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Hyphomycetes and Coelomycetes fungi isolated from affected leaves and twigs of cherry laurel trees

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Abstract

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The aim of this work was to identify fungi occurring on leaves and twigs of *Prunus laurocerasus* L. Mycological observations were carried out on leaves and twigs collected from symptomatic cherry laurels growing in green areas of the Nitra town from spring to autumn in 2009–2010. Six species of microscopic fungi were isolated and microscopically identified from samples during the study period. The occurrence of some microscopic fungi in class Hyphomycetes (*Alternaria alternata* (Fr.) Keissl., *Fusarium oxysporum* Schltdl., *Thielaviopsis basicola* (Berk. & Broome) Ferraris, *Trichothecium roseum* (Pers.) Link) and Coelomycetes (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Phomopsis* sp.) has been confirmed. The most frequently found fungi included: *Alternaria alternata*, *Colletotrichum gloeosporioides* and *Phomopsis* sp. The fungus *Fusarium oxysporum* was found in examined samples relatively commonly. Only sporadical incidence of the fungi *Thielaviopsis* and *Trichothecium* was noticed.

Key words

Alternaria, Coelomycetes, *Colletotrichum, Fusarium,* Hyphomycetes, *Phomopsis, Prunus laurocerasus, Thielaviopsis, Trichothecium*

Introduction

Prunus laurocerasus L., common name cherry laurel, sometimes called English laurel is an evergreen shrub or a small tree in Rosaceae family. Cherry laurel was frequently planted as an ornamental plant in temperate regions worldwide, and has become naturalized widely in some areas. It is often used as a mass landscape and ground cover plant. Cherry laurels growing in urban green areas are susceptible to various pathogens. Infected plants develop discoloration, brown spots and necroses, affecting their aesthetic value. The symptoms of infection, observable from spring to autumn, increase when the plants are in bloom – resulting in dieback and leaf drop. The damage is caused by fungi of the class Hyphomycetes, genera *Alternaria, Fusarium*, *Thielaviopsis* and *Trichothecium* and the class Coelomycetes, genera *Colletotrichum* and *Phomopsis*.

The class **Hyphomycetes** is a class of fungi belonging to the Deuteromycotina. They lack locular fruiting bodies (conidiomata), and sporulation occurs on separate or aggregated hyphae, which may or may not be differentiated; the thallus consists of septate hyphae. The unifying feature of the group is the production of conidia from superficial, exposed conidiogenous cells arising separately from vegetative hyphae (hyphas) or cells. Hyphomycete colonies are conspicuous as black, brown, green, gray, and white growths on substrates.

The Hyphomycetes draw nourishment from living or dead organic matter, having been adapted to grow, reproduce, and survive in a wide range of ecological situations. Many also cause economically important diseases in all types of vascular plants, especially agricultural and forestry crops. Hyphomycetes are primary pathogens of plants and weeds, causing root, stem and leaf necrosis, diebacks, cankers, wilts and blights (ELLIS, 1976; ELLIS and ELLIS, 1987).

The class **Coelomycetes** includes conidial fungi with conidia formed within a cavity lined by fungal or host tissue. The fruiting structures may be spherical with an apical opening (pycnidia) or saucer shaped (acervuli). The production of conidia within a fruiting body distinguishes this group from the Hyphomycetes which have "naked" conidia. Pycnidia may be confused with ascocarps. Fungi with sporulation occurring inside fruiting bodies (conidiomata) arise from a thallus consisting of septate hyphas (CANO et al., 2004).

Coelomycetes are known mainly from temperate and tropical regions. They grow, reproduce, and survive in a wide range of ecological environment and can be categorized as either stress-tolerant or combative species. They are commonly found in and recovered from soils, leaf litter and other organic debris from both natural and manufactured sources and saline and fresh water; and on other fungi and lichens. Coelomycetes are consistently isolated from or associated with disease conditions in all types of vascular plants, often in association with other organisms (SUTTON, 1980).

During our investigation on the mycoflora of cherry laurel trees, isolates of some fungi of Hyphomycetes and Coelomycetes were obtained. The aim of this work was to identify the fungal microorganisms, occurring on affected cherry laurel leaves and twigs which are causal agents involved in health state degradation of *Prunus laurocerasus* L. in urbanized settings of the Nitra town.

