 1. Ecological characteristics of studied forest ecosystems in buffer zone of Slovenský raj National Park (Western Carpathians)

Locality		Hliníky	
Study plot		G1 – clear-cut area	G2 – fir-beech stand
Age		10	80–100
Altitude [m]		960	950
Geographical coordinates		20°32’07’’E	20°32’12’’E
		48°51’51’’N	48°51’49’’N
Exposure		S	SW
Stocking	upper storey	0.6–1.0	0.5
	lower storey	–	0.6–0.7
Canopy	upper storey	60–100	50
	lower storey	–	60–70
Vegetation unit		*Abieti-Fageta inferiora*	
Parent rock		quartz conglomerates	
Soil subtype		Dystric Cambisol	
pH_{H_2O} in 0–5 cm		4.2	5.1
pH_{KCL} in 0–5 cm		3.3	4.1
C : N in 0–5 cm		10.3	10.3

the intercellular CO_2 concentration (ci) were measured on the upper surface of leaves (the middle part of the leaf blade) *in situ* using the portable gas exchange system *LCpro+* (*ADC BioScientific Ltd.*, Hoddesdon, Great Britain). This instrument measures the gas exchange based on method of an open system (Šesták et al., 1966; Holá et al., 2010). Measurement time was set according to the work by Tucci et al. (2010). These physiological characteristics were measured under adjusted light and temperature conditions, the irradiance was 650 µmol m^{-2} s^{-1} of photosynthetically active radiation, the temperature in the measurement chamber was 25 °C, the CO_2 concentration was 420 ± 35 vpm (µmol mol^{-1}), the air flow rate was 205 ± 30 µmol s^{-1} and the duration of the measurement of each sample was 20 min after the establishment of steady-state conditions inside the measurement chamber. The value of VSD (vapour pressure deficit) was 0.85 ± 0.15 kPa. Measured parameters of gas exchange rates were calculated using the following formulas:

Rate of photosynthesis (P_N) [µmol CO_2 m^{-2} s^{-1}]

$$P_N = u_s \cdot \Delta c, \quad (1)$$

where Δc is the difference in concentration of CO_2 on input and output of the chamber [µmol mol^{-1}] and u_s is concentration – air flow per the m^2 leaf area [mol m^{-2} s^{-1}].

Rate of transpiration (E) [mmol H_2O m^{-2} s^{-1}]

$$E = u_s \cdot \Delta W, \quad (2)$$

where ΔW is the difference in concentration water vapor [mol mol^{-1}] and u_s is concentration – air flow per the m^2 leaf area [mol m^{-2} s^{-1}].

Stomatal conductance (g_s) [mol m^{-2} s^{-1}]

$$g_s = 1 : r_s, \quad (3)$$

where r_s is stomatal resistance.

The minimum Chl fluorescence (F0) and the maximum Chl fluorescence (Fm) were also measured *in situ* with the portable 1 Chl fluorometer *ADC:OSI 1 FL* (*ADC BioScientific Ltd.*, Hoddesdon, Great Britain) with 1 s excitation pulse (660 nm) and saturation intensity 3,000 µmol m^{-2} s^{-1} after 20 min dark adaptation of the leaves. The maximum quantum efficiency of Photosystem (PS) II was calculated as Fv/Fm (Fv = Fm – F0).

Gas exchange and chlorophyll fluorescence were always measured from 9:00 to 12:30 h. CET. The natural intensity of PAR of the lower storey and the upper storey was 320 ± 55 µmol m^{-2} s^{-1} and 650 ± 50 µmol m^{-2} s^{-1}, respectively.

The energy content of phytomass (J g^{-1} of dry matter) was determined using an adiabatic calorimeter IKA C-4000 (software C-402). The samples weighing 0.7–1 g and homogenised were pressed into a form of briquette, dried up to a constant weight at 105 °C and burnt in pure oxygen under a pressure of 3.04 MPa (DIN 51900). The ash content was determined gravimetrically, by total oxidation of specimens in a muffle furnace at 500 °C (Javorský et al., 1987).

Statistical analyses were made with the use of a statistics program Statistica 9 software and the variability in mean physiological and energy characteristics of plant species between monitoring plots was tested by anova model. The significance of differences was verified by Fisher-LSD test. The measured characteristics included rate of photosynthesis, rate of transpiration, stomatal conductance, chlorophyll fluorescence, energy and ash accumulation. Average ± standard deviation for the physiological characteristics was within 15–30 individuals while preserving three repetitions. For each plant species, three measurements of energy value were made.

Results

Rate of photosynthesis (P_N)

Rate of photosynthesis of plant species growing in clear-cut area fluctuated in the interval from 4.51 µmol CO_2 m^{-2} s^{-1} (*D. filix-mas*) to 13.29 µmol CO_2 m^{-2} s^{-1} (*F. sylvatica*). In the case of fir-beech stand, photosynthesis was the lowest in ferns (5.74 µmol CO_2 m^{-2} s^{-1}) and the highest in beech leaves (16.31 µmol CO_2 m^{-2} s^{-1}). On studied plots, herb and shrub species showed lower photosynthesis compared to the assimilatory organs of *F. sylvatica*, Fig. 1. Significantly lower photosynthesis was observed in assimilatory organs of beech, male fern, bilberry and raspberry in clear-cut area in comparison with the fir-beech stand (significance level $\alpha < 0.001$). In the case of *F. sylvatica* species, the difference between compared plots was the highest (3.21 µmol CO_2 m^{-2} s^{-1}), Fig. 1.

Rate of transpiration (E)

On studied plots, herb and shrub species showed lower rate of transpiration (0.49–1.65 mmol H_2O m^{-2} s^{-1}) compared to the assimilatory organs of *F. sylvatica* (2.10–2.11 mmol H_2O m^{-2} s^{-1}), Fig. 2. In the case of *F. sylvatica*, *D. filix-mas* and *V. myrtillus* species higher rate of transpiration was found in clear-cut area; *R. idaeus* species showed higher transpiration in the fir-beech stand. Significantly different were only the mean values found for *D. filix-mas* species (significance level $\alpha < 0.001$). For other plant species differences between compared plots were not statistically significant.

Stomatal conductance (g_s)

The values of stomatal conductance on studied plots varied from 0.19 ± 0.05 mol m^{-2} s^{-1} (*D. filix-max*) to 0.57 ± 0.23 mol m^{-2} s^{-1} (*R. idaeus*), Fig. 3. In the case of *F. sylvatica* and *V. myrtillus* species higher values of

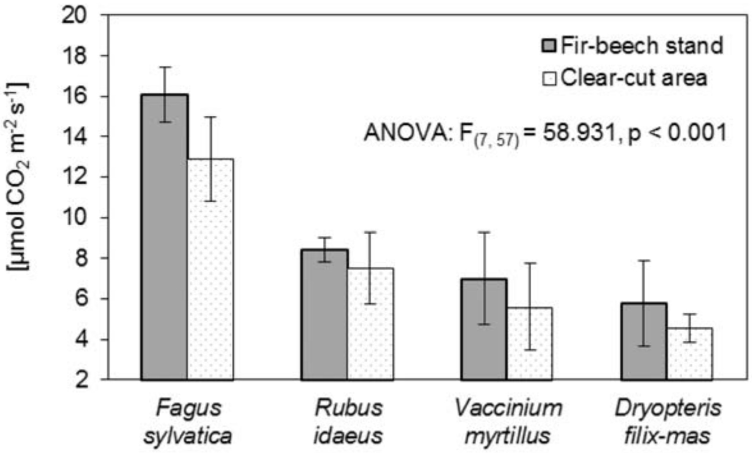

Fig. 1. Rate of photosynthesis (P_N) of assimilatory organs of dominant plant species (mean value ± SD).

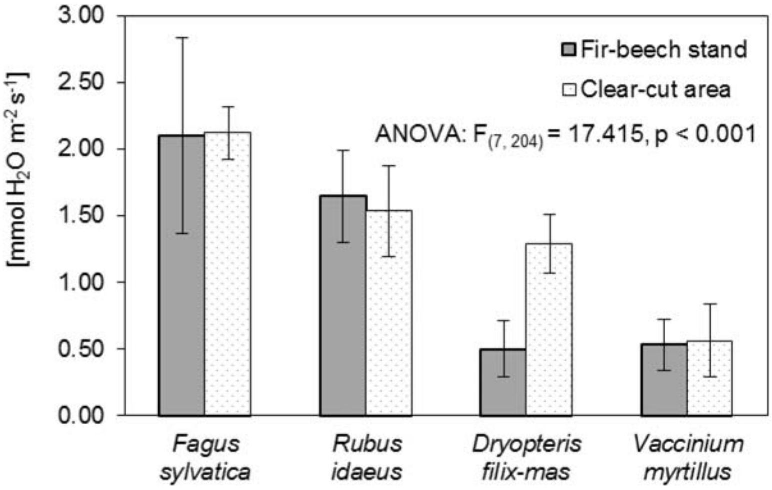

Fig. 2. Rate of transpiration (E) of assimilatory organs of dominant plant species (mean value ± SD).

stomatal conductance were observed in the fir-beech stand; *R. idaeus* and *D. filix-mas* species showed higher values in clear-cut area. Significantly different were only the mean values found out for *F. sylvatica* and *V. myrtillus* species, where the differences between plots were the highest (significance level $\alpha < 0.001$). The rate of gas exchange can be limited not only by stomatal conductance (gs), but also by substomatal CO_2 (ci). The values of substomatal CO_2 varied from 490 ± 35 vpm to 610 ± 35 vpm.

Chlorophyll fluorescence

Studied plant species showed lower values of fluorescence in clear-cut area (range 0.57–0.80) in comparison with the fir-beech stand (range 0.80–0.81). The lowest value of fluorescence was found out for *F. sylvatica* species (0.57 ± 0.02) growing in clear-cut area, the highest for *R. idaeus* species in the fir-beech stand (0.81 ± 0.18). The highest difference between studied plots showed assimilatory organs of *F. sylvatica* (29%), the lowest *R. idaeus* species (1.3%), Fig. 4.

Energy and ash accumulation

Higher values of energy were observed in assimilation organs of beech, male fern and blueberries (range 19,984–20,551 J g^{-1}) in the fir-beech stand; raspberry showed slightly higher value of energy in clear-cut area (18,562 ± 325 J g^{-1}) in comparison with the fir-beech stand (18,511 ± 251 J g^{-1}), Fig. 5. Significantly lower was only the mean calorific value found for *D. filix-mas* species (18,562 ± 310 J g^{-1}) growing in clear-cut area compared with the value in fir-beech stand (19,049 ± 251 J g^{-1}), (significance level $\alpha < 0.05$). Ash content of analysed plants in the fir-beech stand varied in the following order [mg g^{-1}]: 31.6 (*F. sylvatica*) < 41.0 (*V. myrtillus*) < 46.1 (*R. idaeus*)

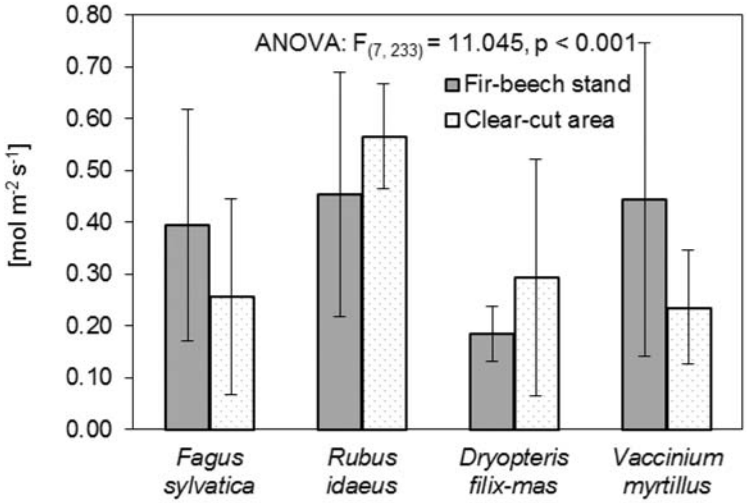

Fig. 3. Stomatal conductance (g_s) of assimilatory organs of dominant plant species (mean value ± SD).

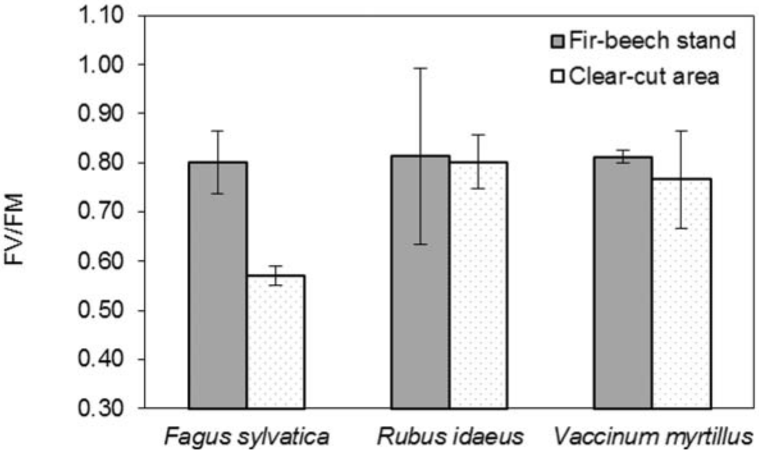

Fig. 4. Potential photochemical efficiency of electron transport in photosystem II of dominant plant species (mean value ± SD).

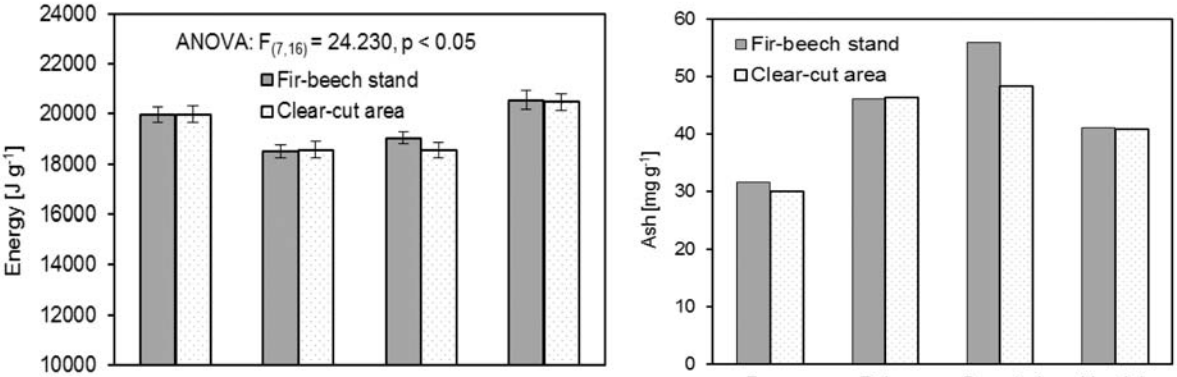

Fig. 5. Energy and ash contents of assimilatory organs of dominant plant species (mean value ± SD).

< 55.9 (*D. filix-mas*); in clear-cut area the values were as follows [mg g^{-1}]: 30.0 < (*F. sylvatica*) < 40.8 (*V. myrtillus*) < 46.4 (*R. idaeus*) < 48.4 (*D. filix mas*). Slightly higher was ash content in the dry matter of assimilatory organs of male fern in the fir-beech stand, only.

Discussion

Plants are in the course of their lives exposed to changing conditions of environment. Adverse environmental conditions (excessive radiation, high temperature, lack of water, lack of nutrients in the soil, etc.) can slow down their vitals, but also damage the organs of plants. Physiological reaction and quality of dry matter of assimilatory organs can also be influenced by acidic precipitations with increasing industrial and agricultural activities of man. The airborne pollutants deposited on the leaf surface can affect the plant´s metabolism by blocking light, obstructing stomatal apertures, increasing leaf temperature and altering mineral contents of the leaf (Hope et al., 1991; Pavlík et al., 2012). For all studied plant species in buffer zone of Slovenský raj NP, decrease of photosynthesis was recorded in clear-cut area (10-year-old stand), where obviously more radiation and thus heat penetrate to stand compared with the 80-100-year-old stand. With inappropriate environmental conditions the rate of photosynthesis decreases (Matyssek et al., 1993). Drought stress or sudden radiation, are considered to be the most important factors, which plants encounter in nature. In the absence of water in the plant, it loses its ability to cool off, in particular sunlit part of its phytomass and this leads to its overheating (Penka, 1985).

Direct irradiation of plant affects not only the plant itself, but also changes the microclimate around it, which in turn may affect its growth (Anderson, 1964). Light intensity in forest stand is lower than in the open area and its quality varies depending on the absorption and reflection of leaves. In submontane beech stands of different stocking in Kremnické vrchy Mts, e.g. Janík (2009) observed at the stage of full foliage values from 2.3 to 8.2% of open area lighting.

In the case of *F. sylvatica* species, the difference in rate of photosynthesis between compared ecosystems was the highest. Deteriorated physiological status of juvenile beech trees on the site with higher immission load is stated by Kmeť and Ditmarová (2001). According to the results of Woo (2009), beech belongs to the sensitive woody plant species on air pollution which is also reflected in the change of rate of photosynthesis. Takagi and Gyokusen (2004) observed higher rate of phytotosynthesis of leaves of the *Ilex* trees in the urban core, whereas in the suburban area it was the lowest. The authors found, that rate of photosynthesis was negatively correlated with sun light conditions and positively with air pollutant concentrations.

According to Farmer (1993), airborne dust in forest ecosystems also influences transpiration rate. *R. idaeus* species showed higher transpiration in the older fir-beech stand, only. All other plant species had higher values on plot with lower stand density (clear-cut area). The most sensitively to file of habitat conditions reacted *D. filix-mas* species, where the difference between compared plots was the highest, which could also be caused by higher input of risk elements from polluted atmosphere (leaching of risk elements captured in fir-beech stand canopy).

Stomatal conductance is one of the important factors affecting the metabolism of plant (Agbaire, 2009). Interspecies differences in stomatal conductance are confirmed by Tomasevic et al. (2005), who observed the influence of pollutants on the characteristics of hazel and horse chestnut. Changes in the values of substomatal CO_2 (ci) depending on environmental factors are stated by Kumaravelu and Ramanujam (1998), Liang, et al. (2008). Differences in stomatal conductance depending on the locality were studied by Nizzetto and Perlinger (2012). According to Dohmen et al. (1990) and Leuzinger and Körner (2007), stomatal conductance usually due to air pollution and the increasing concentration of CO_2 decreases, but the reaction in plants is not always reflected. From measured values of stomatal conductance of plant species in Slovenský raj NP is evident not only effect of site, but also the differences between plant genotype. In assimilatory organs of beech and blueberry, stomatal conductance was higher in the 80-100-year-old ecosystem; in the mature stand, leaves of raspberry and male fern answered more sensitively, which can again be related to airborne pollutants (leaching of risk elements captured in fir-beech canopy). Prasad (1995) for example states, that the plants from the control conditions have higher stomatal conductance in comparison with the plants from the stressed conditions.

Chlorophyll fluorescence is also an important physiological indicator, which appears to be a suitable indicator of plant reaction to stress (Maxvell and Johnson, 2000). Negative impact of stressors on chlorophyll fluorescence was studied by Gamon and Surfus (1999) and Takayama and Nishina (2009). These authors state that not always, these changes are accompanied by visible signs of damage leaves. Studied plant species in Slovenský raj NP showed lower values of fluorescence in clear-cut area in comparison with the fir-beech stand. The most sensitively to file of habitat conditions answered *F. sylvatica*, the least *R. idaeus* species. In the case of C3 plants, photosynthesis cycle as a “normal” is the range from 0.79 to 0.84 (Maxwell and Johnson, 2000). Measured values of potential photochemical efficiences of electron transport in photosystem II (Fv/Fm) showed decrease in this parameter under physiological limit for *F. sylvatica* species growing in clear-cut area.

On studied plots significantly higher was only the mean value of energy found for *D. filix-mas* in the fir-beech stand compared with clear-cut area. This fact was indeed associated with ecological demands of species (semi-shadow, water moderately demanding species). Calorific values for all other species were similar (slightly higher in clear-cut area, where more light was available for production of dry matter) and did not significantly differ between plots. Bobkova and Tuzhulkina (2001) state that combustion heat as a physical parameter is characterized by a relatively high variability, being dependent on plant species, growing conditions, morphological structure, age, period of sampling, and other factors. Bublinec et al. (2011), Schieber and Kováčová (2002) e.g. in mature beech forest in Kremnické vrchy Mts found out lower energy contents (by 5–22%) in herb species compared to plants growing on anthropogenic influenced plot (clear-cut area). Negative effects of pollutants on herb understorey in Bielovodská dolina valley, the High Tatras Mts, were observed by Kukla et al. (2003). In case of blueberry, the authors found higher energy values in undamaged stands opposite those anthropogenic affected areas. Ash contents of plant organs in studied stands are typical for the plants growing on acidic soils, poor in nutrients. On studied plots in Slovenský raj NP, slightly higher value of ash was showed by the dry matter of assimilatory organs of male fern in the fir-beech stand, only.

Conclusions

Evaluation of growth processes occurring in phytocoenoses is an important source of information about the degree of threat to forest ecosystems, depending on the intensity of the negative external environmental impacts. The research conducted in fir-beech ecosystems affected by air pollution, situated in the locality Hliníky (the buffer zone in Slovenský raj NP) showed, that forest stands with its structure, density of canopy of trees and habitat conditions differentiated the measured physiological characteristics. From measured values of gas-exchange parameters is evident not only effect of site, but also the differences between plant genotypes. Environmental conditions (radiation, drought, airborne pollutants) mainly influenced rate of phytotosynthesis and rate of transpiration by relatively lower values of plants in the juvenile stand (clear-cut area). In the case of *F. sylvatica* the difference of photosynthesis between compared ecosystems was the highest. The most sensitively to file of habitat conditions reacted *D. filix-mas* species with significantly lower value of transpiration in the engaged stand, which could be caused by impact of airborne pollutants (leaching of risk elements captured in fir-beech canopy). The results showed significant effect of site, but also plant genotype on stomatal conductance of plants (*V. myrtillus* and *F. sylvatica* answered sensitively with significantly lower values in clear-cut area, *R. idaeus* and *D. filix-mas* in the mature stand). Reduction of the potential photochemical efficiency (FV/FM) under physiological limit was found for *F. sylvatica* species in clear-cut area, where the file of habitat conditions for growth of juvenile beech trees was not probably favourable. Approximately 7–10% higher energy contents on studied plots were showed by assimilatory organs of beech and blueberry compared to raspberry and fern. Significantly higher was only the mean calorific value found for *D. filix-mas* species in the mature stand, where habitat conditions for growth of this species were obviously optimal.

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Selected biotic vectors transmitting beech bark necrotic disease in Central and South-Eastern Europe

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Abstract

Mihál, I., Cicák, A., Tsakov, H. 2014. Selected biotic vectors transmitting beech bark necrotic disease in Central and South-Eastern Europe. *Folia oecol.*, 41: 62–74.

The authors mapped occurrence of three biotic vectors of beech (*Fagus sylvatica* L.) bark necrotic disease: beech lice *Cryptococcus fagi* Bärensp. and moths *Bucculatrix ulmella* Zeller and *Ectoedemia liebwerdella* Zim. in several countries in Central and South-Eastern Europe. The role of these species in European beech pathology is discussed. We have found a massive occurrence of *C. fagi* in all the localities, with frequency values reaching up to 100%. The occurrence of beech lice was not limited by the locality altitude. The butterfly *E. liebwerdella* generally occurred in most localities, with exception of Poland and Romania. In contrast to *C. fagi*, occurrence of *E. liebwerdella* was limited by altitude. The upper occurrence limit in Slovakia was 800 m a.s.l., in Bulgaria it was 1,220 m a.s.l. (32%) and infrequent in 1,380 m a.s.l. (only 4%). The butterfly *B. ulmella* occurred everywhere, apart from Poland. Its frequency was always lower than that of *C. fagi* and *E. liebwerdella*. The maximum value (91%) was found in Slovakia in a beech stand strongly affected with airborne pollutants in the past. Our results show that the upper occurrence limit for this species was 900 m a.s.l. in Slovakia and 1,250 m a.s.l. (4%) in Bulgaria.

Keywords

beech bark disease, biotic vectors, Central Europe, *Fagus sylvatica* L., insects, South-Eastern Europe

Introduction

Over the last three decades, an increasing occurrence of beech bark necrotic disease has been documented in Central and South-Eastern Europe (Cicák and Mihál, 2002; Jančařík, 1992; Rosnev and Petkov, 1996). This disease can be caused and spread by several biotic vectors – insects associated with the European beech (*Fagus sylvatica* L.). The insects transport on their bodies particles of mycelia and spores of various fungi species acting as beech bark disease causal agents: e.g. fungi from genera *Fusarium* Link., *Nectria* (Fr.) Fr., *Ophiostoma* Syd., *Phomopsis* Sacc., *Phytophthora* de Bary, *Valsa* Fr., *Verticillium* Nees. and others (e.g. Houston, 1994; Jančařík, 2000; Jung, 2009; Mihál et al., 2009; Perrin, 1984).

The issue of biotic vectors transmitting beech bark necrosis has been studied by many authors in Central and South-Eastern Europe. The problems of occurrence, spreading and phytopathological importance of *Cryptococcus fagi* has been studied by e.g. Cicák et al. (2006), Chira et al. (2003), Jurášek and Vacek (1983), Karadžić et al. (2003), Marinković and Karadžić (1985), Mihál and Cicák (2001), Mihál et al. (2009), Rojek (2005), Rosnev and Petkov (1996), Suvák (1998). Moreover, the role of moths *Ectoedemia liebwerdella* and *Bucculatrix ulmella* in beech bark disease complex is also studied by Borkowski and Konca (1991), Cicák et al. (2006), Csóka and Kovács (1999), Csóka and Szabóky (2005), Mihál and Cicák (2001), Mihál et al. (2009), Rojek (2005), Stolnicu (2007). Some mutual aspects between xylophagous Coleoptera and beech bark disease complex has been studied by e.g. Jančařík (1992) or Surovec and Novotný (1985).

The aim of our work was to map the occurrence of three selected biotic vectors (*Cryptococcus fagi*, *Bucculatrix ulmella* and *Ectoedemia liebwerdella*) for beech bark necrotic disease in countries of Central and South-Eastern Europe (Czech Republic, Poland, Slovakia, Hungary, Romania, Bulgaria and Serbia).

Materials and methods

Together with assessment of degree of necrotic beech bark disease, we recorded occurrence of biotic vectors transmitting this disease – beech lice *Cryptococcus fagi* Bärensp. (Fig. 1) and moths *Bucculatrix ulmella* Zeller (Fig. 2) and *Ectoedemia liebwerdella* Zim. (Fig. 3). The research ran (April–May or October–November) in selected localities in Slovakia in the years 1995–2007. In the years 2001–2013, we also monitored occurrence of these species in several localities in the Czech Republic, Poland, Hungary, Romania, Serbia and Bulgaria.

Fig. 1. *Cryptococcus fagi* Bärensp. – adult colonies on beech bark (photo A. Cicák).

Fig. 2. *Bucculatrix ulmella* Zeller – pupae on beech bark (photo A. Cicák).

Fig. 3. *Ectoedemia liebwerdella* Zim.– the relict traces (galleries in bark) after the mining (photo A. Cicák).

Occurrence of these biovectors was recorded in each locality on a sample set consisting of 100 trees selected from the local beech parent stand. The sample size in localities with status of a permanent research plot (Prolaz, Troyan, Shipka, Balkanets, Tri Buki) was conformed according to the number of the numbered trees (in general less than 100 trees). We evaluated trees representing all the tree classes (according to Kraft). Occurrence of biotic vectors was recorded on the tree bark, around the whole stem perimeter, from root buttresses up to 2 m above the ground. In case of *C. fagi* we evaluated occurrence of adults, in case of *B. ulmella* occurrence of pupae on beech bark and in case of *E. liebwerdella* the relict traces (galleries in bark) after the mining. Also, isolated occurrence of a target species was considered as a recorded finding. The results were interpreted in percents – expressing frequency occurrence of the target species. A short description of the Slovak localities is in Table 1, the foreign localities in Central and South-Eastern Europe are summarised in Table 2.

Results and discussion

The information about frequency occurrence of the studied biotic vectors in Slovakia is in Table 3, the data for the other countries of Central and South-Eastern Europe are in Table 4.

According to our observations, *Cryptococcus fagi* had the highest frequency occurrence. Almost 80% of Slovak localities showed frequency occurrence values for this biovector exceeding 70%. In Bulgaria, these values were found lower, but also 68% of Bulgarian localities exceeded the value of 70%. In the other lands of Central and South-Eastern Europe the frequency values of this vector were higher than 90%. The only exception was the locality Crucea in Romania where

Table 1. Basic characteristics of research localities in Slovakia

Orographic unit	Locality	Altitude [m a.s.l.]	Exposition	Age of stand [years]	Beech [%] composition
Kremnické vrchy Mts	Boky	370	NW	70–120	55.0
	Kováčová	450	W	90	98.0
	Badínsky prales	760	N	20–150	95.0
	Mláčik	850	SE	95	60.0
Spišsko-gemerský kras karst	Javorníčková	600	SE	90	30.0
	Veľká Stožka	880	NE	65	90.0
	Nemecké lúčky	950	SE	80	70.0
	Vyšná Roveň	1,000	NE	105	95.0
	Červená Skala	1,050	NE	70	60.0
Bukovské vrchy Mts	Havešová	520	SE	120	100.0
	Udava	620	W	120	60.0
	Stužica	800	N	20–180	95.0
	Riaba skala	980	E	160	30.0
Štiavnické vrchy Mts	Žiar nad Hronom	470	NW	70	95.0
	Jalná	610	NW	75	100.0
	Sitno	900	NW	90	70.0
Malé Karpaty Mts	Kačín	320	NE	90	100.0
	Havrania skala	400	N	90	98.0
Nízke Tatry Mts	Korytnica	960	SW	80	88.0
	Lomnistá dolina	1,200	SE	140	50.0
Vihorlatské vrchy Mts	Sninský kameň	560	N	90	100.0
	Kyjov	800	N	20–250	100.0
Krupinská planina plain	Litava	300	NW	90	75.0
Slovenský kras karst	Brzotínske skaly	450	NW	70	65.0
Považský Inovec Mts	Hrádocká dolina	460	NE	60	100.0
Volovské vrchy Mts	Volovec	540	S	70	55.0
Laborecká vrchovina highlands	Výrava	550	SW	40–100	100.0
Ondavská vrchovina highlands	Kačalová	640	SW	60	60.0
Pieniny Mts	Bukový les	660	NW	90	99.0
Revúcka vrchovina highlands	Železník	660	NW	60	98.0
Stolické vrchy Mts	Kohút	680	W	100	95.0
Malá Fatra Mts	Valčianska dolina	680	NE	100	90.0
Slánske vrchy Mts	Oblík	700	SW	170–250	95.0
Pohronský Inovec Mts	Veľký Inovec	720	NE	45	100.0
Tríbeč Mts	Veľký Tríbeč	770	SW	80	50.0
Belianske Tatry Mts	Belianska jaskyňa	780	NE	30	55.0
Čierna Hora Mts	Vysoký vrch	780	NE	90	100.0
Ostrôžky Mts	Bralce	790	E	90	100.0
Biele Karpaty Mts	Veľká Javorina	940	E	80	80.0
Poľana Mts	Hrončecký grúň	950	SE	20–150	40.0
Strážovské vrchy Mts	Strážov	950	SW	80	100.0
Veporské vrchy Mts	Dobročský prales	950	NW	20–250	25.0
Veľká Fatra Mts	Veľká Skalná	1,000	S	60	80.0
Vtáčnik Mts	Škurátka	1,025	SE	100	95.0
Moravsko-sliezske Beskydy Mts	Malý Polom	1,060	NW	90	45.0

Table 2. Basic characteristics of research localities in selected countries of Central and South-Eastern Europe

Country	Orographic unit	Locality	Altitude [m a.s.l.]	Exposition	Age of stand [years]	Beech [%] composition
Czech Republic	Moravsko-sliezske Beskydy Mts.	Hukvaldy	450	E	90	38.0
		Šance	650	SW	60	90.0
		Pustevny	680	SE	115	74.0
Poland	Beskid Sądecki Mts	Kiczera	600	W	65	80.0
	Bieszczady Mts	Przysłop	610	S	70	80.0
	Beskid Nizki Mts	Przełecz Zebrak	825	W	100	95.0
Hungary	Zempéni-Hegység Mts	Telkibánya	300	SE	65	50.0
	Börzöny Mts	Diosjenö	500	N	100	80.0
	Mátra Mts	Parád	600	E	90	100.0
	Bükk Mts	Öserdö	800	SW	20–200	92.0
Romania	Munţii Tibleş Mts	Telciu	420	E	70	90.0
	Munţii Bistriţei Mts	Holda	660	NE	30–120	95.0
	Munţii Stânişoarei Mts	Crucea	690	SW	30–100	90.0
	Munţii Bârgau Mts	Piatra Fântânele	1,020	E	80	98.0
Serbia	Kucheyske planine plain	Yavorak	720	NW	55	100.0
		Velka Brezovitsa	900	N	70	100.0
Bulgaria	Stara planina Mts	Prolaz	300	N	60	90.0
		Shumen	450	E	100	90.0
		Boaza	450	N	70	100.0
		Troyan	480	N	80	100.0
		Vrbitsa	500	W	80	90.0
		Shipkovo	650	NE	70	100.0
		Ichera	700	NW	80	100.0
		Pravets	700	N	50	100.0
		Kotel	700	N	120	100.0
		Etropole	720	NE	125	99.0
		Ticha	750	SW	70	90.0
		Vitinya	970	NE	90	97.0
		Karandila	1,000	SE	135	100.0
		Ribaritsa	1,100	NW	70	100.0
		Shipka	1,100	NE	65	90.0
		Barzia	1,150	NW	110	100.0
		Balkanets	1,250	N	110	100.0
		Govezhda	1,250	N	70	100.0
		Beklemeto	1,300	NE	60	100.0
		Petrohan	1,400	NE	110	100.0
		Dlgi Del	1,450	S	130	100.0
	Rodopi Mts	Debravitsa	550	N	65	50.0
		Semchinovo	700	NW	75	100.0
		Fotinski vodopadi	750	N	70	100.0
		Rozovo	900	NE	65	100.0
		Eleshnitsa	900	NW	65	100.0
		Dobra Voda	950	NW	80	100.0
		Chepino	1,100	E	90	98.0
		Grashevo	1,100	N	80	100.0
		Marino	1,150	E	100	100.0
		Ravnogor	1,200	N	90	100.0
		Ossenovo	1,220	NW	140	100.0

Table 2. Basic characteristics of research localities in selected countries of Central and South-Eastern Europe – continued

Country	Orographic unit	Locality	Altitude [m a.s.l.]	Exposition	Age of stand [years]	Beech [%] composition
Bulgaria	Rodopi Mts	Velingrad	1,250	E	75	100.0
		Rakitovo	1,380	SW	80	100.0
		Aposlovtchark	1,400	E	50	60.0
	Pirin Mts	Razlog	1,150	NW	70	100.0
		Yane Sandanski	1,200	NW	120	100.0
		Popovi livadi	1,350	N	35	95.0
	Rila Mts	Rilski monastir	975	S	90	100.0
		Raduil	1,060	E	90	100.0
		Chaira	1,150	N	90	100.0
		Borovets	1,500	NE	90	90.0
	Sredna Gora Mts	Oborishte	750	W	80	100.0
		Panagyurishte	1,000	S	90	100.0
	Strandzha Mts	Silkosia I	305	NE	56	90.0
		Silkosia II	305	N	100	98.0
	Kraisthe planina Mts	Breznik	975	S	70	100.0
	Ljulin planina Mts	Gorna Bania	900	NW	70	80.0
	Osogovo planina Mts	Tri Buki	1,550	NW	160	100.0
	Vitosha Mts	Dragalevski monastir	1,080	NE	70–130	100.0

Table 3. Occurrence frequency (%) of selected biotic vectors of beech necrotic disease in selected localities in Slovakia

Locality	Altitude [m a.s.l.]	*Cryptococcus fagi*	*Bucculatrix ulmella*	*Ectoedemia liebwerdella*
Litava	300	88.0	62.0	35.0
Kačín	320	76.0	51.0	97.0
Boky	370	74.0	22.0	59.0
Havrania skala	400	79.0	0.0	70.0
Brzotínske skaly	450	85.0	0.0	59.0
Kováčová	450	94.0	8.0	60.0
Hrádocká dolina	460	91.0	12.0	71.0
Žiar nad Hronom	470	100.0	91.0	100
Havešová	520	100.0	3.0	70.0
Volovec	540	90.0	9.0	0.0
Výrava	550	89.0	6.0	80.0
Sninský kameň	560	3.0	0.0	29.0
Javorníčková	600	94.0	5.0	9.0
Jalná	610	89.0	0.0	96.0
Udava	620	88.0	3.0	0.0
Kačalová	640	100.0	11.0	70.0
Bukový les	660	48.0	0.0	0.0
Železník	660	86.0	13.0	92.0
Valčianska dolina	680	98.0	5.0	84.0
Kohút	680	88.0	7.0	44.0
Oblík	700	99.0	8.0	77.0

Table 3. Occurrence frequency (%) of selected biotic vectors of beech necrotic disease in selected localities in Slovakia – continued

Locality	Altitude [m a.s.l.]	*Cryptococcus fagi*	*Bucculatrix ulmella*	*Ectoedemia liebwerdella*
Veľký Inovec	720	99.0	14.0	91.0
Badínsky prales	760	99.0	15.0	3.0
Veľký Tríbeč	770	99.0	14.0	100.0
Belianska jaskyňa	780	39.0	5.0	0.0
Vysoký vrch	780	97.0	2.0	2.0
Bralce	790	99.0	5.0	89.0
Kyjov	800	99.0	4.0	0.0
Stužica	800	93.0	5.0	0.0
Mláčik	850	80.0	0.0	0.0
Veľká Stožka	880	95.0	11.0	0.0
Sitno	900	94.0	2.0	12.0
Veľká Javorina	940	87.0	0.0	0.0
Strážov	950	47.0	0.0	17.0
Hrončecký grúň	950	33.0	0.0	0.0
Dobročský prales	950	52.0	1.0	1.0
Nemecké lúčky	950	75.0	1.0	0.0
Korytnica	960	79.0	2.0	0.0
Riaba skala	980	2.0	0.0	3.0
Vyšná Roveň	1,000	83.0	0.0	0.0
Veľká Skalná	1,000	13.0	5.0	0.0
Škurátka	1,025	10.0	1.0	0.0
Červená skala	1,050	83.0	0.0	0.0
Malý Polom	1,060	25.0	2.0	0.0
Lomnistá dolina	1,200	93.0	0.0	0.0

Table 4. Occurrence frequency (%) of selected biotic vectors of beech necrotic disease in selected countries of Central and South-Eastern Europe

Country	Locality	Altitude [m a.s.l.]	*Cryptococcus fagi*	*Bucculatrix ulmella*	*Ectoedemia liebwerdella*
Czech Republic	Hukvaldy	450	100.0	17.0	95.0
	Šance	650	100.0	2.0	14.0
	Pustevny	680	99.0	2.0	22.0
Poland	Kiczera	600	100.0	0.0	0.0
	Przysłup	610	99.0	0.0	0.0
	Przelecz Zebrak	825	100.0	0.0	0.0
Hungary	Telkibánya	300	97.0	27.0	52.0
	Diosjenö	500	100.0	19.0	63.0
	Parád	600	99.0	18.0	60.0
	Öserdö	800	100.0	3.0	4.0
Romania	Telciu	420	98.0	0.0	0.0
	Holda	660	90.0	2.0	0.0
	Crucea	690	77.0	3.0	0.0
	Piatra Fântânele	1,020	99.0	1.0	0.0

Table 4. Occurrence frequency (%) of selected biotic vectors of beech necrotic disease in selected countries of Central and South-Eastern Europe – continued