Material and methods

From spring to autumn of the years 2009 and 2010, there were collected leaves and twigs of *Prunus laurocerasus* with the symptoms of discoloration, brown spots or necroses from affected plants in parks, private gardens and settlement greenery of the town of Nitra. Visual characteristics of necrotic and chlorotic leaves and damaged twigs were examined with a stereomicroscope SZ51 (Olympus). Investigation of fungal structures (conidia, conidiophores, pycnidia, acervuli) immersed in water was performed with using a clinical microscope BX41 (Olympus) under 400× and 1,000× magnification.

The leaf and twig segments cut from the diseased plants were surface-sterilized with a 3% sodium hypochlorite solution for 20 min, rinsed in sterile distilled water (2–3 times) and dried carefully with filter paper. After the surface sterilization, the tissue samples were cut to small pieces (4–5 mm), placed on potato-dextrose agar (PDA) and subsequently incubated in Petri dishes. Subsequent cultivation in a versatile environmental test chamber MLR-351H (Sanyo) at 24 ± 1 °C temperature, 45% humidity and photoperiod of 12/12 hours and isolation on potato-dextrose agar (PDA) was performed. Pure cultures were obtained after multiple purifications.

The colonies of fungi were identified using various keys for identification: ANDERSEN et al. (2002), ARX (1970, 1981), BURGESS et al. (1988), ELLIS and ELLIS (1987), HANLIN (1973), MORDUE (1971), SAMSON et al. (2002), SIMMONS and ROBERTS (1993) and SUTTON (1980), based on micro- and macroscopic symptoms of isolates. The samples of material have been deposed at the Institute of Forest Ecology of the Slovak Academy of Sciences, Branch for Woody Plants Biology in Nitra.

Results and discussion

Many fungal diseases cause damage to fruit and ornamental tree species in the genus *Prunus*, including cherry laurel. Among pathogenic fungi, microscopic pathogens isolated and identified from the affected leaf and twig tissues include hyphomycetous fungi in the genera *Alternaria, Fusarium*, *Thielaviopsis* and *Trichothecium* and coelomycetous fungi in the genera *Colletotrichum* and *Phomopsis*.

Class Hyphomycetes

Alternaria alternata (Fr.) Keissl. has been recorded causing leaf spot and other diseases on over 380 host species. It is an opportunistic pathogen on numerous hosts causing leaf spots, rots and blights on many plant parts (SILVA and MELO, 1999). Our study and morphological identification has showed that *A. alternata* is the quite common pathogenic fungus associated with affected cherry laurel leaves isolated frequently in examined samples. Early symptoms appeared as small, circular to oval, light brownish spots (25–38 mm), 2–6 per leaf, scattered at the tip, margin, and midrib of the leaves. Subsequently, the spots enlarged and usually developed into a concentric ring. At the advanced stage, the spots became dark brown to blackish in colour, gradually coalesced, and irregular in shape (TIMMER et al., 2003; MAITI et al., 2007). According to PERES and TIMMER (2006), the disease (*Alternaria* brown spot) produces black necrotic lesions on young leaves, twigs and fruit. On leaves, lesions may expand easily, due to the production of a host-specific toxin by the pathogen, resulting in leaf drop and twig dieback, in most cases.

Teleomorph of this fungus is unknown. Anamorph includes pale brown to olive brown conidiophores, 25–60 × 3–3.5 μm in size, straight or flexuous. Individual conidiophores arise directly from substrate forming bushy heads consisting of 4–8 large catenate conidia chains. Secondary conidiophores are generally short and 1-celled. Conidia are pale brown to light brown, obclavate to obpyriform or ellipsoid, short conical beak at the tip, or beakless, surface smooth to verruculose 20–63 × 9–18 μm in size, with 3–7 transepta and 1–5 longisepta inside (Simmons and Roberts, 1993). In our observations, the pale brown spores clavate in shape contained 3–8 transverse and 1–4 vertical septa (Figs. 1a, 1b). The fungus produced on potato-dextrose agar abundant brownish to dark brown or black mycelium (Fig. 1c). Characteristics of conidia from cultures were similar to those of conidia isolated from infected plants. Based on the morphological characters, the organism was identified in accordance with mycological keys such as *Alternaria alternata* (Andersen et al., 2002; Simmons and Roberts, 1993).