Country	Locality	Altitude [m a.s.l.]	*Cryptococcus fagi*	*Bucculatrix ulmella*	*Ectoedemia liebwerdella*
Serbia	Yavorak	720	100.0	6.0	88.0
	Velka Brezovitsa	900	82.0	4.0	44.0
Bulgaria	Prolaz	300	97.5	10.0	0.0
	Silkosia I	305	62.0	14.0	16.0
	Silkosia II	305	96.0	2.0	56.0
	Boaza	450	65.0	5.0	0.0
	Shumen	450	100.0	12.0	98.0
	Troyan	480	40.0	10.0	97.5
	Vrbitsa	500	100.0	14.0	56.0
	Debravitsa	550	98.0	6.0	2.0
	Shipkovo	650	30.0	10.0	55.0
	Ichera	700	100.0	12.0	90.0
	Kotel	700	100.0	0.0	75.0
	Pravets	700	49.0	62.0	7.0
	Semchinovo	700	98.0	46.0	100.0
	Etropole	720	83.0	25.0	17.0
	Ticha	750	96.0	0.0	92.0
	Oborishte	750	96.0	22.0	68.0
	Fotinski vodopadi	750	56.0	2.0	8.0
	Gorna Bania	900	62.0	2.0	5.0
	Rozovo	900	98.0	4.0	94.0
	Eleshnitsa	900	34.0	0.0	0.0
	Dobra Voda	950	100.0	5.0	98.0
	Vitinya	970	75.0	22.0	97.0
	Breznik	975	44.0	1.0	2.0
	Rilski monastir	975	36.0	0.0	1.0
	Karandila	1,000	100.0	23.0	100.0
	Panagyurishte	1,000	100.0	0.0	100.0
	Shipka	1,000	87.5	0.0	100.0
	Chepino	1,000	68.0	0.0	0.0
	Grashevo	1,000	92.0	0.0	42.0
	Raduil	1,060	78.0	8.0	0.0
	Dragalevski monastir	1,080	50.0	6.0	0.0
	Ribaritsa	1,100	12.0	2.0	2.0
	Barzia	1,150	20.0	0.0	4.0
	Marino	1,150	96.0	4.0	42.0
	Razlog	1,150	97.0	0.0	97.0
	Chaira	1,150	84.0	0.0	0.0
	Ravnogor	1,200	100.0	0.0	38.0
	Yane Sandanski	1,200	100.0	0.0	0.0
	Ossenovo	1,220	86.0	0.0	32.0
	Balkanets	1,250	87.5	0.0	0.0
	Govezhda	1,250	92.0	0.0	0.0
	Velingrad	1,250	94.0	4.0	2.0

Table 4. Occurrence frequency (%) of selected biotic vectors of beech necrotic disease in selected countries of Central and South-Eastern Europe – continued

Country	Locality	Altitude [m a.s.l.]	*Cryptococcus fagi*	*Bucculatrix ulmella*	*Ectoedemia liebwerdella*
Bulgaria	Beklemeto	1,300	88.0	0.0	0.0
	Popovi livadi	1,350	54.0	0.0	0.0
	Rakitovo	1,380	100.0	0.0	4.0
	Aposlovtchark	1,400	66.0	0.0	0.0
	Petrohan	1,400	84.0	0.0	0.0
	Dlgi Del	1,450	8.0	0.0	0.0
	Borovets	1,500	78.0	0.0	0.0
	Tri Buki	1,550	12.5	0.0	0.0

we observed *C. fagi* occurring with a 77% frequency. On the other hand, not in a single locality from the studied ones in Central and South-Eastern Europe, *C. fagi* was absent. This result corresponds well to the fact that several authors consider *C. fagi* as the most important and widest spread species acting as a biotic vector transmitting beech bark necrotic disease.

Bionomy, distribution and importance of *C. fagi* in European forests have been documented by Pfeffer (1954). Lonsdale and Sherriff (1982) isolated from colonies of *C. fagi* conidia of the fungi *Nectria coccinea* (Pers.) Fr., *Nectria viridescens* C. Booth and *Verticillium lecanii* Viegas that may cause beech bark necrosis. Spores of the fungi belonging to the genera *Nectria, Alternaria* Nees and *Verticillium* on bodies of *C. fagi* in conditions in vitro were determined by Suvák (1998). Rojek (2005) refers to *C. fagi* as a biotic vector of beech bark necrotic disease in Poland and Karadžić et al. (2003), Marinković and Karadžić (1985) in Serbia. Chira et al. (2003) describe *C. fagi* in the Romanian Carpathians as the primary vector of beech bark necrotic disease.

From the Lepidoptera, we focussed on two species, *Bucculatrix ulmella* and *Ectoedemia liebwerdella.* The highest, 91% frequency value of *B. ulmella* was found in case of the Slovak locality Žiar nad Hronom. Up to the recent past, the beech stand in this locality was strongly influenced by airborne pollutants. The second highest – 62% frequency value of *B. ulmella* was also observed in Slovakia – locality Litava. The beech stand in this locality was the lowest-situated – at 300 m a.s.l., from all the Slovak localities. The same, 62% frequency, we also found in the submountain beech monoculture in the Bulgarian locality Pravets. From the total number of 45 Slovak localities, 13 were found without occurrence of *B. ulmella*. Most localities in which the butterfly is absent are situated at an altitude exceeding 900 m a.s.l. Situation was similar in Bulgaria where we did not record occurrence of *B. ulmella* in 24 localities from the total number of 50 ones. Most of these sites are situated above 1,100 m a.s.l. (except two localities situated in 1,150 and 1,250 m a.s.l. – identically 4% occurrence of *B. ulmella*). It seems that 900 m a.s.l. in Slovakia and about 1,000 m a.s.l. in Bulgaria are natural altitudinal limits for occurrence of *B. ulmella.* Relatively high frequency values of *B. ulmella* occurrence were also recorded in Hungarian localities. With exception of one locality (Öserdö), in which we observed a 3% frequency value, the other three localities showed values ranging from 18 to 27%. *B. ulmella* generally occurs in oak trees in Hungary (Csóka and Szabóky, 2005). Moreover, this species is also frequent in oak forests in Slovakia (Patočka et al., 1999). For two Czech localities, we obtained 2% and 17%, respectively. Very low frequency values (1 to 3%) were found in Romania. In Southern Poland we have not recorded presence of this moth. The interesting results about occurrence of *B. ulmella* in beech forest stand under strong immission impact in Central Slovakia have been presented by Kulfan et al. (2002).

In case of *E. liebwerdella* we found considerably higher frequency values in comparison with *B. ulmella*. Several values obtained in Slovakia and in Bulgaria reached 100%. Lower than 100% these values were in Czech, Serbian and Hungarian localities. We have found that the upper altitudinal limit for *E. liebwerdella* occurrence in Slovakia is 800 m a.s.l. From the total number of 18 localities situated at and above 800 m a.s.l., we identified four with presence of *E. liebwerdella*. The values obtained for these localities were incomparably lower (representing 1 to 17%) than the values obtained for the localities situated lower than at 800 m a.s.l. The upper altitudinal occurrence limit for this species in Bulgaria is shifted much higher, in comparison with Slovakia – up to the altitude of 1,250 m a.s.l. (32%) and infrequent 1,380 m a.s.l. (only 4% of *E. liebwerdella* occurrence). Relatively high values of *E. liebwerdella* occurrence have been recorded in Serbia – 44% and 88%. The general presence of *E. liebwerdella* in damaged Slovak beech forests attacked by necrosis has been referred by Kodrík and Suvák (1999). These authors implemented the method in vitro and found that this moth is a vector transporting spores of the fungi of the genus *Nectria* (Fr.) Fr. *E. liebwerdella* is considered to be a common species in beech forest stands in Hungary (Csóka and Kovács, 1999) and Slovakia (Kulfan et al., 2011). Without occurrence of *E. liebwerdella* the localities in Romania and in Poland were found. This, however, does not mean that this butterfly does not occur in these countries. This fact has also been confirmed with the most recent data about its occurrence in beech stands in boundary areas in the Polish Sudeten Mts (Borkowski and Konca, 1991). The former occurrence of *E. liebwerdella* in Poland has also been confirmed with an ancient finding determined in the locality Jesienia in 1947 by Adamczewski (in Schönherr, 1958). Rojek (2005) assigns to this butterfly a special importance in association with beech bark necrotic disease. Occurrence of the relative species *Ectoedemia albifasciella* (Heinemann) and *E. heringii* (Toll) has been reported by Stolnicu (2007) in Eastern Romania and from the near Moldavia.

It is evident, that the interval of altitudinal zone suitable for living and occurrence of particular Lepidoptera species is very important not only for conditions in Central Europe, as well as for ones in South-Eastern Europe. For example, Kulfan (1990) observed that highest index of species diversity of butterflies (4.96) has been recorded in the localities situated in the valleys and foothills of the Malé Karpaty Mts in western Slovakia. On the other hand, the lowest index of ones (2.91) has been recorded in the localities situated on higher altitudes on the mountain ridges. The vertical gradient is very important for composition of butterfly fauna. This fact was confirmed by Kulfan and Kulfan (1997), who observed that from 118 species of butterflies recorded in the Malá Fatra Mts in northern Slovakia, total 94 species of ones have been found on various biotopes situated up to 500 m a.s.l. On the contrary, in the mountain biotopes, from 1,000 m a.s.l., only 8 species of butterflies occurred steady. The decreasing of butterflies fauna on the biotopes situated in higher altitudes, opposite to the biotopes situated in lowest altitudes, is the native phenomenon, which is typical mainly for higher mountains.

On the other hand, the situation of occurrence and spreading of butterflies within the conditions in South-Eastern Europe seems to be different from conditions in Central Europe. For example, van Swaay et al. (2007) have been studied lepidopterofauna in 9 various localities in eastern Serbia. Total 117 species of butterflies have been determined, only 25 species of ones have been recorded in the localities situated in 400–420 m a.s.l. in the Rtnaj Mts. On the contrary, the most species of butterflies (61) have been recorded in the locality situated from 980 up to 1,600 m a.s.l. in the Stara planina Mts. Moreover, in the Croatian Karst in Dalmacia the species diversity of butterflies has been investigated by Mihoci et al. (2011). The researched localities have been situated on vertical gradient, from coast of the Adriatic Sea to the elevated plateau in the inland. The authors found that in the localities situated from 0 up to 500 m a.s.l. the index of species diversity of butterflies was the lowest (0.84), in the localities situated from 500 up to 1,000 m a.s.l. the index of ones was 0.86 and within the zone up to 1,000 m a.s.l. index was the highest (0.92).

By the comparing these data obtained from Slovakia (Central Europe) and from Croatia and Serbia (Southern Europe) we can see that the climatic conditions in the continental Central Europe are supporting the declining of species diversity of butterflies towards the higher altitude biotopes. On the other hand, the butterflies occurred abundantly and steady also on the biotopes situated in higher altitudes in the climatic more suitable areas in South-Eastern Europe, in the Balkan Peninsula and Mediterranean area generally, probably due to high temperature and more suitable climatic conditions in the higher situated localities.

Other important and potential biotic vectors spreading beech bark necrotic disease are listed in the Table 5. The least investigated vectors include the species of the Acarina order. Other species from other groups, e.g. *Fagocyba cruenta* (Herrich-Schäffer), *Phyllapsis fagi* L. and *Mikiola fagi* (Hartig) have been indicated as important pests of beech by Hartmann et al. (1995) and Strout and Winter (1994). Butterflies and moths represent a significant group of biotic vectors for beech bark necrotic disease. Masarovičová et al. (1999) mention a massive occurrence of caterpillars of the butterfly *Calliteara pudibunda* (L.) in a beech stand seriously attacked by necroses. The stand has been, moreover, heavily loaded with airborne pollution of fluorine type. The high degree of necrotic damage to this stand has also been confirmed by Cicák and Mihál (2002). In the same stand, Šušlík and Kulfan (1993) observed a high abundance of caterpillars of the species *Ennomos quercinaria* (Hufnagel), *Agriopsis aurantiaria* (Hübn.) Regular at Kinloch and *Erannis defoliaria* Cl. Topp et al. (1998) mention a massive occurrence of the species *Operophthera fagata* Scharf. and *Erannis defoliaria* Cl. in beech forests in surroundings of the Rhine River in Germany. A potential vector from Lepidoptera can also be *Argyresthia semitestacella* Curt. Little known data on occurrence and bionomy of this species can be found in Patočka (1998). The author reports occurrence of this species in submountain beech stands. The species was locally abundant, and the author considers it to be a local pest and vector of fungal diseases of beech.

Another significant group of pests of beech and of biotic vectors of beech diseases consists of beetles. Table 5 gives a list of species living in tree bark or directly in tree wood mass, and can also be classified

Table 5. Overview of significant and potential biotic vectors of beech necrotic disease of tracheomycotic type by selected literary sources

Order	Family	Species	Literary source
Acarina	Eriophyidae	*Eriophyes stenaspis* Nal.	Hartmann et al. (1995), Pfeffer (1954), Szmidt (1990)
Homoptera	Cicadellidae	*Fagocyba cruenta* (Herrich-Schäffer)	Hartmann et al. (1995)
	Coccidae	*Cryptococcus fagi* Bärensp.	Chira et al. (2003), Hartmann et al. (1995), Jurášek and Vacek (1983), Krabel and Petercord (2000), Kulfan et al. (2011), Lonsdale and Sheriff (1982), Lunderstädt (1998), Pfeffer (1954), Rojek (2005), Strout and Winter (1994), Suvák (1998), Szmidt (1990)
Sternorrhyncha	Callophididae	*Phyllapsis fagi* L.	Hartmann et al. (1995), Kulfan et al. (2011), Strout and Winter (1994), Szmidt (1990)
Diptera	Cecidomyiidae	*Hartigiola annulipes* (Hartig)	Hartmann et al. (1995), Kulfan et al. (2011), Strout and Winter (1994)
		Mikiola fagi (Hartig)	Hartmann et al. (1995), Kulfan et al. (2011), Strout and Winter (1994), Szmidt (1990)
Lepidoptera	Bucculatricidae	*Bucculatrix ulmella* Zeller	Csóka and Szabóky (2005), Kulfan et al. (2002, 2011), Stolnicu (2007), Šušlík and Kulfan (1993), Topp et al. (1998)
	Geometridae	*Operophthera fagata* Scharf.	Kulfan et al. (2011), Szmidt (1990), Šušlík and Kulfan (1993), Topp et al. (1998)
	Lymantriidae	*Calliteara pudibunda* (L.)	Kulfan et al. (2011), Masarovičová et al. (1999), Šušlík and Kulfan (1993), Topp et al. (1998)
	Nepticulidae	*Ectoedemia liebwerdella* Zim.	Borkowski and Konca (1991), Csóka and Kovács (1999), Kodrík and Suvák (1999), Kulfan et al. (2011), Rojek (2005)
	Yponomentidae	*Argyresthia semitestacella* Curt.	Kulfan et al. (2011), Patočka (1998), Topp et al. (1998), Šušlík and Kulfan (1993)
Hymenoptera	Ichneumonidae	Erannis defoliaria Cl.	Hartmann et al. (1995), Kulfan et al. (2011), Pfeffer (1954), Šušlík and Kulfan (1993), Topp et al. (1998)
	Tenthredinidae	*Caliroa annulipes* (Klug.)	Kulfan et al. (2011), Strout and Winter (1994)
Coleoptera	Buprestidae	*Agrillus viridis* (L.)	Jančařík (1992), Surovec and Novotný (1985)
	Curculionidae	*Rhynchaenus fagi* L.	Hartmann et al. (1995), Innes (1992), Strout and Winter (1994), Szmidt (1990)
	Lymexylidae	*Hylecoetus dermestoides* L.	Hartmann et al. (1995), Surovec and Novotný (1985), Szmidt (1990)
	Scolytidae	*Ernoporicus fagi* (Fabr.)	Surovec and Novotný (1985)
		Taphrorychus bicolor (Herbst.)	Jančařík (1992), Surovec and Novotný (1985), Zach et al. (2002)
		Xylosterus domesticus L.	Carlier et al. (2006), Surovec and Novotný (1985), Szmidt (1990)

As other biotic vectors of beech necrotic disease can also be considered scale insects (*Pulvinaria* sp., *Phenacoccus aceris* Signoret), ants (Formicidae), birds (Certhiidae, Picidae, Sittidae), mammals (Rodentia, Artiodactyla) and, last but not least, the human itself too.

as important ones. Surovec and Novotný (1985) regard these species as important biotic vectors transmitting beech tracheomycoses in Slovakia. In a similar way, Jančařík (1992) discussing the species *Taphrorychus bicolor* (Herbst.) and *T. villifrons* Duffaeur, does not exclude the possibility of transport of spores of fungi belonging to the genus *Ophiostoma*. Potential vectors of beech bark necrotic disease can also be almost all the species of the family Scolytidae, whose life cycle is associated with wood (Zach et al., 2002). During our research on beech bark necrosis in Slovakia, we found occurrence of the species *Phyllobius argentatus* L. and *Xyloterus domesticus* L. attacking beech trees with severe necrotic symptoms. Carlier et al. (2006) observed in species *Xyloterus domesticus* and *X. signatus* transport of spores of *Ophiostoma arduennense* Carlier, Decock, K. Jacobs & Maraite, sp. nov.

Apart from xylophages, transmission of beech bark necrotic disease is also shared by phylophagous beetles. An important representative of this group is *Rhynchaenus fagi*, in outbreaks causing impaired health stand of beech stands. Innes (1992) suggests that there are links between changes in leaf colouring (chlorosis – yellowing) and leaf necrosis (browning) on one and synergic effect of *R. fagi* and fungus *Nectria ditissima* Tul. et C. Tul. on the other side. Probability of conidia transport of the species *Nectria galligena* Bres. sensu Strasser by leaf-eating insects, e.g. *R. fagi*, has been evaluated by Downing (1989).

The obtained results concerning occurrence of the discussed insect species suspected to act as biotic vectors of beech bark necrotic disease in selected countries of Central and South-East Europe reveal the following facts:

- Massive occurrence of the beech lice *Cryptococcus fagi*, in all the studied localities and countries, sometimes reaching frequency value of 100%. The occurrence of beech lice was not limited by altitude.
- Increased occurrence of *Ectoedemia liebwerdella,* except Poland and Romania. In several cases, the frequency reached 100%. In contrast to *C. fagi*, the occurrence of *E. liebwerdella* was limited by altitude. The upper limit in Slovakia is 800 m a.s.l., in Bulgaria it is 1,220 m a.s.l. (32%) and infrequent also in 1,380 m. a. s. l. (only 4%).
- Increased occurrence of *Bucculatrix ulmella*, except Poland. The maximum frequency value (91%) was obtained in Slovakia, in a locality strongly influenced by fluorine airborne pollutants in the past. The maximum frequency value of occurrence of *B. ulmella* in Bulgaria was 62%. The obtained frequency values show that the upper occurrence limit for this species is 900 m a.s.l. in Slovakia and 1,250 m a.s.l. in Bulgaria.

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Effect of altitude on phenology of selected forest plant species in Slovakia (Western Carpathians)

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Abstract

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Phenological response of selected forest plant species to different ecological conditions along the altitudinal gradient were studied during the period of 5 years (2007–2011). Leafing as well as flowering phenophases were investigated within two herb species (*Dentaria bulbifera and Galium odoratum*) and three shrubs (*Prunus spinosa, Rubus idaeus* and *Sambucus nigra*), respectively. Altitudinal gradient was expressed by different altitude sites situated in Burda Mts (200 m a.s.l.), in Kremnické vrchy Mts (500 m a.s.l.) and in Poľana Mts (≥900 m a.s.l.) belonging to Western Carpathians. On average, the earliest onset of the phenophases was found in the low-lying sites and vice versa. For vegetative phenophases, phenological gradient expressing the shift in onset of the phenophases along the gradient reached the mean values from +2.7 to +3.6 days per 100 m. In the case of generative phases (flowering) within the species, the gradient moved from +3.0 to +4.3 days per 100 m of an altitude increase. Interannual variability in onsets of phenophases was also determined.

Keywords

Dentaria bulbifera, *Galium odoratum*, flowering, leafing, *Prunus spinosa, Rubus idaeus, Sambucus nigra,* vertical phenological gradient

Introduction

Recently, the phenology standing at the edge of ecology and meteorology has been a dynamically developing science. The current changes (local and global) in the environment influence the life processes of the individuals as well as entire ecosystems. In terms of phenology, these changes are observed at the species level – species-level phenology, and at the ecosystem level – ecosystem- and global-scale phenology (Maak and Storch, 1997; Totland and Alatalo, 2002; Zhang et al., 2003; Badeck, 2004; Bednářová and Merklová, 2007; Inouye, 2008; Škvareninová et al., 2008; McEwan et al., 2011). In addition to exogenous factors such as increase in average air temperature, precipitation regime change, the increase of CO_2 concentration, also internal – biological factors, e.g. genetic variability, play an important role in the phenological response of the species (Chesnoiu et al., 2009). The initial development of the plants in spring is related to the development of the assimilation system. This period is crucial for its subsequent successful growth. Late frosts often damage leaves and plant must expend more energy to regenerate itself again. If such events occurred frequently, fatal consequences for populations of several species would be observed (Hufkens et al., 2012). Flowering is a part of plant life, when the preconditions for its generative reproduction are created. This is the basis of genetic diversity, which plays an important role in the adaptation of species to changing environmental conditions. It is known that in areas of the temperate zone most of the plant species blooms in spring or in early-summer. The beginning of flowering signalises the onset of some daily mean temperature (DMT), which can be defined as the onset of growing season, e.g. flowering of blackthorn and linden signalises the onset of DMT 10 °C and DMT 15 °C, respectively. Weather course varies considerably during the year at the temperate zone (Fitter et al., 1995; Tyler, 2001; Lu et al., 2006). This variability is related to variability in the onset and course of the phenophases. It can be found at particular species during the year. The reason for the variability may be the different phytoclimate within the same stand, or the different bioclimatic conditions along the vertical gradient (Schieber, 1996, 2008, 2013). Shift (positive or negative) in the onset of phenophases along the gradient indicates indirectly the differences in environmental conditions.

There is relatively little information concerning plant phenology along the altitudinal gradient in the literature (Levesque et al., 1997; Blionis et al., 2001; Dittmar and Elling, 2006; Giménez-Benavides et al., 2007; Ziello et al., 2009; Schieber, 2013; Schuster et al., 2013). However, the results of these studies can be used in prognosis of the physiological responses of plants to changing environmental conditions due to ongoing climate change, which can already interfere significantly with the functioning of forest ecosystems.

Therefore the aim of this paper is to assess the variability in the onset and course of selected vegetative and generative phenological phases – leafing and flowering of five widespread species in forest ecosystems along the altitudinal gradient in South and Middle Slovakia.

Material and methods

Phenological research was conducted on five species (*Dentaria bulbifera* L., *Galium odoratum* (L.) Scop., *Prunus spinosa* L., *Rubus idaeus* L. and *Sambucus nigra* L.) in forest ecosystems in three neo-volcanic mountains located south of the main line of the climate in Slovakia – Burda Mts, Kremnické vrchy Mts and Poľana Mts (Fig. 1). The Burda Mts. lie in the south-eastern corner of the lower Danube Plain between the rivers Danube, Hron and Ipeľ and their height is relatively little differentiated (113–405 m a.s.l.). The area belongs to the warm climate, dry district with mild winter. Observations were done at site located north of the village of Chľaba (Helemba). The locality is situated at the beginning of the Veľká dolina valley (200 m a.s.l.). The Kremnické vrchy Mts are situated in the central part of Slovak Republic. Phenological observations were carried out on an Experimental Ecological Stationary Kremnické vrchy Mts (500 m a.s.l.), which is located in the SE part of the above orographic unit. The locality belongs to the temperate climate zone, slightly damp to wet district. The Poľana Mts are the highest volcanic mountain range in Slovak Republic. Southern foothills of the Poľana Mts belong to the temperate climate, humid district. The other mountain territory is classified into a cold climate zone, slightly cool to cold mountain district (Lapin et al., 2002). Observations were done at five localities. The lowest-lying area, within this mountain chain, is situated above the village of Priehalina (900 m a.s.l.), the second location is Javorinka (1,000 m a.s.l.). The other sites are located at Huklová (1,200 m a.s.l.), Predná Poľana (1,300 m a.s.l.) and Zadná Poľana (1,400 m a.s.l.). Supplemental characteristics of research sites are given in Table 1.

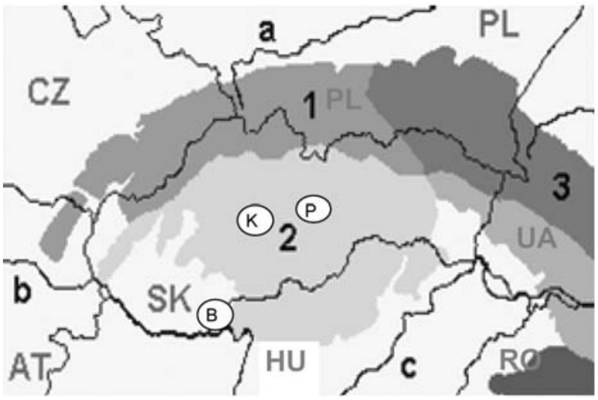

Fig. 1. Location of the research plots in Slovak Republic (B, Burda Mts; K, Kremnické vrchy Mts; P, Poľana Mts; 1, Outer Western Carpathians; 2, Inner Western Carpathians; 3, Outer Eastern Carpathians; a, Vistula River; b, Danube River; c, Tisza River).

Methodology of observations is based on the methodological prescription for phenological observation used in phenological monitoring by Slovak Hydrometeorological Institute (SHMI) Bratislava (Braslavská and Kamenský, 1996). Phenophases were defined according to the BBCH scale (Hess et al., 1997). Observations were conducted weekly during the growing season for five years (2007–2011). For herbaceous species, there were observed vegetative and generative phenophases: germination (BBCH 09), first leaves (BBCH11), first flowers (BBCH60) and flowering 50% (BBCH65). In the case of (semi-)shrubs the following phenophases were evaluated: first leaves (BBCH11), three leaves (BBCH13), first flowers (BBCH60) and flowering 50% (BBCH65). The onset of phenophases was expressed as a sequence of days counted from 1st January – day of year (DOY). Climate data were taken from the databases of SHMI and Hungarian National Meteorological Service and supplemented by own measurements (smart sensor Minikin, Mini32 software, EMS Brno, Czech Republic).

Results

Vegetative phenophases

Onset of vegetative phenological phases for all species along the altitudinal gradient in the period 2007–2011 is described in Tables 2–3. As for herb species *Dentaria bulbifera*, the average onset of the both BBCH09 and BBCH11 phenophases was recorded on 75th and 81st day of the year (DOY), i.e. 15th March and 21st March at the lowermost site (200 m a.s.l.), respectively. Onset of the phenophases delayed progressively with increasing altitude, the 39 days delay (i.e. 24th April and 30th April) was recorded at the highest altitude (1,300 m a.s.l.). Phenological response of *Galium odoratum* species was similar, the average onset of both BBCH09 and BBCH11 phenophases was recorded on 102nd and 110th DOY, i.e. 12th April and 20th April at the altitude of 500 m a.s.l., respectively. The 22–23 days delay (i.e. 4th May and 13th May) was recorded at the highest altitude (1,300 m a.s.l.). The average length of the interval between both BBCH09 and BBCH11 phenophases varied within the range of 4.8–6.0 days and 7.4–9.4 days for *Dentaria bulbifera* and *Galium odoratum*, respectively.

Table 1. Characteristics of research sites along the altitudinal gradient

Localities	Burda Mts	Kremnické vrchy Mts	Poľana Mts				
Altitude [m a.s.l.]	200	500	900	1,000	1,200	1,300	1,400
Exposition	NE	WSW	SE	SE	E	SE	SW
AAT $_{1951–1980}$ [°C]	9.3	6.9	5.6	5.1	4.0	3.4	2.9
AT$_{IV.-IX.1951–1980}$ [°C]	15.9	13.6	11.6	11.0	10.1	9.5	8.8
AAP$_{1951–1980}$ [mm]	600	780	890	930	1,040	1,100	1,160
AP$_{IV.-IX.1951–1980}$ [mm]	355	420	500	530	575	610	640

AAT, average annual air temperature; AT$_{IV.–IX.}$, average air temperature IV.–IX.; AAP, average annual precipitation; AP$_{IV.–IX.}$, average precipitation IV.–IX.

Table 2. The onset of vegetative phenophases of herb species at different altitudes

Altitude [m a.s.l.]		200		500		900		1,000		1,200		1,300	
Phenophase (BBCH)		09	11	09	11	09	11	09	11	09	11	09	11
Mean$_{2007–2011}$ [DOY]	*Dentaria bulbifera*	75	801	87	92	96	101	100	105	109	115	114	120
	Galium odoratum	–	–	102	110	110	118	114	121	119	128	124	133
SD [± days]	*Dentaria bulbifera*	3.9	4.8	5.9	5.8	4.4	4.3	4.7	4.7	4.2	3.5	3.8	3.4
	Galium odoratum	–	–	5.0	4.4	3.9	4.3	4.4	5.2	2.9	4.3	4.2	4.2
CV [%]	*Dentaria bulbifera*	5.2	6.0	6.8	6.3	4.6	4.2	4.7	4.5	3.8	3.1	3.3	2.9
	Galium odoratum	–	–	4.9	4.0	3.6	3.6	3.9	4.3	2.4	3.4	1.5	3.2

DOY, day of year; SD, standard deviation; CV, coefficient of variation; BBCH09, emergence: shoot/leaf breaks through soil surface; BBCH11, first true leaf / leaf pair of whorl unfolded.

Table 3. The onset of vegetative phenophases of shrub species at different altitudes

Altitude [m a.s.l.]		200		500		900		1,000		1,200		1,300		1,400	
Phenophase (BBCH)		11	13	11	13	11	13	11	13	11	13	11	13	11	13
Mean$_{2007–2011}$ [DOY]	*Prunus spinosa*	105	113	121	126	–	–	–	128	136	–	–	–	–	–
	Rubus idaeus	–	–	103	117	115	127	118	130	127	138	132	143	140	149
	Sambucus nigra	–	–	103	130	118	140	122	145	–	–	–	–	–	–
SD [± days]	*Prunus spinosa*	1.9	2.3	5.7	3.6	–	–	6.2	5.9	–	–	–	–	–	–
	Rubus idaeus	–	–	1.9	1.7	2.0	2.3	1.7	2.1	2.8	4.3	3.7	5.1	4.0	5.4
	Sambucus nigra	–	–	2.3	6.7	4.0	5.5	4.7	6.0	–	–	–	–	–	–
CV [%]	*Prunus spinosa*	1.8	2.1	4.7	2.9	–	–	4.9	4.3	–	–	–	–	–	–
	Rubus idaeus	–	–	1.8	1.5	1.7	1.8	1.5	1.6	2.2	3.1	2.8	3.6	2.9	3.6
	Sambucus nigra	–	–	2.2	5.1	3.4	3.9	3.9	4.1	–	–	–	–	–	–

DOY, day of year; SD, standard deviation; CV, coefficient of variation; BBCH 11, first true leaf unfolded; BBCH 13, three true leaves.

Vertical phenological gradient, which reflects the difference in onset of the phenophases among the sites calculated to 100 meters of altitude increase, reached the values of 3.5 (for BBCH09) and 3.6 (for BBCH11) in the case of *Dentaria bulbifera*. As for *Galium odoratum*, the gradient reached the values of 2.7 and 2.9 days per 100 m, respectively (Fig. 2).

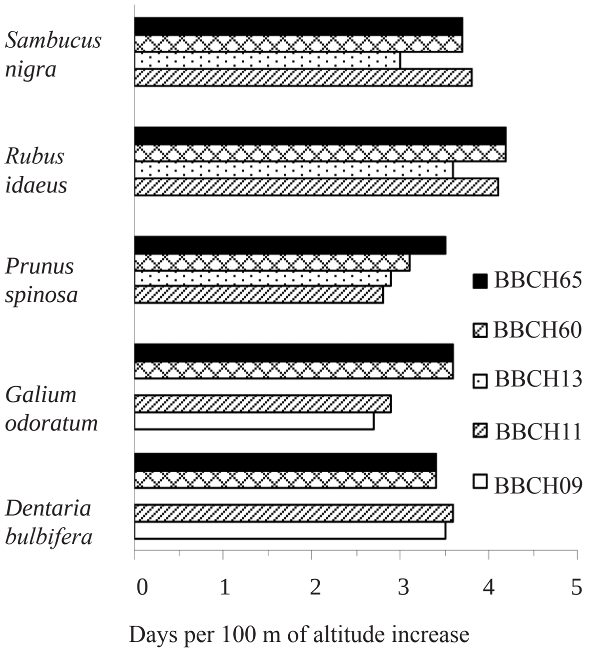

Fig. 2. Average shifts of the onset of phenophases along the altitudinal gradient during the years 2007–2011. BBCH09–BBCH65, see Tables 2–5.

On average, the onset of both BBCH11 and BBCH13 phenophases of *Prunus spinosa* was recorded on 105th and 113rd DOY (i.e. 15th April and 23rd April) at the lowermost site (200 m a.s.l.), respectively. Onset of these phenophases delayed with increasing altitude along the altitudinal gradient, the 23 days delay (i.e. 8th May and 16th May) was recorded at the altitude of 1,000 m a.s.l. As for the other shrub species, the onset of vegetative phenophases for *Rubus idaeus* was observed on 103rd and 117th DOY, for *Sambucus nigra* it was on 103rd and 130th DOY, respectively. The average length of the interval between onset of both BBCH11 and BBCH13 phenophases varied within the range of 5.4–8.0 days and 22.2–26.6 days for *Prunus spinosa* and *Sambucus nigra*, respectively. Vertical phenological gradient for shrub species moved from 2.8–2.9 days (*Prunus spinosa*) to 3.6–4.1 days (*Rubus idaeus*) per 100 m of altitude increase (Fig. 2).

Dating the vegetative phenophases varied not only depending on the altitude, but also between the compared years. For herb species, the smallest year to year variability in the onset of phenophases was found out within the higher mountain ranges (>1,200 m a.s.l.), on the other hand, higher variability was observed at the lower sites (<500 m a.s.l.). As for shrub species, the variability increased with the altitude increasing in the case of *Prunus spinosa*. The variability in onset of phenophases of the other shrubs was relatively stable along the gradient, only for *Rubus idaeus* higher variability at the altitudes >1,200 m a.s.l. was recorded.

Generative phenophases – flowering

Onset and course of generative phenophases (flowering) of the species along the gradient is described in Tables 4–5. The average onset of both BBCH60 and BBCH65 phenophases of *Dentaria bulbifera* was recorded on 108th and 114th day of the year (DOY), i.e. 18th April and 24th April at the lowermost site (200 m a.s.l.), respectively. Increasing altitude caused the delay of the onset of the phenophases, the 37–38 days delay (i.e. 26th May and 31st May) was recorded at the highest altitude (1,300 m a.s.l.). As for *Galium odoratum* species, the average onset of the phenophases was recorded on 125th and 132nd DOY, i.e. 5th May and 12th May at the altitude of 500 m a.s.l., respectively. The 28–29 days delay (i.e. 2nd June and 10th June) was recorded at the highest altitude (1,300 m a.s.l.). The average length of the interval between both BBCH60 and BBCH65 phenophases, which represents the dynamics of flowering of plants along the gradient, varied within the range of 4.8–6.0 days and 6.6–8.2 days for *Dentaria bulbifera* and *Galium odoratum*, respectively.

In the case of *Dentaria bulbifera*, vertical phenological gradient reached the same values of 3.4 days for both BBCH60 and BBCH65 phenophases, for *Galium odoratum* it was similar, the gradient reached the values of 3.6 days per 100 m (Fig. 2).

On average, the onset of both BBCH60 and BBCH65 phenophases of *Prunus spinosa* was recorded on 96th and 100th DOY (i.e. 6th April and 10th April) at the altitude 200 m a.s.l., respectively. The shift in onset along the gradient was 24–28 days (i.e. 1st May and 7th May) at the altitude of 1,000 m a.s.l. The onset of generative phenophases for *Rubus idaeus* was observed on 145th and 151st DOY, for *Sambucus nigra* it was on 145th and 157th DOY, respectively. The average length of the interval between both BBCH60 and BBCH65 phenophases varied within the range of 3.4–6.6 days and 11.4 days for *Prunus spinosa* and *Sambucus nigra*, respectively. Vertical phenological gradient for shrub species moved from 3.0–3.5 days (*Prunus spinosa*) to 4.2–4.3 days (*Rubus idaeus*) per 100 m of altitude increase (Fig. 2). Interannual variability in onset of generative phenophases was also found. As for herb species, the smallest year to year variability in onset of generative phenophases was found out within the lowest site (200 m a.s.l.) with tendency of its increasing to the altitude 900 m a.s.l. Variability at higher altitudes was

Table 4. The onset of generative phenophases of herb species along the altitudinal gradient

Altitude [m a.s.l.] Phenophase (BBCH)		200		500		900		1,000		1,200		1,300	
		60	65	60	65	60	65	60	65	60	65	60	65
Mean$_{2007–2011}$ [DOY]	*Dentaria bulbifera*	108	114	115	120	129	135	138	144	143	148	146	151
	Galium odoratum	–	–	125	132	135	144	143	149	150	157	153	161
SD [± days]	*Dentaria bulbifera*	2.3	3.7	3.9	4.3	5.9	6.7	5.0	5.1	5.3	5.3	6.3	5.6
	Galium odoratum	–	–	4.8	3.3	5.1	6.2	5.7	6.4	5.4	5.3	5.4	6.1
CV [%]	*Dentaria bulbifera*	2.1	3.3	3.4	3.6	4.6	4.9	3.6	3.5	3.7	3.6	4.3	3.7
	Galium odoratum	–	–	3.8	2.5	3.8	4.3	4.0	4.	3.6	3.4	3.5	3.8

DOY, day of year; SD, standard deviation; CV, coefficient of variation; BBCH60, first flowers open; BBCH65, full flowering: 50% of flowers open.

Table 5. The onset of generative phenophases of shrub species along the altitudinal gradient

Altitude [m a.s.l.] Phenophase (BBCH)		200		500		900		1,000		1,200		1,300		1,400	
		60	65	60	65	60	65	60	65	60	65	60	65	60	65
Mean$_{2007–2011}$ [DOY]	*Prunus spinosa*	96	100	110	114		121	127	–	–	–	–	–	–	–
	Rubus idaeus	–	–	145	151	159	167	164	171	172	178	176	182	183	189
	Sambucus nigra	–	–	145	157	158	170	164	175	–	–	–	–	–	–
SD [± days]	*Prunus spinosa*	4.0	3.7	5.3	5.0	–	–	6.5	7.1	–	–	–	–	–	–
	Rubus idaeus	–	–	5.3	5.2	5.3	5.7	4.8	4.5	6.0	4.5	5.9	4.3	4.0	3.4
	Sambucus nigra	–	–	4.7	5.8	4.3	3.9	5.4	3.5	–	–	–	–	–	–
CV [%]	*Prunus spinosa*	4.1	3.7	4.8	4.4	–	–	5.4	5.5	–	–	–	–	–	–
	Rubus idaeus	–	–	3.6	3.4	3.4	3.4	2.9	2.6	3.5	2.5	3.4	2.3	2.2	1.8
	Sambucus nigra	–	–	3.2	3.7	2.7	2.3	3.3	2.0	–	–	–	–	–	–

DOY, day of year; SD, standard deviation; CV, coefficient of variation; BBCH60, first flowers open; BBCH65, full flowering: 50% of flowers open.

relatively stable. Interannual variability in flowering of *Prunus spinosa* increased with the altitude increasing, but the other shrubs flowered relatively stable along the gradient.

Discussion

Onsets of phenophases varied depending on the altitude, but also between the compared years. It is known that the temperature is the main factor affecting the dynamics of the plant development. Our investigations revealed that the earliest onset of all phenophases was observed in the lowest altitudes and vice versa. This fact was affected by decreasing in temperature due to the increasing altitude. Considering that the vertical temperature gradient in spring reached –0.6 °C per 100 m of altitude increase (Fig. 3), the drop in temperature of 1 °C was associated with delayed onset of 5.7–6.0 days and 4.5–6.0 days for *Dentaria bulbifera* and *Galium odoratum*, respectively. The delay in onset of vegetative and generative phenophases within the shrubs reached the following values: 4.6–5.8 days for *Prunus spinosa*, 5.0–6.3 days for *Sambucus nigra* and 6.0–7.2 days for *Rubus idaeus* per 1 °C of temperature decrease. These results correspond to those reported by Vitasse et al. (2009) or Cornelius et al. (2013). Kurpelová (1972) stated phenological gradient of 3 days and 5 days per 100 m along the elevation gradient in central Slovakia within the blackthorn and elder phenophases, respectively.