Fusarium oxysporum Schltdl. belongs to a large genus of filamentous fungi massively occurring in soil and in association with plants where can cause root rot and seedling blight. The first indication of this disease is yellowing and dropping the lower leaves. This symptom often occurs only on one side of the plant or on one single shoot. Successive leaves turn yellow, wilt and die, often before the plant has reached maturity. As the disease progresses, growth is typically stunted, and little or no fruit develop (Guarro and Gene, 1992). In our study, *F. oxysporum* was isolated from cherry laurel leaves at a high frequency and the fungus was identified as relatively common pathogenic fungus from all inspected samples.

Fusarium wilt is a warm-weather disease, most prevalent on acid, sandy soils. The pathogen is soilborne and remains in infested soils for up to ten years. Soil and air temperatures of 28 °C are optimum for the disease. If soil temperature is optimum but air temperature is below optimum, the pathogen extends into the lower stem parts; the plants, however, do not exhibit external symptoms (Nelson et al., 1994; Wong, 2003).

Mycelia of *F. oxysporum* are delicate white to pink, often with purple tinge, sparse to abundant in occurrence. According to Nelson et al. (1981), *Fusarium oxysporum* forms three types of asexual spores: macroconidia, microconidia and chlamydospores. Leslie and Summerell (2006) observed that macroconidia are formed from monophialides on branched conidiophores in sporodochia, and to a lesser extent from monophialides on hyphae. According to Jones (2000) the macroconidia are four to eight celled, sickle-shaped, thin-walled and delicate, with foot-shaped basal attenuated apical cells. Microconidia, abundantly borne by false heads on short monophialides, are one or two celled, oval to kidney shaped. Burges et al. (2008) observed microconidia small, usually non-septate and formed in false heads on very short phialides. According to Wong (2003) microconidia are borne on simple phialides, arising laterally in large amounts, oval to ellipsoid, straight to curved, 5–12 × 2.2–3.5 μm in size, nonseptate. Macroconidia, sparse to abundant, are borne on branched conidiophores or on the surface of sporodochia. They are thin walled, three- to five-septate, fusoid-subulate and pointed at both ends, having pedicellate base.

The typical dimensions of the macro- and microconidia are (typically) 27–55 × 3.3–5.5 μm and 5–16 × 2.4–3.5 μm. Chlamydospores are thick-walled, asexual, globose spores, large 7–11 μm. They occur usually singularly or in pairs, but may be also found in clusters or short chains. The critical morphological features of this fungus include: production of microconidia on false heads on short phialides formed on hyphae, production of chlamydospores, and the shape of the macro- and microconidia (Jones, 2000; Leslie and Summerell, 2006).

Three-septate conidia are 27–46 × 3–5 μm in size, while five-septate conidia measure 35–60 × 3–5 μm. Macroconidia, fusoid, gradually becoming slender and pointed towards the ends, with an undifferentiated foot cell, are usually three-septate (Fig. 2a), rarely four-septate (Fig. 2b), as it is demonstrated also in our microscopical observations. Chlamydospores (Fig. 2c), smooth and thick-walled, ellipsoidal to globose or round in shape are abundant and form terminally or on an intercalary basis. They generally develop in chains of 2 and 3 and rarely of 4 and up to 6. No perfect stage is known. *F. oxysporum* in culture produced aerial floccose, white and light-pink mycelium often with darker shade of pink or purple on PDA (Fig. 2d).

Thielaviopsis basicola (Berk. & Broome) Ferraris (syn. *Chalara elegans* Nag Raj & W.B. Kendr., *Torula basicola* Berk. & Broome, *Trichocladium basicola* (Berk. & Broome) J.W. Carmich.) is a soil inhabitant – the cause of black root rot that attacks more than 200 plant species in 33 families (Shew and Meyer, 1992). Black root rot is a widespread and destructive root disease, most prevalent under conditions stressful to the host plant. General symptoms are: root rot, foliar discoloration, foliar necrosis and branch dieback. Cherry laurel infected with black root rot displays aboveground symptoms typical for other root rotting diseases – including stunting and/or wilting of plants and foliar chlorosis. In early stages of the disease, the roots, normally white, develop dark spots or bands. Early symptoms may be more prevalent in secondary feeder roots. In advanced cases, the root system becomes black and water-soaked. In our field monitoring, similar disease symptoms on cherry laurel leaves were noticed. The disease caused by the fungus *Th. basicola* can be especially destructive during the late summer months when the temperature is high.

The pathogen may survive for years in form of chlamydospores in absence of host plants or parasitically on plants different from the hosts. Symptoms on hosts are: black cortical rot of the roots that stunts plant growth, thus delaying development and reducing yield. Generally this disease is a chronic problem, rarely killing the host (Avanzato and Rothrock, 2010). During the study period, fungus *T. basicola* was found as a fungal pathogen with infrequent incidence. Its presence was noticed sporadically in damaged leaf tissues.