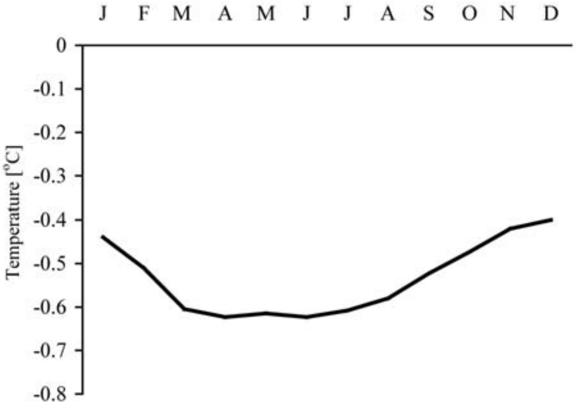

Fig. 3. Average temperature gradient (per each 100 m) throughout the year in the period 2007–2011.

Blionis et al. (2001) found a shift from 2 to 3 days per 100 m of altitude increase in flowering of different species of the genus *Campanula* along the vertical gradient in the mountain areas. Melo (2006) reported that blossoming of lime tree was delayed on average of 4.1 days per 100 m of altitude increase in various localities in Slovakia. Remišová and Vinceová (2007) evaluated the first flowering of European hazel during the period of 20 years in Slovakia. They found the delay of 3 days per 100 of altitude increase. Pellerin et al. (2012) determined for the trees in the Alps the delays of spring phenophases ranging from 2.4 to 3.4 days per 100 m.

Interannual variability in onset of vegetative phenophases for herb species was the smallest within the higher mountain ranges (>1,200 m a.s.l.), on the other hand, higher variability was observed at the lower sites (<500 m a.s.l.). As for shrub species, the variability in onset of vegetative phenophases increased with the altitude increasing. Interannual variability in onset of generative phenophases was slightly different. As for herb species, the smallest year to year variability in onset of generative phenophases was found out within the lowest site (200 m a.s.l.) with tendency of its increasing to the altitude of 900 m a.s.l. Variability at higher altitudes was relatively stable. Interannual variability in flowering of *Prunus spinosa* increased with the altitude increasing, but the other shrubs flowered relatively similarly along the gradient. Based on the above findings, it seems that climate variability did not affect onsets of the phenophases of herbal species so significantly at high-altitude environments (>1,200 m a.s.l) compared to low-lying sites. Likewise, the onset of generative phenophases for *Rubus idaeus* was associated with the lowest variability at the highest altitude (1,400 m a.s.l.).

Increased attention is devoted to study how changes in environmental conditions are caused by global changes, which are classified as climate changes. Impact of the increasing volatility of the main climatic elements running throughout the year occurs subsequently to different phenological response. For a more objective understanding of these processes, it is therefore important to know the response of plants on the edges of their existence, for example along the vertical gradient. The results of phenological studies on plants may contribute to the expansion of knowledge regarding their phenological responses as bio-indicators of changing environmental conditions.

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Accumulation of C_t and N_t in humus and mineral soil layers: the effect of change of tree species composition in nudal beech forests

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Abstract

Šimková, I., Kuklová, M., Kukla, J. 2014. Accumulation of C_t and N_t in humus and mineral soil layers: the effect of change of tree species composition in nudal beech forests. *Folia oecol.*, 41: 82–91.

The effect of change of tree species composition on the content of carbon and nitrogen in surface humus and mineral soil layers was studied in the Kremnické vrchy Mts, in the phytocoenoses of forest type group *Fagetum pauper*. Research was conducted in six forest stands, which represented the natural mature beech stand and five changed stands – birch, spruce, larch, pine and beech pole stage. The research results showed that change of tree species composition had a partial influence on the content of C_t and N_t in soil layers of analyzed phytocoenoses. It was reflected the most in surface humus and topsoil 0–5 cm layers. The highest C_t content was found in surface humus of coniferous stands; in the case of N_t it was in humus horizons of deciduous trees. In the mineral soil layers significantly higher contents of C_t and N_t were found in coniferous stands, in comparison with deciduous stands. Forest stands with changed tree species composition showed a higher content of studied elements in humus horizons as well as in mineral soil layers. In deciduous stands higher C/N ratio was found in mineral layers in comparison with coniferous stands. Stands with changed tree species composition had higher C / N ratio in the subhorizons Ool, and towards to the depth profiles it decreased. Statistically significant differences were found out only in the 0–5 cm soil layers.

Keywords

cambisols, carbon, nitrogen, nudal beech forest, tree species edificator

Introduction

Trees are the basic attribute of the forest communities. They produce the main part of the phytomass. It is organic matter – dropped leaves and needles, which has very important role as natural fertilizer and it is significant for forest existence and for forest soil (Šály, 1988). When dropped leaves and needles die back, the decomposition process begins. Decomposition is very important in the biochemical nutrient cycle, where nutrients are mineralized and provided to vegetation again. Speed of decomposition and nutrient release from organic material are influenced by climate conditions and quality of litter fall (Vesterdal et al., 1995). This fact is reflected in the properties of surface humus and upper soil layers (Binkley and Valentine, 1991; Binkley, 1995; Neirycnk et al., 2000; Augusto et al., 2002, Thorn et al., 2004).

Carbon and nitrogen contents belong to the most important macroelements that determine fertility of the soils (Johnson and Curtis, 2001). Content of these two elements has attracted the attention of many authors (Johnson and Curtis, 2001; Berger et al., 2002; Jandl et al., 2007; Menšík et al., 2009; Guenet et al., 2013). In general the amount of carbon contained in soil is twice higher than in the atmosphere, and three times higher than in the vegetation (Schimel, 1995; Guenet et al., 2013). That is the reason why the soil environment is considered as the most important reservoir of carbon, because it is very dynamic and it belongs to the most significant environment in the existing carbon cycles (Bielek, 2007).

Nitrogen as the most important macroelement is in N_2 form in the highest amount contained in atmosphere (78%). N_2 form is unavailable for plants. Exceptions are plants, which have symbiotic relationship with nitrogenfixing microorganisms. Primary organisms can receive nitrogen in the nitration form (NO_3), or in ammonium salts (NH_4NO_3). Content of these compounds is the result of the biological process in soil (Pelíšek, 1964). In forest ecosystems nitrogen is considered to be one of the limited factors and it has impact on the primary production. Lack of N in the soil is reflected with stunted growth of plants, light green leaves and premature leaves shedding. On the other hand, excess of nitrogen prolonged period of shoot growth until the frosts that deplete immature shoots (Kuklová et al., 2011).

Nitrogen and carbon content is determined by the difference between input and output speed from soil. Therefore tree species are considered to be one of the significant factors that manage the rate of biochemical processes in the soils (Vesterdal et al., 2008; Augusto et al., 2002). With this background, the present study was undertaken with the objective to compare the effect of tree species composition on carbon content (C), nitrogen content (N) and C/N ratio in the humus and mineral soil layers in natural and changed phytocoenoses of forest type group *Fagetum pauper*.

Material and methods

Study site

Geobiocenological plots were situated in the middle Slovakia in the southeast of orographic unit Kremnické vrchy Mts (48°41'N; 18°44'E). Research plots belong to cadastre municipalities of Tŕnie and Sliač, in 580–690 m above sea level, on the SW and SE exposure with 10–20° slopes (Table 1). Research plots are represented by original 80–100 years old beech forest (*Fagus sylvatica* L.) (G1) and changed phytocoenosis segments: 30-year-old birch forest (*Betula pendula* var. *carelica* Hämet-Ahti) (G2), spruce forest (*Picea abies* (L.) H. Karst) (G3), larch forest (*Larix decidua* Mill.) (G4), beech forest (*Fagus sylvatica* L.) (G5) and pine forest (*Pinus sylvestris* L.) (G6). All stands belong to forest type group (FTG) *Fagetum pauper.* The studied forest ecosystems are formed under influence of permanent ecological conditions and a result of interspecific competition. For the purpose of the study geobiocoenological typology was used (Zlatník, 1976). Based on present soil properties, bedrock, climate data and floristic composition, every plot was assigned to a correspondent altitudinal vegetation zone and trophic range (geobiocoenoses G1, G4 – 3rd oak-beech zone, G2, G3, G5, G6 – 4th beech zone). From the edaphic-ecological point of view studied forest stands belong to the mesotrophic range of geobiocoenoses.

The annual mean precipitation reported from the nearest weather station is 690 mm and annual mean temperature is 7.3 °C, available at: http://www.emsbrno.cz/p.axd/sk/Boky.Sever.pri.Budci.TUZVO.html [cited 2013-10-02]. Soils of study stands are dystric cambisols (G1, G2, G5, G6) and skeli-dystric cambisols (G3, G4), created from neo-volcanic andesite tuffs. The average value of the active soil reaction (pH_{H_2O}) ranges from 5.77 to 5.36, exchange reaction (pH_{KCl}) from 4.09 to 4.57 (Šimková et al., 2013). In the soil profiles of study stands dominated silt fraction, the size of 0.01–0.05 mm. Proportion of these fractions represented 30–40%. According to triangle texture diagram (Bedrna et al., 2000), medium-deep soils occurred there, mostly clay loam (G1–G5), partly loam (G1). In the case of site G6 (pine stand), it is covered by sandy loam soil (Table 2) (Šimková, 2013).

Table 1. Basic characteristics of study forest ecosystems

Site / stand age	Tree species edificator	Location	Altitude [m]	Slope [°]	Exposure	Canopy closure [%]
G1 (80–100 y.)	*Fagus sylvatica*	48°37′38" 19°01′40“	645	20	SW	90
G2 (30 y.)	*Betula pendula* var. *carelica*	48°37′04" 19°01′26“	635	10	SE	90
G3 (30 y.)	*Picea abies*	48°37′18" 19°01′35“	625	10	E	90–100
G4 (30 y.)	*Larix decidua*	48°37′07" 19°01′20“	630	15	SW	80
G5 (30 y.)	*Fagus sylvatica*	48°38′91" 19°02′44“	690	10	S	100
G6 (30 y.)	*Pinus sylvestris*	48°36′49" 19°02′15“	580	20	SW	80–90

Table 2. Ecological characteristics of study soils

Site / Edificator	Soil subtype*	Soil reaction $pH_{(H_2O)}$	Soil reaction $pH_{(KCl)}$	Layer [cm]	Skeleton % vol	Fine earth fraction [mm]			Soil class
						Clay [%]	Silt [%]	Sand [%]	
G1 / 80–100 y. *Fagus sylvatica*	Dystric Cambisols	5.38	4.09	0–5	0	27.03	30.33	42.54	Loam
				10–20	10–20	31.71	27.70	40.49	
				20–30	10–20	30.62	33.48	35.81	Clay loam
G2 / 30 y. *Betula pendula* var. *carelica*	Dystric Cambisols	5.77	4.57	0–5	0	30.7	42.63	26.61	Clay loam
				10–20	5–10	34.34	36.02	29.57	
				20–30	5–10	36.27	35.15	28.5	
G3 / 30 y. *Picea abies*	Dystric Cambisols	5.37	4.09	0–5	+	32.66	34.77	32.47	Clay loam
				10–20	10	34.49	30.45	35.01	
				20–30	30–40	40.55	37.39	22.05	
G4 / 30 y. *Larix decidua*	Skeli-Dystric Cambisols	5.36	4.31	0–5	0	31.64	37.97	30.37	Clay loam
				10–20	20–30	30.23	30.68	38.87	
				20–30	60	29.15	28.04	42.68	
G5 / 30 y. *Fagus sylvatica*	Dystric Cambisols	5.42	4.12	0–5	10	32.5	38.60	28.85	Clay loam
				10–20	20	37.5	38.67	23.79	
				20–30	20	37.77	35.71	26.46	
G6 / 30 y. *Pinus sylvestris*	Dystric Cambisols	5.37	4.43	0–5	10	22.23	26.31	51.43	Sandy loam
				10–20	30–40	24.36	20.47	55.14	
				20–30	30–40	24.36	24.50	51.14	

*(WRB, 1994).

Methods

The research was conducted from June to July 2009. Samples of surface humus (subhorizon Ool – leaf litter: from leafs, cupules, sticks, bark and the residue of forest plants without intensive decomposition; subhorizon Oof – fermentation horizon, with partial plant residue decomposition but with distinguishable original structure) were collected from square miniplots (0.1 m^2) in three random repetitions on each plot. The dry weight was obtained by drying the samples during 48-hours at a temperature of 80 °C to a constant weight with a precision 0.002 g. The material was homogenized in a planetary micro mill (<0.001 mm).

Mineral soil samples were taken from 0–5 cm, 10–20 cm and 20–30 cm layers. The soil samples were air-dried and passed through a sieve with a mesh size of 2 × 2 mm. The adjusted samples of surface humus and mineral soil were prepared for analysis and the results converted into the percentage of dry matter in the samples. The Flash 112 analyzer was used to determinate the total carbon and nitrogen contents in samples according to standards STN ISO 10 694 (for carbon) and STN ISO 13 878 (for nitrogen). Ratio C / N was calculated as a nitrogen and carbon total contents.

To characterize the basic information about the studied variables (mean, standard deviation) the descriptive statistics in program SAS was used (StatSoft, Inc., 2010). The effect of change of tree species edificator on the contents of total carbon, nitrogen and ratio C / N in surface humus and mineral soil layers was analyzed by one-way anova. The Fisher LSD test was used to detect significant differences between study plots, groups of tree species (coniferous and deciduous) and also between natural and changed tree-species composition.

Results

Carbon content

A result of carbon content in surface humus and mineral soil samples proved that C_t content decreased with depth of the soil profiles. Content of C_t in the subhorizont Ool ranged from 47.08% in 30-year-old birch stand to 54.89% in 30-year-old larch stand. This difference was 14.22%. In adult beech stand content of C_t in subhorizon Ool represented 52.12% (Fig. 1).

Carbon content was lower in subhorizons Oof than in subhorizons Ool. This was probably associated with gradual release during decomposition process. The highest carbon content was detected in 30-year-old larch forest (51.6%) and the lowest was in 30-year-old birch stand (46.96%). This difference represented 9.15%. In adult beech forest content of C_t in subhorizon Oof represented 49.46%. Statistical evaluation of results showed that values of carbon contents in Ool differed from mature beech forest only with 30-year-old birch stand ($F_{(1,4)}$ = 10.84; p = 0.031). In subhorizons Oof there were not found out any significant differences.

On average, surface humus of deciduous stands contained lower carbon content than humus of coniferous stands. The difference between compared tree

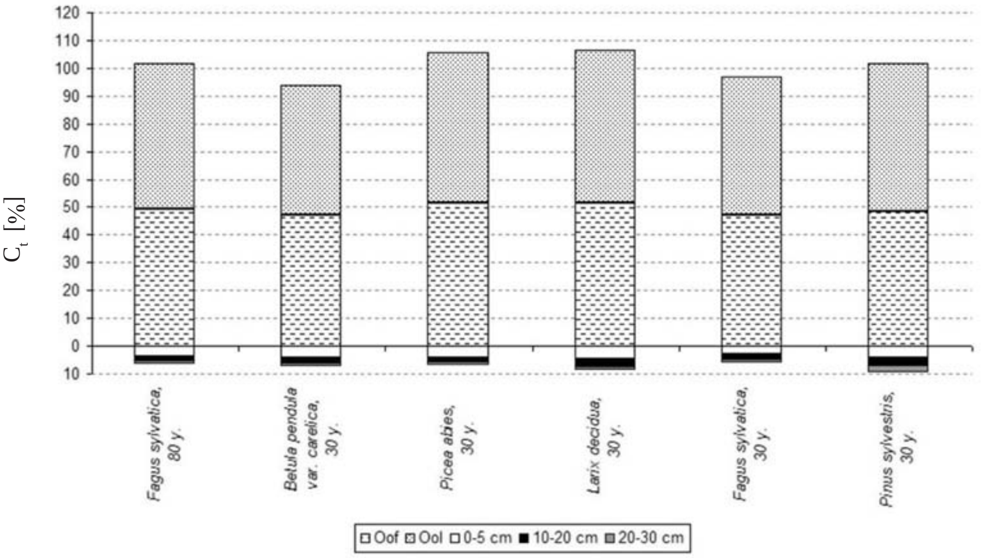

Fig. 1. Content of carbon in soils of studied forest stands.

species groups was 6.8% and it was statistically significant ($F_{(1,34)} = 7.726$; $p = 0.011$). In subhorizon Ool the carbon content was higher in stands with altered species composition than in the original species composition. On the contrary, this result has not been demonstrated in the subhorizont Oof. Differences between compared subhorizons were statistically significant ($F_{(1,16)} = 3.251$; $p = 0.04$ (Ool); $F_{(1,16)} = 3.585$; $p = 0.043$ (Oof); Table 3).

The highest carbon accumulation was detected in 30-year-old larch forest stand in the 0–5 cm depth (4.8%). The lowest one was in the beech forest (3.23%). Similar results were found in the 10–20 cm and 20–30 cm soil layers, too. The highest carbon content was accumulated in the pine forest (2.61%, respectively 2.42%), and the lowest in 30-year-old beech forest (1.34 respectively 1.23%). The most significant differences were found in the case of 80–100-year-old beech forest opposite larch stand (0–5 cm, 10–20 cm) and pine stand (10–20 cm layers) (Table 3).

Mineral soil layers in deciduous stands contained less carbon, than mineral soil in coniferous stands but differences were significant in the 0–5 cm ($F_{(1,16)} = 14.279$; $p = 0.017$) and 10–20 cm ($F_{(1,16)} = 10.475$; $p=0.046$) soil layers, only. Higher carbon content in soil layers was demonstrated in forest stands with altered tree species composition than in the natural beech forests. Differences were statistically significant in the 0–5 cm soil layer, only ($F_{(1,16)} = 63.183$; $p = 0.021$, Table 3).

Nitrogen content

The nitrogen content accumulated in subhorizon Ool ranged from 1.02% in the 30-year-old pine stand to 1.92% in 30-year-old birch stand (Fig. 2). The difference between phytocoeonoses represented 46.3%. Amount of N_t contained in subhorizon Oof ranged from 1.13% to 1.71%. The minimum values were found in pine stand, maximum in birch stand. The difference represented 33.9% and was statistically significant ($F_{(1,4)} = 22.42$; $p = 0.009$ (Ool); $F_{(1,4)} = 9.73$; $p = 0.035$ (Oof)).

Based on the results, we can conclude, that on average, higher content of N_t in Oo horizons was in the case of deciduous stands in comparison with coniferous stands. This difference was statistically significant ($F_{(1,34)} = 13.385$; $p = 0.048$).

Depending on originality of woody plant on studied plots, higher content of N_t in Oo horizons was recorded in the stands with altered species composition in comparison with beech stands, but without significant difference (Table 3).

The highest value of N_t in organo-mineral soil layer 0–5 cm was recorded in the 30-year-old birch stand (0.35%), the lowest in adult beech phytocoenosis (0.18%). In the 10–20 cm layer there was the largest content of nitrogen observed again in the birch stand (0.21%), the lowest in the adult beech stand (0.10%). The differences between the compared phytocoenoses were statistically significant ($F_{(1,4)} = 70.61$; $p = 0.0018$ (0–5 cm); $F_{(1,4)} = 25.9$; $p = 0.007$ (10–20 cm)). In the 20–30 cm mineral soil layers content of N_t ranged from 0.08% (adult beech phytocoenosis) to 0.18% (30-year-old pine stand). In the layer 0–5 cm statistically significant differences were observed in the case of 80–100-year-old beech stand opposite birch, pine, spruce and pine edificators. In the layer 10–20 cm it was observed between mature beech stand opposite

Table 3. Mean (±SD) C_t, N_t content (%) and C/N ratio in the surface humus and mineral soil layers

Subhorizonts	Ool – horizon			Oof – horizon			0–5 cm			10–20 cm			20–30 cm		
Plot/Tree species edificator	C [%]	N [%]	C/N	C [%]	N [%]	C/N	C [%]	N [%]	C/N	C [%]	N [%]	C/N	C [%]	N [%]	C/N
G1/Beech	52.12^{bc} (0.93)	1.49^{ac} (0.21)	35.51^{ab} (4.85)	49.46^{ab} (2.58)	1.43^{ab} (0.15)	35.92^{ab} (4.87)	3.73^{bc} (0.34)	0.18^{c} (0.02)	20.04^{b} (3.61)	1.37^{a} (0.26)	0.10^{a} (0.03)	16.22^{a} (6.22)	1.3^{a} (0.27)	0.08^{a} (0.02)	15.7^{a} (4.6)
G2/Birch	47.08^{a} (0.75)	1.92^{c} (0.23)	24.73^{a} (4.07)	46.96^{a} (12.9)	1.71^{a} (0.31)	27.3^{b} (3.62)	4.42^{ab} (0.25)	0.35^{a} (0.02)	12.25^{a} (1.54)	1.75^{a} (0.06)	0.21^{b} (0.02)	9.35^{ab} (1.35)	1.23^{a} (0.05)	0.12^{ab} (0.01)	10.47^{ab} (0.82)
G3/Spruce	53.75^{bc} (1.67)	1.31^{a} (0.06)	40.98^{ab} (1.83)	51.64^{ab} (2.02)	1.17^{b} (0.13)	44.71^{c} (1.69)	4.41^{ab} (0.06)	0.33^{ab} (0.02)	13.21a (0.88)	1.4^{a} (0.11)	0.14^{ab} (0.02)	10.39^{a}b (1.77)	1.34^{a} (0.06)	0.11^{a} (0.03)	8.69^{b} (2.09)
G4/Larch	54.89^{b} (0.81)	1.55^{bc} (0.10)	35.47^{ab} (2.84)	51.69^{b} (0.30)	1.51^{ab} (0.11)	34.32^{ab} (2.03)	4.8a (0.35)	0.3^{ab} (0.01)	14.57^{ab} (0.7)	2.37^{b} (0.22)	0.18^{ab} (0.01)	11.75^{ab} (0.45)	1.35^{a} (0.18)	0.13^{ab} (0.02)	9.18^{ab} (0.73)
G5/Beech	49.45^{c} (2.12)	1.37^{ab} (0.11)	36.44^{ab} (4.44)	47.33^{ab} (1.66)	1.57^{ab} (0.13)	30.32^{b} (3.27)	3.23^{c} (0.25)	0.26^{bc} (0.02)	13.3^{a} (2.03)	1.34^{a} (0.11)	0.17^{ab} (0.03)	8.14^{b} (0.75)	1.33^{a} (0.06)	0.14^{ab} (0.02)	9.15^{ab} (1.74)
G6/Pine	53.42^{bc} (3.1)	1.02^{a} (0.23)	54.44^{b} (15.4)	48.36^{ab} (2.69)	1.13^{b} (0.02)	42.69^{ac} (2.77)	4.43^{ab} (0.32)	0.31^{ab} (0.04)	13.67^{a} (0.99)	2.61^{b} (0.16)	0.19^{ab} (0.05)	12.71^{ab} (2.41)	2.42^{a} (0.17)	0.18b (0.02)	12.73^{ab} (1.03)
Broadleaf	49.55* (5.28)	1.59* (0.30)	32.22* (4.35)	47.91* (6.82)	1.57* (0.22)	31.93** (4.71)	3.79* (0.44)	0.26 (0.07)	15.19 (4.26)	1.6* (020)	0.15 (0.04)	11.24 (4.43)	1.28 (0.14)	0.11 (0.03)	10.80 (3.93)
Conifer	54.02 (1.92)	1.29 0.26)	43.62 (11.55)	50.57 (6.52)	1.26 (0.27)	46.48 (7.16)	4.49 (0.27)	0.32 (0.03)	14.05 (1.47)	2.13 (0.55)	0.18 (0.04)	11.60 (1.81)	1.48 (0.65)	0.14 (0.04)	10.21 (2.62)
Natural composition	50.78* (2.07)	1.42 (0.16)	35.97 (4.19)	49.05* (2.70)	1.49 (0.14)	33.12 (4.81)	3.55* (0.3)	0.22* (0.04)	16.66* (4.52)	1.54 (0.23)	0.14 (0.04)	12.19 (5.38)	1.29 (0.18)	0.11 (0.03)	12.47 (4.79)
Change composition	55.72 (3.5)	1.45 (0.37)	40.73 (11.28)	46.46 (6.52)	1.52 (0.29)	31.49 (7.16)	4.43 (0.27)	0.32 (0.03)	13.60 (1.47)	2.03 (0.51)	0.18 (0.04)	11.04 (1.94)	1.43 (0.56)	0.13 (0.03)	10.27 (2.27)

a, b, c, different letters indicate significant differences between tree species edificator ($\alpha < 0.05$). Significant differences ($*\alpha < 0.05$; $**\alpha < 0.01$) for the tree species groups and type of tree species composition.

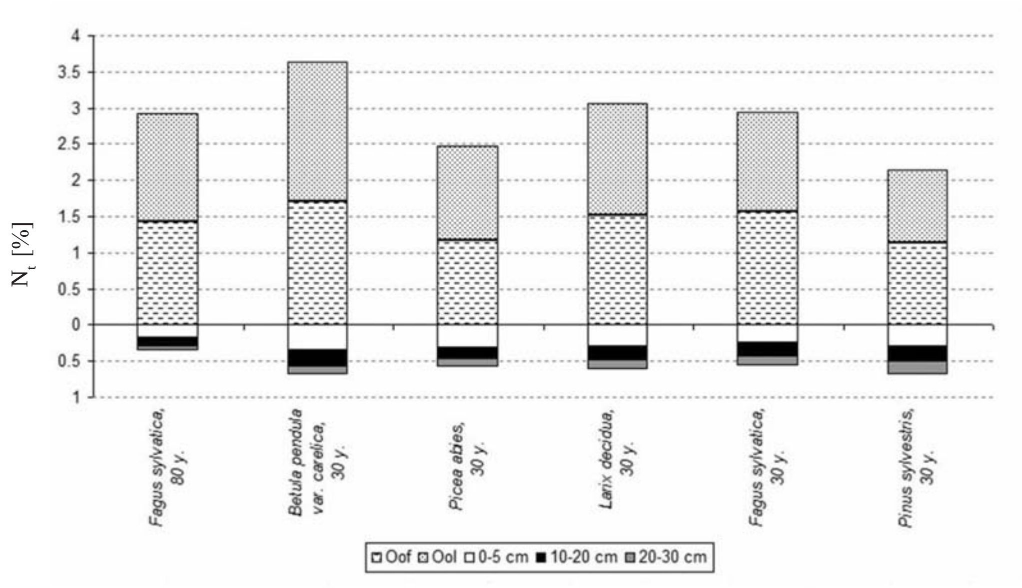

Fig. 2. Content of nitrogen in soils of studied forest stands.

birch stand; in 20–30 cm layer between mature beech and pine stand (Table 3).

Mineral layers of soils in broadleaf stands contained more nitrogen, than mineral soils in coniferous stands but differences were not significant. On average, a higher content of N_t was observed in forest stands with altered species composition, as compared to natural tree species composition, but with significant differences only in the 0–5 cm ($F_{(1,16)} = 36.280$; $p = 0.047$) layer of soils (Table 3).

C/N ratio

The C/N ratio in subhorizon Ool ranged from 24.74 (birch stand) to 54.44 (pine stand). In subhorizon Oof it ranged from 27.3 (birch stand) to 44.71 (30-year-old spruce stand) (Fig. 3). The differences between the compared phytocoenoses were statistically significant ($F_{(1,4)} = 10.12$; $p = 0.033$ (Ool); $F_{(1,4)} = 19.68$; $p = 0.011$ (Oof)). In subhorizon Oof, significant differences were found in case of natural beech phytocoenosis opposite of 30-year-old spruce stand. The lower C/N ratio in humus horizons (Oo) was found in broadleaf forest stands. Based on the results, we can conclude, that higher C/N ratio was observed in subhorizonte Ool in the forest stands with altered tree species composition, in the case of subhorizonts Oof in the stands with natural tree species composition (Table 3). However, these differences were not statistically significant.

More significant differences in the C/N ratios were found in the 0–5 cm layer of soils, where the values ranged from 12.25 (30-year-old birch stand) to 4.20 (adult beech phytocoenosis). In the 10–20 cm and 20–30 cm layers of mineral soils, the highest ratio was found in adult beech stand (16.22, respectively 15.7), the lowest in the 30-year-old beech pole stage (8.14, respectively 8.69), Fig. 3. Differences between C/N ratio of adult beech stand (in layers 0–5 and 10–20 cm) were statistically significant opposite birch, spruce, pine and young beech stand. In the layers of 10–20 cm and 20–30 cm significant differences were found in the case of adult stand opposite spruce stand (Table 3).

In the mineral soil layers of broadleaf stands, slightly higher C/N ratio was found, than in the mineral soils of coniferous stands but differences between groups of tree species were not statistically significant. The stands with changed tree species composition showed lower C/N ratio in the soil layers but differences were statistically significant only in the case of 0–5 cm layer ($F_{(1,16)} = 4.718$; $p = 0.049$).

Discussion

Woody tree species are considered to be one of the important factors that influence individual components of the ecosystems (Wulf and Naaf, 2009). Hüttl and Schaaf (1995) state that the supply of nutrients in forest soils is affected by many forest-silvicultural activities and other influences, such as the choice of edificator tree species, biomass removal, historic use of forest stands, change of tree species composition and finally acidic and nitrogen depositions. Based on our results, we can conclude that the 30-year-old stands of spruce, pine, larch, birch and beech growing on sites of natural beech stands had a partial influence on the content of carbon and nitrogen in surface humus and mineral soil layers. The same results were also concluded by the

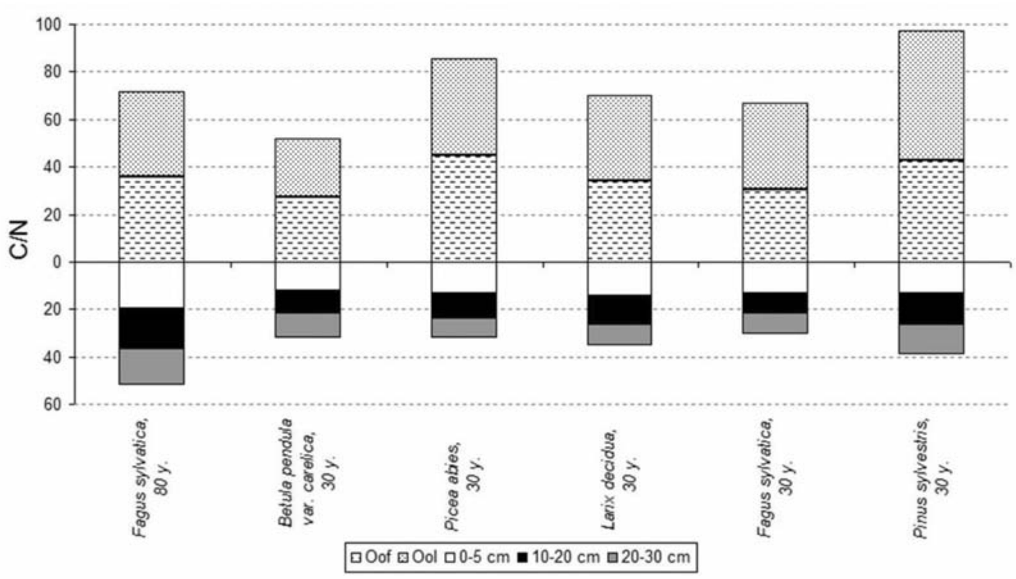

Fig. 3. C : N ratio in soils of studied forest stands.

authors Vesterdal et al. (2008), who observed effect of six European tree species on C_t and N_t contents in forest soils. Binkley and Valentine (1991) state that the chemical properties of soils can change after 50 years of growing stands, which confirms work of Fischer et al. (2002), too. The authors observed differences in soil properties between stands of different tree species at the age of 60 years. Hagen-Thorn et al. (2004) investigated the impact of six European tree species on the chemistry of mineral top soils in forest plantations. Authors did not confirm significant differences in carbon contents between the studied species after 40 years of their cultivation.

Effect of change of tree species edificator in Kremnické vrchy Mts was the most significant in the layers of surface humus and topsoil 0–5 cm, suchlike conclusions were confirmed in the work by Augusto et al. (2002), too. The changes mainly concern to increased accumulation of C_t in soil layers of coniferous stands – European larch (*Larix decidua*), Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). Gärdenäs (1998) in his work also indicates higher stock of soil carbon in spruce and pine stand in comparison with deciduous stands. Similar results reached Vesterdal and Rasmunssen (1998); Fischer et al. (2002); Augusto et al. (2002) and Schulp et al. (2008). The highest carbon content in studied stands of Kremnické vrchy Mts was recorded in surface humus, and its contents towards the depth of the soil profiles decreased, which was probably related with the gradual release of nutrients during decomposition processes. Similar results are reported in the works by Labudová et al. (2009); Menšík et al. (2009) and Lesná and Kulhavý (2003).

Growing of non-native forest stands of different tree species edificators in the Kremnické vrchy Mts had a statistically significant impact on carbon contents in surface humus and 0–5 cm mineral soil layers. In sites with altered tree species composition, a higher accumulation of C_t, was observed than in natural beech stand. Kreutzer (1989) in their work evaluated changes in soil properties caused by growing two generations of Norway spruce on a site before formed with *Quercus* and *Tilia* species. Growing of spruce stands caused increased accumulation of C in the soil profile to 1 m depth.

Effect of tree species edificators on nitrogen content was confirmed in the 0–5 cm mineral soil layers, where statistically significant differences were found out. The highest amount of nitrogen was observed in the surface humus of studied soils, and contents towards the depth of soil profiles decreased, which was similar as in the case of C_t.

Sýkora (2011) indicated in soil layers of successional stages of beech stands a decreasing trend of biogenic elements towards the depth of the soil profiles, too. Similar results were observed by Berger et al. (2002). Lesná and Kulhavý (2003) found higher N content in subhorizon Oof, in comparison with Ool. In lower subhorizons authors observed a decrease of nitrogen content, which is in agreement with our results. Rey et al. (2008) and Sýkora (2011) found out a decrease in the nitrogen content from 0–5 cm soil layer to 10–20 cm layer of almost 50 %.

The highest significant differences in nitrogen content were found in surface humus of deciduous stands compared to the coniferous. In the mineral soil layers, higher N_t contents were reported in the coniferous stands. The results found in Kremnické vrchy Mts are consistent with findings of Lesná and Kulhavý (2003), who found higher nitrogen content in surface humus of deciduous stands, in mineral soils in coniferous stands. On the other hand, KLEMMENDSON (1987) in his work indicated insignificant differences in concentrations of soil nitrogen between coniferous and deciduous stands, significant differences were only observed in its vertical distribution.

The total nitrogen content in the surface humus and mineral soils of studied geobiocenoses in Kremnické vrchy Mts formed following order: pine stand < spruce stand < beech forest < larch stand < birch stand. ŠÁLY (1978) in his work indicated the following order of N content in litter fall: pine < larch < birch < spruce < beech. For example, RANGER et al. (1994) found that litter fall of *Fagus sylvatica* species has approximately about 12% higher content of nitrogen in comparison with litter of *Pinus sylvestris* species. In comparison with our results, this difference was even slightly higher.

Higher N_t content in soil samples was found in the stands of changed tree species composition in comparison with the sites with natural beech composition. A statistically significant difference was found only in the 0–5 cm layer of mineral soil. KREUTZER (1989) also observed significant differences in concentrations of N in soil samples, when on the sites with changed tree species composition, higher N contents were found in the humus layers and the lower in the upper mineral soil layers (0–50 cm).

The C/N ratio is considered to be one of the main factors controlling decomposition rate of soil organic matter (COTE et al., 2000). In surface humus of studied soils, a higher C/N ratio was found in the coniferous stands (45.2), compared with deciduous (32.07). Based on the results from Kremnické vrchy Mts, ratios C/N in litterfall of analyzed stands formed following order: birch < beech < larch < spruce < pine. WITTICH (1952) in his work indicates the following order: birch < beech < spruce < pine < larch. BUBLINEC (1994) evaluated the effect of litterfall beech, oak and hornbeam tree species. Based on his results, beech belongs among tree species with the most unfavourable effect on litterfall decomposition.

The ratios of C/N in mineral soil layers in Kremnické vrchy Mts decreased downwards in the soil profiles. Similar results were confirmed by LESNÁ and KULHAVÝ (2003). The highest ratio was found in a group of deciduous stands, but without statistically significant differences.

In the European forest soils C/N ratio ranges from 10 to 100. The C/N in organic horizons ranges between 20 and 40, in mineral horizons from 10 to 30. In Kremnické vrchy Mts, the ratio C/N in organic horizons ranged from 24.73 (birch stand) to 54.44 (pine stand). In the mineral horizons values varied from 8.14 (30-year-old beech stand) to 4.20 (80–100-year-old beech forest). EMMETT et al. (1998) in his work indicates value 24 as the critical C/N ratio for coniferous stands, when at the ratio >24 less than 10% of nitrogen is washed out from ecosystem. At the C/N ratio < 24, more than 10% of total nitrogen is washed out from ecosystem. ŠÁLY (1978) states, that the ratio C/N higher than 30 leads to slow decomposition, which is reflected in the slow release of nutrients, secondary impoverishment and subsequent soil degradation (LESNÁ and KULHAVÝ, 2003). According to WARRING and RUMMING (1998) net mineralization occurs at C/N ratio lower than 20 and net immobilization at value higher than 30.

Conclusion

The research results showed that change of tree species composition on the sites of natural occurrence of beech had a partial influence on the content of C_t and N_t in the surface humus and mineral soil layers, after three decades of their planting. It was reflected the most in the surface humus and topsoil 0–5 cm layers. This concerned mainly C_t contents while N_t contents did not show so many significant differences between the compared stands. The highest amounts of carbon and nitrogen were recorded in the surface humus of the studied stands. These contents towards the depth of the soil profiles decreased. The highest C_t content was found in surface humus of coniferous stands; in the case of N_t it was in deciduous stands. In the mineral soil layers significantly higher contents of C_t and N_t were found in the coniferous stands, in comparison with deciduous ones. Stands with changed tree species composition showed a higher content of elements in surface humus as well as in mineral soil layers. C/N ratio in the surface humus was higher and statistically significant in the case of coniferous stands in comparison with deciduous stands. In the mineral soils higher C/N ratio was found in the deciduous stands compared with coniferous stands. The difference between compared groups of stands was insignificant.

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Soil structure and soil organic matter of different ecosystems

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Abstract

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In this study, the soil structure of six soils of different ecosystems in Slovakia was compared. The stability of organic matter inside of aggregates was assessed through the carbon parameters – the index of carbon lability (LI_c), carbon pool index (CPI), and carbon management index (CMI). The soil structure of different ecosystems was compared through the proportion of water-resistant macro-aggregates (WMA) and the parameters of soil structure – the coefficient of vulnerability (K_v), aggregates stability index (S_w), critical soil organic matter content (S_t), and index of crusting (I_c). The quality of soil structure was decreasing in the following order: forest ecosystem (FE) > agro-ecosystem (AE) > meadow ecosystem (ME) > grassy urban ecosystem (UE). In the FE, the WMA of the 1–3 mm size fraction had the highest proportion and in case of AE the highest proportion had WMA of the 0.5–1 mm size fraction. The highest content of labile carbon was incorporated into aggregates of the FE and the highest stability of organic matter was in the aggregates of the ME. According to parameter CMI, labile components are better protected in the smaller aggregates. An important indicator for the assessing of the ecosystem impact is WMA of the 0.5–1 mm size fraction. Increased proportion of this aggregate fraction refers to the deterioration of soil structure.