According to morphological studies of Hanlin (1973) and Abbas et al. (2007), the fungus grows poorly on synthetic media. The optimum temperature range for growth in culture is between 22 °C and 30 °C. Mycelium produced by *T. basicola* can be cinnamon to light brown or gray to light black. The septate hyphae are hyaline at first and become pigmented with age. The sexual state has not been observed in *T. basicola*.

The fungus reproduces asexually by the production of two types of conidia, endoconidia and aleurioconidia (chlamydospores). Endoconidia are clear and cylindrical, produced in larger numbers than chlamydospores. These hyaline, cylindrical spores with slightly rounded ends, markedly vary in size (8–30 × 3–5 μm). Chlamydospores are dark, thick-walled (25–65 × 10 –12 μm), often produced in chains (two to eight spores per chain).

Based on morphological features, the fungus was identied as *T. basicola* after reference to Ellis (1976) and Punja and Sun (1999). In microscopic examination in our laboratory presence only of unicellular thick-walled brown chlamydospores in chains of 5–6 was observed (Fig. 3). No endoconidia were observed.

Trichothecium roseum (Pers.) Link (syn. *Cephalothecium roseum* Corda, *Sphaeria rosea* Pers., *Trichoderma roseum* Pers.) is a filamentous mitosporic fungus word-widely distributed. The fungus is mostly saprophytic or weakly parasitic (Barnett and Hunter, 1972). It was found as laboratory contaminant, was previously recorded on felled trunks and fallen branches of *Acer, Corylus, Fagus, Prunus, Quercus* and *Ulmus* (Ellis and Ellis, 1985). Our survey has resulted in finding that *T. roseum* is the rare pathogenic fungus isolated from inspected leaf tissues in connection with attacked cherry laurel trees.