Keywords

ecosystem, labile carbon, soil structure, water-resistant aggregates

Introduction

Soil quality is defined as the ability of soil to play the role within the ecosystem, so that its biological productive ability and environmental quality were preserved, and also it should support healthy growth of plants and animals (Karlen et al., 1997). Larson and Pierce (1991) propose the organic carbon, and soil structure as the indicators that are sensitive to the land use, for the monitoring of the soil quality. Soil organic matter (SOM) is important for the conservation of favourable physical, chemical, and biological properties of soils (Johnsen et al., 2013). The SOM is regarded as an essential element in the formation of aggregates (Zeytin and Baran, 2003), and the formation of aggregates contributes to the stabilisation of organic matter through the physical protection of the aggregates (Balabane and Plante, 2004; Tobiašová, 2010). Different fractions of organic matter participate in the formation and stabilization of soil aggregates by various ways (Roberson et al., 1991; Tobiašová, 2011). Stabilization through the physical-chemical protection, in hierarchical soil aggregates, is crucial for the conservation of carbon and nitrogen sources (O'Brien and Jastrow, 2013). Particularly important are labile fractions of SOM, because they are more sensitive to soil disruption and play important role in carbon and nutrient turnovers (Tian et al., 2013). Labile SOM is a sensitive indicator of changes in land use and soil management practices (Wang and Wang, 2011). Therefore the objectives of this study were as follows: (i) to compare the differences in the composition of water-resistant aggregates and in a stability of organic matter inside them, depending on the ecosystem, and (ii) to assess the suitability of the fractions of water-resistant aggregates as an indicator of the soil structure deterioration.

Material and methods

The studied areas are located in the West of Slovakia. The localities Močenok (48°13'N, 17°55'E) with Haplic Chernozem, Horná Kráľová (48°14'N, 17°54'E) with Mollic Fluvisol, and Šaľa (48°09'N, 17°52'E) with Eutric Fluvisol are situated on the northern border of the Danube lowland. Region is formed by strata of Neogene, mainly of claystones and sandstones, which are covered with younger quaternary rocks represented by different fluvial and aeolian sediments (Pristaš et al., 2000). The natural vegetation consists mostly of ash-oak-elm-alder forests, and along the river, there are willow-poplar and floodplain forests. In the elevated areas and dunes, xerophilous communities of oak-elm forests are dominant (Korec et al., 1997).

The locality Veľké Zálužie (48°18'N, 17°56'E), with Haplic Luvisol is situated in the Danubian Hills (Upland) formed by quarternary sediments – loess and loess loam. Neogene bedrock consists of lake brackish sediments (clays, gravels, and sands), Hók et al., 2001. In the lower parts of area dominate oak forests and in the higher parts, there are mixed beech forests.

The locality Pružina (49°00'N, 18°28'E) with Rendzic Leptosol and Eutric Cambisol is situated at the north-eastern foot of the hill Strážov, in the valley of the river Pružina. Region belongs to core mountains of the outer arc of the Central Western Carpathians. A substantial part of the Strážovské vrchy Mts is composed of the nappes with highly variable resistance of rocks. The core is formed with the crystalline slates, granites, amphibolites, and in the south and southeast, there are mesozoic dolomites, limestones and slates, which are folded and stored in the form of nappe debris (Pristaš et al., 2000). In the forests dominate beech and oak, in the higher parts with the admixture of fir and higher number of other conifers.

The experiment included four types of ecosystems, which present different land use and management (forest ecosystem, meadow ecosystem, grassy urban ecosystem, and agro-ecosystem) on six soil types (Haplic Chernozem, Mollic Fluvisol, Eutric Fluvisol, Rendzic Leptosol, Eutric Cambisol, and Haplic Luvisol). These are the soils of lowlands and uplands, which have the largest proportions in Slovakia and are intensively agriculturally used. The forest ecosystems are close to nature and managed; the meadow ecosystems were created by man 30 years ago; and the urban ecosystems are affected by human activity lawns. The studied agro-ecosystems were located in different farms under real production conditions.

The soil samples for chemical and physical properties determination were collected in three replicates from a layer of 0.0–0.3 m. Soil samples were dried at constant room temperature (25 ± 2 °C) and then divided by the sieving (dry and wet sieving) to fractions of the net aggregates. The aggregate stability index (S$_w$) (Henin et al., 1969), the coefficient of vulnerability (K$_v$) (Valla et al., 2000), the index of crusting based on textural composition and soil organic matter (I$_c$), Lal and Shukla, 2004, and the critical soil organic matter content (S$_t$) according to Pieri (1991) were also calculated. The particle size distribution, which was used for the calculation of soil structure parameters, was determined after dissolution of CaCO$_3$ with 2 mol dm^{-3} HCl and oxidation of the organic matter with 30% H$_2$O$_2$. After repeated washing, samples were dispersed using Na$_4$P$_2$O$_7$.10H$_2$O. Silt, sand, and clay fractions were determined according to the pipette method (Day, 1965). In the water-resistant aggregates, the TOC by wet combustion (Orlov and Grišina, 1981) and the labile carbon (C$_L$) by KMnO$_4$ oxidation (Loginov et al., 1987) were determined. Non-labile carbon (C$_{NL}$), lability of carbon (L$_C$), index of carbon lability (LI$_C$), carbon pool index (CPI), and carbon management index (CMI) were also calculated (Blair et al., 1995).

The obtained data were analysed using Statgraphic Plus statistical software. A multifactorial ANOVA model was used for individual treatment comparisons at $P < 0.05$, with separation of the means by Tukey multiple-range test.

Results and discussion

Proportion of water-resistant aggregates in soils of studied ecosystems

The highest amount of water-resistant macro-aggregates of the 0.5–1 mm size fraction was statistically significant in agro-ecosystem (Table 1). Emadi et al. (2009) also reported that a higher amount of the micro-aggregates and small macro-aggregates (<0.5 mm) remains in ploughed soils and according to Whalen and Chang (2002), the increased proportion of the smaller aggregates (<1.2 mm) is an important indicator of soil degradation. In the other ecosystems, the content of this aggregate fraction was relatively balanced. The values found in the meadow and grassy urban ecosystems were slightly higher than in the forest ecosystem. The richest sources of organic substances occur primarily on the soil surface in the forest ecosystem. The result of their decomposition is represented by substantially higher amounts of the mobile acids than in the arable land, in which there is a greater mixing of the organic portion with mineral portion of the soil. These acidic components later get into deeper parts of the soil profile through leaching, and support the leaching of carbonates that act as the cementing agents in aggregates, as well as acidification of soil. Iron and aluminium, which are the cement agents in the smaller aggregates, are also mobilised (Barral et al., 1998; Duiker et al., 2003). From the organic components, mainly the stabilized forms of organic matter have the function of cementing agents in smaller aggregates having the lowest proportion in the soil of forest ecosystem. The lowest proportion of the macro-aggregates of the 0.5–1 mm size fraction was in soils of the meadow and grassy urban ecosystems. In both cases there were grasses which points to the significant influence of the vegetation cover, which is higher than the impact of the anthropogenic factors, which influence the soil properties in the grassy urban ecosystem. According to Cantón et al. (2009), the type of vegetation influences the aggregates, the size of which is larger than 4–8 mm. The highest proportion of agronomically the most valuable water-resistant macro-aggregates was on average in the agro-ecosystem (56.85%), where the farmyard manure, which is a source of the stabile forms of humus substances and of the large number of micro-organisms, was added. Stabile forms of the organic matter support the formation of stabile soil aggregates and carbohydrates of microbial origin resist degradation better than carbohydrates of plant origin do (Debosz et al., 2002). The second highest proportion of these aggregates was in the forest ecosystem (47.39%). Dynamics of soil aggregates is the reflection of the chemical composition of plant residues. In the forest litter, the phenols and polyphenols, which are the precursors for the formation of humus substances, are dominant (Martens, 2000) and of the lignin, which supports the aggregate formation (Magill and Aber, 1998). The lowest contents of agronomically the most valuable aggregates were in meadow (39.72%) and in grassy urban (39.65%) ecosystems. In both cases occurred grasslands, in which the root exudates are an important source of labile fraction of soil organic matter. According to Tisdall and Oades (1982), the polysaccharides are easily mineralizable and play the role of temporary components at the formation of soil aggregates. As it can be seen, the proportion of agronomically the most valuable size fraction of 0.5–3 mm was significantly influenced not only by the quantity, but also the quality of organic substance inputs into the soil (Tobiašová, 2011). However, if we take only agronomically valuable aggregates of the 1–3 mm size fraction, it means without the size fraction 0.5–1 mm, its significantly higher proportion was in the forest ecosystem (35.45%). In the other ecosystems, their proportions were relatively balanced, with the proportion of 31.59% in the agro-ecosystem, 30.04% in urban ecosystem, and 29.64% in meadow ecosystem. Emadi et al. (2009) presented, that deforestation, and ploughing of a meadow decreased mainly the content of water-resistant macro-aggregates of the 24.75 mm size fraction (4.5-times) and the size fraction of

Table 1. Statistical evaluation of differences among contents of water-resistant aggregate fractions in different soils and ecosystems

Fraction of aggregates	2–3 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	<0.25 mm
			[%]		
Soil					
HC[a]	11.72ab	18.65b	18.25a	11.51ab	21.52bc
MF[b]	12.87ab	18.10b	19.89a	19.00b	15.74abc
EF[c]	14.78bc	16.76ab	15.06a	8.55a	14.84ab
RL[d]	21.50c	19.68b	13.67a	8.84a	8.68a
EC[e]	18.23bc	21.27b	15.61a	9.04a	8.19a
HL[f]	6.23a	10.01a	20.95a	29.19c	25.91c
Ecosystem					
FE[g]	17.58a	17.87a	11.94a	10.07a	8.44a
ME[h]	14.87a	14.77a	10.08a	10.45a	14.71a
UE[i]	16.32a	13.72a	9.61a	7.20a	11.47a
AE[j]	12.70a	18.89a	22.26b	18.19a	19.03a

[a]HC, Haplic Chernozem; [b]MF, Mollic Fluvisol; [c]EF, Eutric Fluvisol; [d]RL, Rendzic Leptosol; [e]EC, Eutric Cambisol; [f]HL, Haplic Luvisol; [g]FE, forest ecosystem; [h]ME, meadow ecosystem; [i]UE, urban ecosystem; [j]AE, agro-ecosystem; different letters (a, b, and c) show statistically significant differences – Tukey test ($P < 0.05$).

1–2 mm (1.9-time). Both fractions are also larger, than the mentioned size fraction of 0.5–1 mm. This fact also points to the increased proportion of water-resistant macro-aggregates of the 0.5–1 mm size fraction as the negative rather than the positive state. It follows that an increase of water-resistant macro-aggregates of the 0.5–1 mm size fraction is caused mainly by the changes in soil management, which also predisposes this aggregate fraction to become an important indicator of land use impact on the soil structure.

From the values given in the Table 2, the soil structure of the forest ecosystem can also be considered as the best. This assessment is based on the proportion of agronomically the most valuable water-resistant macro-aggregates of the 1–3 mm fraction (the fraction of 0.5–1 mm is not taken into consideration). Soils below natural vegetation have the highest stability of soil aggregates also according to Barreto et al. (2009). Closest to the values of aggregate stability index (S_w) and coefficient of vulnerability (K_v) of forest ecosystem were the values found in agro-ecosystem. According to Borie et al. (2008), the addition of limestone or dolomite powder to the soil has a considerable impact on the formation of soil aggregates, because divalent cations Ca^{2+} and Mg^{2+} are main polyvalent cations, which not only stabilize the organic matter, but also improve the aggregation. In the agro-ecosystem, the proportion of crops can influence also the extent and frequency of wet and dry periods, which influence the stability of soil aggregates (Materechera et al., 1994). Through suitable land use, it is possible to improve the soil structure state in the agro-ecosystem and bring it near to a level that is in the forest ecosystem, in spite of the higher proportion of the size fraction of 0.5–1 mm. However, the values of the critical content of soil organic matter (S_t) and the index of crusting (I_c) clearly point at a more favourable state of soil structure in the forest ecosystem. These two parameters are significantly influenced by the amount of organic matter and particle size distribution of soil (Lal and Shukla, 2004). Given, that the statistically significant differences in particle size distribution between the ecosystems were not recorded, the positive impact can be attributed mainly to the soil organic matter. This suggests that in spite of the lower inputs of organic matter into the soil in the agro-ecosystem, one of the possibilities for improving of the soil structure can be its stabilization through the binding to polyvalent cations and mineral components of the soil.

Soil organic matter in the fractions of water-resistant aggregates

Christensen (2001) and Oades (1984) described that if the particles of labile organic matter become the core of aggregates, they can be this way physically stabilized inside the aggregates, thereby supporting the formation of stabile soil structure. In the surface soil layer of the forest ecosystem, there is a higher source of particular organic matter in the soil aggregates which was also confirmed by the results of Freixo et al. (2002). Our results (Fig. 1) show, that the highest content of labile carbon in the water-resistant macro-aggregates

Table 2. Statistical evaluation of differences among individual parameters of the soil structure of different soils and ecosystems

Parameter of soil structure	S_w[k]	K_v[l]	S_t[m] [%]	I_c[n]
Soil				
HC[a]	1.05a	1.18b	4.87ab	1.03ab
MF[b]	1.54b	0.31a	6.22b	0.82a
EF[c]	1.08a	1.47b	5.13ab	1.01ab
RL[d]	1.21a	1.04b	3.92a	1.37bc
EC[e]	1.13a	1.12b	4.27ab	1.66b
HL[f]	1.25a	1.39b	4.29ab	1.06ab
Ecosystem				
FE[g]	1.50a	0.79a	7.51b	0.75a
ME[h]	1.28a	1.10ab	5.65a	0.95ab
UE[i]	0.90a	1.62b	4.18a	1.23a
AE[j]	1.41a	0.67a	5.27a	0.99ab

[a]HC, Haplic Chernozem; [b]MF, Mollic Fluvisol; [c]EF, Eutric Fluvisol; [d]RL, Rendzic Leptosol; [e]EC, Eutric Cambisol; [f]HL, Haplic Luvisol; [g]FE, forest ecosystem; [h]ME, meadow ecosystem; [i]UE, urban ecosystem; [j]AE, agroecosystem; [k]S_w, aggregate stability index; [l]K_v, coefficient of vulnerability; [m]S_t, critical soil organic matter content; [n]I_c, index of crusting; different letters (a, b, and c) show statistically significant differences – Tukey test ($P < 0.05$) .

was recorded in the forest ecosystem, which means that the labile fractions become a part of these aggregates. In the meadow ecosystem, the source of labile forms of organic matter is also large, but only its lower amount is incorporated into the aggregates. Substances that support a richer representation of microflora are mainly root exudates. A considerable part of this labile carbon is thus decomposed before its incorporation into the aggregates, as can be seen in case of the lowest content of labile carbon in the fractions of water-resistant macro-aggregates in our study. In the case of the forest ecosystem, substantially higher amount of labile carbon is bound into the water-resistant macro-aggregates of the 0.25–3 mm size fraction, in comparison with meadow ecosystem.

The stability of soil organic matter in water-resistant macro-aggregates is better described by the parameters of the carbon (Table 3). Lability of carbon (L_C) in the aggregates was clearly highest in the forest ecosystem. In this ecosystem, there was not only the highest amount of total organic carbon (TOC) in the aggregates, but also its labile forms. In the forest ecosystem, the values of pH are lower and according to Tobiašová (2010), at lower pH there is a higher amount of the carbon in active form. However, if we compare the stability of organic matter in aggregates of the individual ecosystems with the control variant, which is in our case forest ecosystem, the higher values of the index of carbon lability (LI_C) were in the grassy urban ecosystem and agro-ecosystem than in the meadow ecosystem, where the changes in organic matter of these aggregates, according to carbon management index (CMI), were slower.

Higher values of LI_C show a higher amount of the carbon in active form and therefore less resistance of the soil organic matter against decomposition (Blair et al., 1995). In the meadow ecosystem, the highest content of labile carbon was in the root zone, but its content in the aggregates was the lowest in comparison to other ecosystems (Table 3). Higher values of LI_C do not mean immediately a lower stability of soil aggregates. This means that the physical stabilization of soil organic matter plays an important role in the stability of soil aggregates. According to Santos et al. (1997), this is the result of protective action of the mineral particles of soil, particularly of clay, which inhibits the decomposition of organic matter within the aggregates. The values of L_C show that the lability of carbon is decreasing with reducing of the aggregate size and the values of carbon pool index (CPI) also show that the smaller aggregates in the ecosystems, the larger the amounts of TOC in them, in comparison to aggregates of the forest ecosystem. It is known that the smaller aggregates there are, the less organic matter is in them (Six et al., 2000). It follows that the labile components in smaller aggregates are better protected, especially through the physical stabilization in aggregates, which is confirmed by the values of CMI. These were the lowest in the case of larger aggregates, which indicates more rapid changes in the organic matter. The values of CPI and CMI in the micro-aggregates are a little smaller, but according to Six et al. (1998), the macro-aggregates are more influenced through the land use than the micro-aggregates. In the case of ecosystems, larger changes in the meadow ecosystem are supported by higher sources of easily decomposable substances such

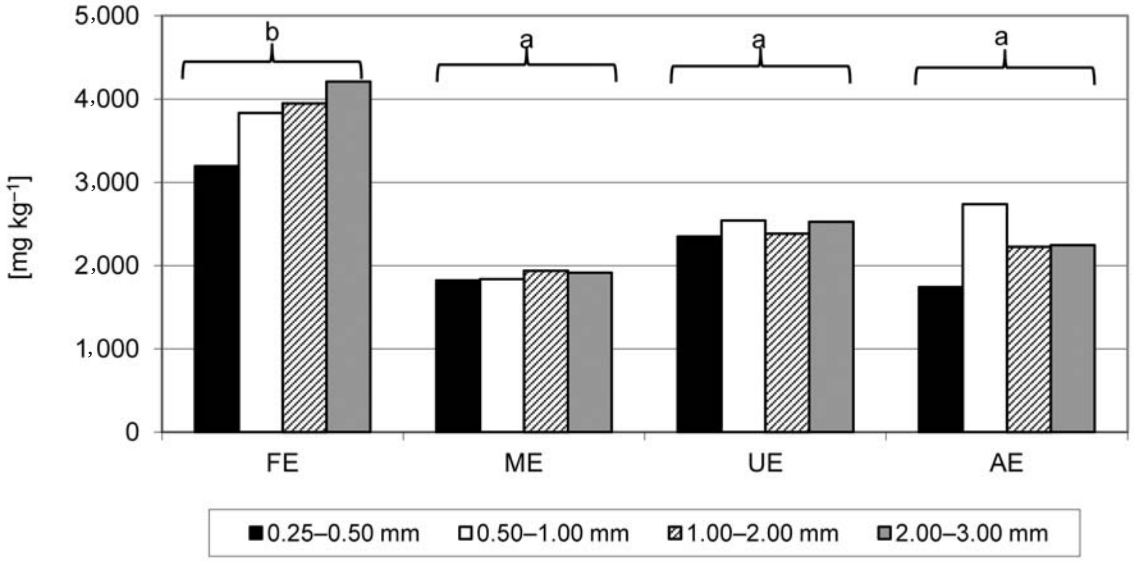

Fig. 1. Contents of labile carbon (C_L) in fractions of water-resistant macro-aggregates in soils of different ecosystems. FE, forest ecosystem; ME, meadow ecosystem; UE, urban ecosystem; AE, agro-ecosystem; different letters (a and b) between the factors show statistically significant differences – Tukey test ($P < 0.05$).