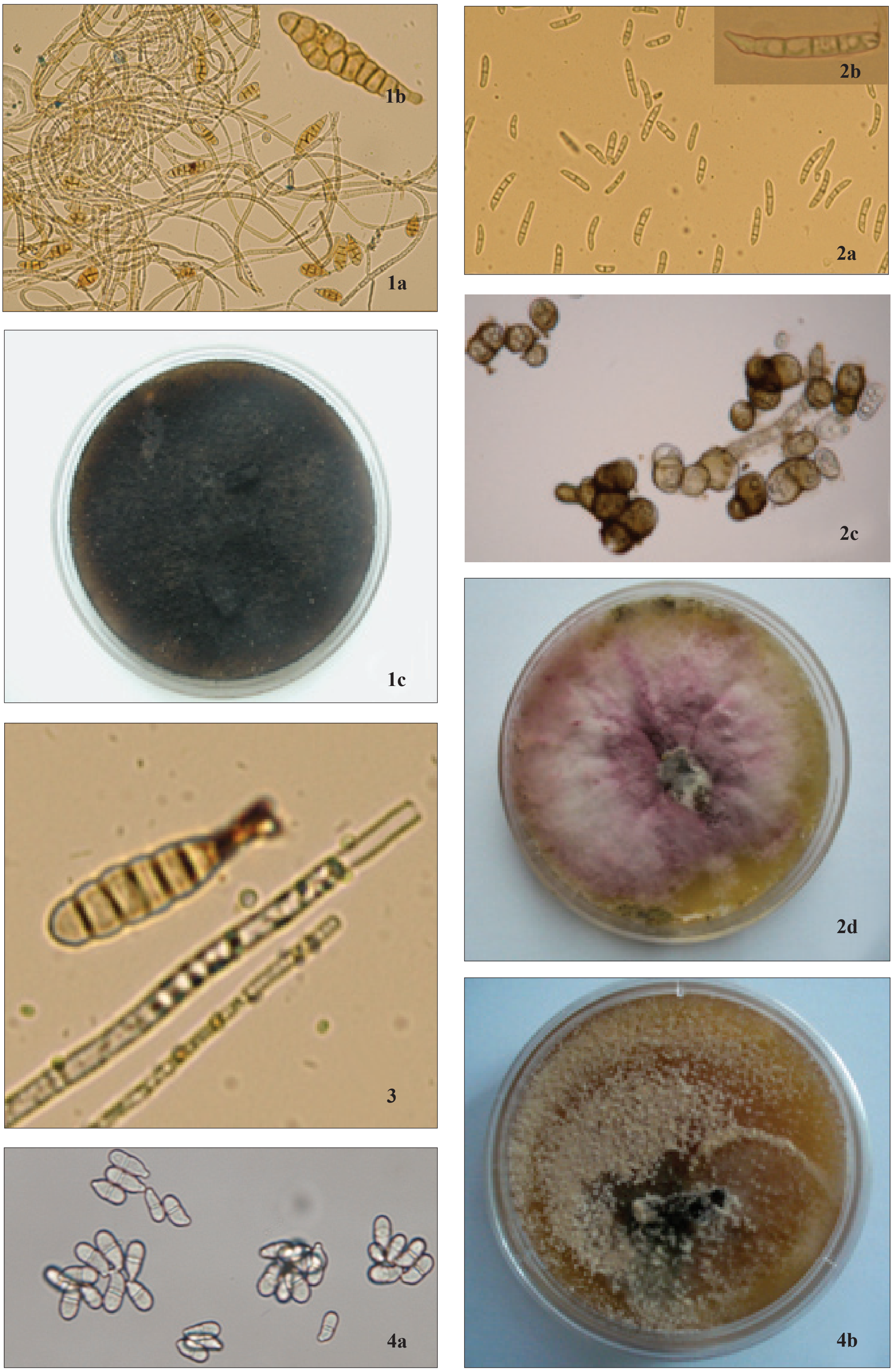
Colonies of *Trichothecium* grow rapidly. At 25 °C and on potato-dextrose agar, colonies are flat, granular and powdery. From the front, the colour is white initially and becomes pale pink to peach-coloured, reverse is pale. The conidiophores are indistinguishable from the septate hyaline vegetative hyphae until the first conidium is produced. They are long, erect, unbranched, often septate near the base, more or less rough-walled, bearing basipetal zig-zag (alternating) chains of conidia at the apex. According to Shamsi and Sultana (2008) conidiophores are up to 147 × 3.0–4.5 μm, hyaline, often slightly swollen at their tips. Conidia are two celled, ellipsoidal to pyriform, with an obliquely truncate basal scar, hyaline to lightly coloured, pink in mass, smooth to delicately roughened and slightly thick-walled, 8–10 × 12–18 μm or 13.5–27 × 8–11 μm, often clustered. Wright et al. (2007) observed several diseases on *Rosa* species, associated with pruning or harvest wounds. They obtained pure, salmon-coloured fungal colonies of *T. roseum* developed within 72 hours. Hyaline, two-celled, ovoid to ellipsoid conidia formed in chains at the apex of simple, long, slender, septate conidiophores.

According to our observations, conidia with one double septum were ellipsoidal to pyriform, hyaline, thick-walled, each with a flattened protuberance at the base, often forming a cluster (Fig. 4a). Colonies of *T. roseum* moderately fast growing, flat, suede-like to powdery, initially white but becoming rosy, pink or salmon-pink with age mycelium were observed on PDA (Fig. 4b).

Class Coelomycetes

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc., teleomorph *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk is known to infect a wide variety of hosts. Fungus is a common saprophyte and secondary invader of damaged tissue. It also causes anthracnose of stems and leaves, dieback, root rot, leaf spot, blossom rot, fruit rot (dieback and ripe rot) and seedling blight on a large variety of plants (Arx, 1970; Farr et al., 1989; Sutton, 1980, 1992). Environmental conditions favouring the pathogen are high temperature (28 °C being optimal) and high air humidity. Spores are released only from acervuli in presence of abundant moisture. The disease is strongly controlled by weather – the fungus is very low active in dry weather. Primary inoculum can be disseminated by wind or rain. Fungi can overwinter on plant foliage, debris and rotten fruits. Fungi of the *Colletotrichum* species naturally produce micro-sclerotia rendering them dormant and inactive in soil during the winter or under stressful conditions. These micro-sclerotia can survive for many years (Pring et al., 1995; Roberts et al., 2009). Initial infection by *Colletotrichum* species involves a series of processes including the attachment of conidia to plant surface, germination of conidia, production of adhesive appressoria, penetration of plant epidermis, growth and colonization of plant tissue, production of acervuli and sporulation (Trujillo and Obrero, 1969; Martínez et al., 2009).