Table 3. Statistical evaluation of differences among individual carbon parameters in soils, ecosystems and water-resistant aggregate fractions

Parameter of carbon	[k]TOC	[l]C_L	[m]C_{NL}	[n]L_C	[o]LI_C	[p]CPI	[r]CMI
	[mg kg^{-1}]						
Soil							
HC[a]	20,827b	3,026b	17,801b	0.170c	72.95ab	0.813c	58.90b
MF[b]	18,958ab	1,709a	17,249ab	0.096a	83.03abc	1.162d	102.20c
EF[c]	19,323ab	2,681ab	16,642ab	0.157bc	85.03abc	0.899c	81.87bc
RL[d]	22,330b	2,064ab	20,266b	0.092a	60.09a	0.262a	15.26a
EC[e]	24,199b	2,996b	21,202b	0.136b	86.35bc	0.532b	46.11ab
HL[f]	12,891a	2,223ab	10,668a	0.209d	101.42c	1.170d	110.99c
Ecosystem							
FE[g]	27,380b	3,647b	23,733b	0.168c	–	–	–
ME[h]	17,092a	1,791a	15,301a	0.117a	69.26a	0.799a	55.26a
UE[i]	18,536a	2,369a	16,167a	0.144b	88.28b	0.873a	85.14b
AE[j]	16,010a	1,992a	14,018a	0.144b	86.89b	0.743a	67.27ab
Aggregate fraction							
3–2 mm	20,599a	2,725a	17,874a	0.151a	71.58a	0.760a	57.57a
2–1 mm	20,896a	2,624a	18,271a	0.144a	78.18a	0.784a	59.51a
1–0,5 mm	20,332a	2,529a	17,803a	0.147a	82.24a	0.798a	66.05a
0.5–0.25 mm	18,881a	2,208a	16,674a	0.140a	85.64a	0.886a	84.38a
<0.25 mm	18,066a	2,164a	15,902a	0.135a	89.76a	0.795a	78.60a

[a]HC, Haplic Chernozem; [b]MF, Mollic Fluvisol; [c]EF, Eutric Fluvisol; [d]RL, Rendzic Leptosol; [e]EC, Eutric Cambisol; [f]HL, Haplic Luvisol; [g]FE, forest ecosystem; [h]ME, meadow ecosystem; [i]UE, urban ecosystem; [j]AE, agro-ecosystem; [k]TOC, soil organic carbon; [l]C_L, labile carbon; [m]C_{NL}, non-labile carbon; [n]L_C, lability of carbon; [o]LI_C, index of carbon lability; [p]CPI, carbon pool index; [r]CMI, carbon management index; different letters (a, b, c and d) show statistically significant differences – Tukey test ($P < 0.05$).

as polysaccharides derivable from the root exudates and microorganisms. In the case of agro-ecosystem, larger changes are supported by an application of the farmyard manure.

It follows that the quality of soil structure was decreasing in the following order: forest ecosystem > agro-ecosystem > meadow ecosystem > grassy urban ecosystem.

The water-resistant macro-aggregates of the 1–3 mm size fraction had the highest proportion in the forest soil, whereas of 0.5–1 mm fraction in the soil of agro-ecosystem.

The highest content of the labile carbon is incorporated into the aggregates of the forest ecosystem and the highest stability of the organic matter was in the aggregates of the meadow ecosystem. The carbon management index shows, that the labile components are better protected in the smaller aggregates.

An important indicator, for assessing of the ecosystem influence, seems to be the water-resistant macro-aggregates of the 0.5–1 mm size fraction. This is only one size fraction, at which statistically significant differences between the ecosystems were recorded, and at which differences between the soil types were not recorded. Increased proportion of this aggregate fraction refers to the deterioration of soil structure.

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Natural hybridization in the genus *Abies*: II. Mitochondrial variation in the hybridogenous complex *Abies alba – A. borisii-regis – A. cephalonica*

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Abstract

Voleková, M., Krajmerová, D., Paule, L., Zhelev, P., Gömöry, D. 2014. Natural hybridization in the genus *Abies*: II. Mitochondrial variation in the hybridogenous complex *Abies alba – A. borisii-regis – A. cephalonica*. *Folia oecol*., 41: 100–105.

Twenty nine fir populations originating from the putative zones of interspecific hybridization in southern Balkans were studied using a mitochondrial *nad5-4* gene marker. The populations were classified to three taxa based on their geographical distribution and an overall taxonomical assessment at the population level based on needle and twig morphology: *Abies alba* Mill., *Abies cephalonica* Loud. and *Abies borisii regis* Mattf. Three haplotypes were found: 230 bp in Calabrian *A. alba* populations, 150 bp in Bulgarian and Macedonian *A. alba* populations, and 341 bp in *A. cephalonica* populations. Populations from central and northern Greece, classified as *A. borisii regis*, shared the 150 bp and 341 bp haplotypes with their closest neighbours, whereby haplotype frequencies changed clinally along the latitudinal gradient. This geographical distribution of mtDNA haplotypes supports the hypothesis that *A. borisii regis* represents a relatively recent hybrid swarm.

Keywords

Abies alba Mill., *Abies borisii regis* Mattf., *Abies cephalonica* Loud., hybrid swarm, hybridization zone

Introduction

Hybridization has long been considered a lapsus of nature. Interspecific hybrids have traditionally been supposed to be sterile or apomictic and restricted to specific sites. This prejudice was based on the experience with mammals, where interspecific hybrids are mostly sterile. In trees, some emblematic hybrids actually do occur in specific environments. For example, the occurrence of hybrid swarms of two widely distributed pine species *Pinus mugo* and *P. sylvestris* is restricted to peat bogs and similar sites (Kormuťák et al., 2008, Staszkiewicz and Tyszkiewicz, 1969). Nevertheless, with the advent of molecular methods it was demonstrated that hybridization is quite common in plants. Almost one-quarter of plant taxa has probably been involved in hybridization (Mallet, 2005).

In the former Czecho-Slovakia, there has been a long tradition in the study of interspecific hybridization within the genus *Abies* (Kantor and Chira, 1972; Kormuťák, 1985; Greguss, 1984; Janeček and Kobliha, 2007), which focused not only on obtaining artificial hybrids, but also on the processes of spontaneous hybridization. Taxonomy of the genus is an issue of controversies. The state-of-the-art taxonomy comprises 59 species organized in two subgenera and 14 sections (Farjon, 2010). However, many taxa recognized as separate species are considered hybrids or subspecies by different authors (see Farjon and Rushforth, 1989). There are several hybrid zones within the distribution range of the genus, e.g., *A. procera* × *A. lasiocarpa* in North America, *A. sibirica* × *A. nephrolepis* in East Asia or *A. cephalonica* × *A. nordmanniana* (= *A.* ×*bornmuelleriana*) in Turkey (Klaehn and

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Winieski, 1962). From the European perspective, the most interesting hybrid zone is located in the southern Balkans, at the contact of the ranges of two basal species: *A. alba* Mill. and *A. cephalonica* Loud. The hybridogenous taxon was named *A. borisii regis* Mattf. (Mattfeld, 1925). The range of this taxon is fragmented, as it is restricted to high-elevation sites. In contrast to *A. alba*, which is a typical climax species, *A. borisii regis* is capable to colonize free sites close to the upper tree limit. On the lower distribution limit, it forms mixtures mainly with beech (*Fagus sylvatica*). According to the original Mattfeld's description, the taxon differs from both putative parental species mainly by vegetative-organ traits. This may be the reason for a high uncertainty in the delimitation of its range: according to, e.g., Liepelt et al. (2010) it covers the whole central and northern Greece, Macedonia and southern Bulgaria, on the other hand, only the populations in the north Pindos mts. and Thessalia were considered *A. borisii-regis* by Fady (1993).

The origin of *A. borisii-regis* is similarly unclear as its distribution. Mattfeld (1930) proposed two mutually exclusive hypotheses (with several subvariants): either *A. borisii-regis* represents an ancient taxon from which *A. alba* and *A. cephalonica* developed, or it is a product of hybridization between *A. alba* and *A. cephalonica*. Both versions clearly suggest that all three taxa represent a single evolutionary branch, either bifurcated (eventually multifurcated) or reticulated.

The knowledge of variation patterns in cytoplasmic genes with maternal inheritance could substantially contribute to the elucidation of gene exchange among parental and hybridogenous taxa. Such genes are transferred exclusively by seeds and thus allow making inferences about migration (Taberlet et al., 1998). In the Pinaceae family, mitochondrial DNA is maternally inherited (Mogensen, 1996). Liepelt et al. (2010) studied the variation at the mitochondrial *nad5-4* locus in Mediterranean firs, and found variation within *A. alba* as well as its differentiation from *A. cephalonica*. Their study also included one population of *A. borisii regis*, even located in southern Macedonia (FYROM), where occurrence of this taxon is disputable. In any case, such a small sample is insufficient to resolve phylogenetic relationships. Therefore, our study focused on the broader transition zone between *A. alba* and *A. cephalonica* in more detail based on substantially larger sample size. As phylogenetic proximity was found in other species between southern Balkans and southern Apennine peninsula (Gömöry et al., 1999, Musacchio et al., 2006), we also included Calabrian *A. alba* populations. The objectives were (i) describing geographical trends of mtDNA variation in the transition zone and (ii) making inference about the origin of *A. borisii regis*.

Materials and methods

We sampled indigenous silver fir populations from Bulgaria, Macedonia, Greece and Calabria (Italy) (Table 1). Taxonomical determination of the samples at the tree level was impossible, because the sampled trees often represented morphological transitions among morphotypes described by Mattfeld (1930) or Fady (1993). However, at the population level, the prevailing morphotypes were in accordance with a preliminary classification based on distribution ranges according to Fady (1993). Therefore, for operational purposes, the samples from the Peloponesos and the Kefalonia and Euboia islands were initially classified as *A. cephalonica*, Bulgarian and Macedonian (FYROM) populations as *A. alba*, and the populations from the central and northern Pindos and Thessalia as *A. borisii-regis*.

Twigs with 2nd-year needles were collected from approx. 30 trees per population and dried in plastic bags with silica gel until the analysis. Total genomic DNA was extracted from the needles using a modified CTAB protocol following Doyle and Doyle (1987). DNA concentration was measured spectrophotometrically.

The assessment of the mtDNA variation followed Liepelt et al. (2002) with slight modifications. The PCR mixture contained 1 × PCR buffer (Invitrogen, Frankfurt a.M., Germany), 1.75 mM $MgCl_2$, 0.2 µM forward and reverse primer (for primer sequences, see Liepelt et al., 2002), 0.2 µM each dNTP, 0.2 unit *Taq* DNA polymerase and 25 ng of template DNA. The cycle profile consisted of an initial denaturation at 94 °C for 3 minutes, followed by 30 cycles of denaturation at 93 °C for 1 min, annealing at 52.5 °C for 1 min, and extension at 72 °C for 1 min 20 sec, and a final extension step at 72 °C for 8 min. The amplified fragments were separated by electrophoresis in a 1.2% agarose gel for 2 hours at 4.5 V/cm (8 µl of each PCR product).

Haplotypic diversity was calculated according to Pons and Petit (1995) using the program HaploDiv (http://www.pierroton.inra.fr/genetics/labo/Software/Haplodiv/index.html). As the evolutionary history of mitochondrial haplotypes under study cannot be reconstructed from allele sizes, measures for unordered alleles were used. For the estimation of haplotypic diversity, unbiased estimates following Nei (1987) were used. The distribution of haplotypic diversity along the latitudinal gradient was modelled using the Gaussian curve (procedure NLIN, SAS 2009):

$$h = h_{\max}\frac{e^{-[(l-c)/\sigma]^2}}{\sigma\sqrt{2\pi}},$$

where $h_{\max}$ is the maximum attainable diversity (height of the peak), l is the population latitude, c is the latitude

Table 1. Geographical coordinates and mitochondrial haplotype frequencies of the analyzed populations within the *A. alba/A. cephalonica* complex

Population	Country	Classification[1)]	Sample size	Longitude [°]	Latitude [°]	nad 5-4 haplotype frequency		
						150 bp	230 bp	341 bp
Serra San Bruno	IT	A	37	16.3506	38.5736		1.000	
Gariglione	IT	A	38	16.5919	39.0859		1.000	
Aspromonte	IT	A	39	15.8434	38.1683		1.000	
Rilski monastir	BG	A	30	23.3416	42.1323	1.000		
Bansko	BG	A	30	23.4592	41.8012	1.000		
Borovec	BG	A	30	23.6137	42.2631	1.000		
Slavjanka	BG	A	30	23.6406	41.4180	1.000		
Yundola	BG	A	30	23.8589	42.0620	1.000		
Ribaritsa	BG	A	30	24.3337	42.8211	1.000		
Trigrad	BG	A	30	24.3828	41.5995	1.000		
Devin	BG	A	30	24.3951	41.7416	1.000		
Paranesti	BG	A	30	24.4877	41.5093	1.000		
Pamporovo	BG	A	30	24.6945	41.6503	1.000		
Asenovgrad	BG	A	30	24.8511	41.9219	1.000		
Zhenda	BG	A	30	25.1556	41.7910	1.000		
Kirkovo	BG	A	30	25.3716	41.2861	1.000		
Mavrovo	MK	A	30	20.8074	41.7052	1.000		
Pelister	MK	A	30	21.1041	41.0632	1.000		
Olympos	GR	B	30	22.2733	40.1861	1.000		
Anilio	GR	B	30	21.1807	39.7534	1.000		
Pertouli	GR	B	30	21.4857	39.5555	1.000		
Tymfristos	GR	B	30	21.9099	38.9114	0.033		0.967
Rentina	GR	B	22	21.9743	39.0657	0.318		0.682
Komi Evoia	GR	C	11	24.2262	38.6565			1.000
Taygetos	GR	C	10	22.2000	37.1000			1.000
Tithorea	GR	C	8	22.6630	38.5715			1.000
Vytina	GR	C	30	22.1951	37.6500			1.000
Veria	GR	C	30	22.5566	37.1875			1.000
Kefalonia	GR	C	12	20.6238	38.1590			1.000

[1)] A, *A. alba*; B, *A. borisii-regis*; C, *A. cephalonica*; re-classification based on Fady (1993).

where haplotypic diversity attains maximum (center of the peak), and σ is the standard deviation (width of the peak).

Results and discussion

The marker is located within the fourth intron of the mitochondrial NAD dehydrogenase subunit 5 gene (*nad5-4*). As this is a non-coding region, there is a potential for large re-structurations without any effects for fitness. Length differences between alleles are enormous. Liepelt et al. (2002) found in a rangewide study of *A. alba* two variants of the amplified fragment, differing by an 80 bp insertion/deletion. In a later study covering Mediterranean fir species, Ziegenhagen et al. (2005) identified in *A. cephalonica, A. nordmanniana* and their transitional taxa another, very long allele (341 bp). Liepelt et al. (2010) further found another low-frequency allele in their *A. borisii regis* population.

Our results were partially in concordance with these findings (Table 1). In Calabrian populations, exclusively the haplotype characteristic for the western *A. alba* lineage (230 bp) was found. The Bulgarian and Macedonian populations of *A. alba* contained purely the haplotype with allele size of 150 bp (eastern *A. alba* lineage sensu Liepelt et al., 2002). In spite of technical problems associated probably with damaged samples and leading to reduced sample size, all trees originating from populations were initially classified as

A. cephalonica contained solely the 341 bp haplotype. This is in contrast with the findings of Ziegenhagen et al. (2005), who found in their *A. cephalonica* material a mixture of 150 bp and 341 bp alleles. Nevertheless, their material was not sampled in situ but taken from a provenance test, which may already be a source of technical errors. Moreover, they did not exactly specify the location of the analyzed provenances, but they seem to be identical with those used in the study of Liepelt et al. (2010). If so, geographical coordinates indicate that the *A. cephalonica* provenance must have been located somewhere close to Tymfristos, which means that it should be classified as *A. borisii regis* rather than *A. cephalonica*.

Our analyzed populations initially classified as *A. borisii regis* were heterogeneous. The northern ones contained only the eastern *A. alba* haplotype (150 bp), whereas two southernmost populations Tymfristos and Rentina were mixed and contained predominantly the *A. cephalonica* allele (341 bp), with a lower proportion of the 150 bp allele. This means that *A. borisii regis* shares the mitochondrial haplotype with the geographically most proximate populations of *A. alba* and *A. cephalonica*, respectively.

As mentioned in the Introduction, Mattfeld (1930) formulated two hypotheses about the origin of *A. borisii regis*: this taxon is either an ancient one, from which *A. alba* and *A. cephalonica* diverged, or a recent one, product of ancient or recent hybridization of both parental taxa, but anyway younger than they from the evolutionary point of view. In the former case, *A. borisii regis* is expected to be more diverse than the other two taxa and to contain all haplotypes present in the whole taxonomical complex. This proved to be not true, the allele 230 bp was not found in any of the Balkan populations. Of course, a more complicated scenarios are thinkable, where the western *A. alba* haplotype occurred through insertion after *A. alba* diverged from the ancestral population close to *A. borisii regis* or got lost from *A. borisii regis* through genetic drift. Such scenarios are, however, less likely. First, the locus under study is rather conservative, as shown by the studies of Ziegenhagen et al. (2005) and Liepelt et al. (2010). Second, mitochondrial variation within *A. borisii regis* could be geographically structured even if it was an ancestral taxon, but there would be no reason for a concordance of this geographical structure with that of daughter species. The divergence of *A. alba* and *A. cephalonica* must have been an ancient event; the separation of mitochondrial lineages within *A. alba* must have appeared at latest during the Eemian (Liepelt et al., 2009), which means that the separation of species must have occurred earlier. It is utterly improbable that a latitudinal cline in haplotype frequencies that we observed within *A. borisii regis* could have persisted over several glacial/interglacial cycles.

The within-population diversity component is close to zero when all populations are considered ($h_S = 0.018$; Table 2). As already mentioned, the intron under study is conservative, each variant was probably formed by a single mutation event. The range of the genus *Abies* in the Mediterranean area has very probably always been highly fragmented during the Pleistocene, so except the recent hybrid zones, opportunities for gene flow by seeds were extremely rare. Therefore, recent populations are predominantly formed of individuals belonging to a single genetic lineage only. In the absence of mixed populations, no wonder that differentiation is almost complete ($G_{ST} = 0.965$). Of course, our sampling does not completely and regularly cover the species' ranges (especially for *A. alba*), but previous studies (Liepelt et al., 2002; Gömöry et al., 2004) demonstrated that mixed populations are restricted to very narrow hybrid zones in the north-western Balkans and the Ukrainian Carpathians. This means that a more complete and more regular sampling would bring the same outcome.

Table 2. Haplotypic diversity and differentiation measures within the *A. alba/A. cephalonica* complex (mean ± S.D.)

Diversity component	All populations	*Abies borisii regis*
h_S	0.018 ± 0.016	0.103 ± 0.089
h_T	0.519 ± 0.076	0.443 ± 0.141
G_{ST}	0.965 ± 0.029	0.766 ± 0.073

Within *A. borisii regis* itself, differentiation is smaller, but most diversity resides among, not within populations (Table 2). Haplotype frequencies exhibit a latitudinal cline, nevertheless, an extremely narrow one. Consequently, the peak of the diversity distribution along the latitudinal gradient is narrow as well, peak width is only 0.113°, which represents 12.49 km (Fig. 1).

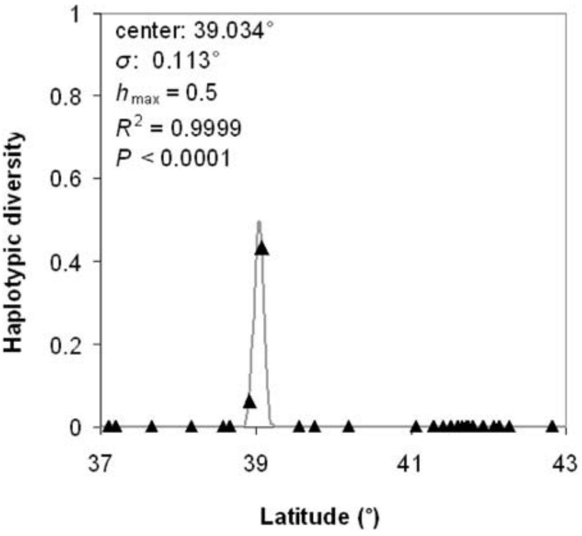

Fig. 1. Distribution of haplotypic diversity along the latitudinal gradient fitted to the Gaussian function.

All known facts support thus the latter Mattfeld's (1930) hypothesis, namely that *A. borisii regis* is a hybridogenous taxon, a product of a relatively recent bidirectional hybridization. Hybrids among phylogenetically close fir species are generally fertile (Klaehn and Winieski, 1962), so that *A. borisii regis* may represent a hybrid swarm. However, nuclear markers are necessary for the assessment of the extent of introgression. In any case, whatever concept of *A. borisii regis* distribution is adopted, the taxon contains both the eastern *A. alba* and the *A. cephalonica* haplotype, distributed along a geographical gradient connecting the ranges of both parental species. This pattern can most plausibly be explained by restricted seed migration from opposite sources.

As artificial hybrids between *A. alba* and *A. cephalonica* generally exhibit hybrid vigour in growth and belong to the most prospective ones (Greguss, 1984), the natural hybrid *A. borisii regis* may be interesting from the point of view of commercial forestry. Silver fir in central Europe suffers from periodically appearing syndrome of fir decline with unknown etiology (Larsen, 1986). *A. borisii regis* may become an option for the replacement of silver fir. Nevertheless, transplanting experiments are needed to verify whether this option is viable.

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