The first symptoms of anthracnose are round, water-soaked, and sunken spots. Lesions may become as large as 5 cm in diameter. Pinkish-orange areas are formed by conidial masses covering the lesion centre. Symptoms also may appear as irregular to circular spots of 1 to 10 mm in diameter, sharply defined, occasionally slightly depressed and reddish-brown in colour. Invaded leaves prematurely wither and fall down. Waxy acervuli created in the infected tissue are subepidermal, typically with setae; conidiophores are simple, short and erect. The fungus was observed producing hyaline, one-celled, ovoid to oblong, slightly curved conidia, 10–17 μm in length and 3.5–7 μm in width (Dickman and Alvarez, 1983), or 9–24 × 3–4.5 μm (Gangadevi and Muthumary, 2008). According to Kim et al. (2001) conidia of *C. gloeosporioides* isolated from anthracnose lesions on perilla plants [*Perilla frutescens* Britton var. *japonica* (Hassk) Hara] are straight, cylindrical, obtuse or round at ends, similar in dimensions: 12–18 × 4–6 μm. Masses of conidia appear pink or salmon coloured.



Figs 1–4. Hyphomycetous fungi isolated from affected leaves and twigs of *Prunus laurocerasus*:

1. *Alternaria alternata*: 1a, Spores with septa (400×); 1b, Conidium in detail (1,000×); 1c, Culture on PDA.
2. *Fusarium oxysporum*: 2a, 3-septate macroconidia (400×); 2b, 4-septate conidium in detail (1,000×); 2c, Chlamydospores (400×); 2d, Culture on PDA.
3. *Thielaviopsis basicola*: Chlamydospores (1,000×).
4. *Trichothecium roseum*: 4a, Conidia in clusters (1,000×); 4b, Culture on PDA.

Our study shows that *C. gloeosporioides*, a common coelomycetous fungus isolated from infected leaves of symptomatic cherry laurel trees, was noticed regularly during microscopic examination. In our observation, *C. gloeosporioides* produced *in vivo* subepidermal acervular conidiomata occurring in the affected tissue in aboveground plant parts. Acervuli were small pinkish, dark-gray or black pustules 0.5 mm in diameter (Fig. 5a). Profuse production of straight, cylindrical, 1-celled conidia with obtuse apex and truncate base, 12–17 × 3.5–6 μm was observed in our microscopical examinations (Figs. 5b, 5c). Sclerotia did not develop. Production of appressoria failed. The fungus formed flattened dark-gray mycelium on PDA (Fig. 5d). These morphological characters correspond to the description of *C. gloeosporioides* published by Cano et al. (2004).

Phomopsis (Sacc.) Bubák is a large coelomycetous genus including over 1,000 species described primarily on the basis of their plant host (Uecker, 1988). *Phomopsis* sp., the imperfect stage of the genus *Diaporthe*, is a more and more common fungal pathogen of the genus *Prunus*, to which it causes an economically important disease called *Phomopsis* dieback.

Ascospores and/or conidia of *Diaporthe* and *Phomopsis* are dispersed in splashing and windblown water droplets. When the pycnidial substrate is moist, the conidia in a mucilaginous matrix extrude from the fruiting body. *Diaporthe* and *Phomopsis* survive winter in form of mycelium and often as fruiting bodies in dead bark and sometimes in leaves. The infection may be caused by either conidia or ascospores. Some species can penetrate intact young leaves or shoots (Sinclair and Lyon, 2005).

Arsenijević (2003) gives the main characteristics of conidiomata and the signs designated of the *Phomopsis* strains isolated from various woody plants in Serbia. Cankered cherry laurel branches were infected and damaged by *Phomopsis perniciosa* Grove. According to Sinclair and Lyon (2005), *Diaporthe ambigua* and *D. perniciosa* (*Phomopsis ambigua* and *P. prunorum*, respectively) cause canker and dieback on *Prunus* (cherry and peach). *Phomopsis perniciosa* is a pathogen causing stem canker on twigs of some *Prunus* species and other hardwood plants (Kokubun et al., 1994). This fungus has also been found causing rot and branch canker of *Prunus armeniaca* L. in Serbia (Garić and Arsenijević, 1990), *P. domestica, P. persica* and *P. spinosa* (Ellis and Ellis, 1997).

Typical symptoms noticed on *Prunus laurocerasus* in this study included brown, round lesions surrounded by pale yellow areas on the leaves and brown to black lens-shaped lesions on the first three to four internodes on green shoots. Lesions often became numerous and covered large areas of leaves. Severely infected leaves became torn, yellow and wither. Small black dots, the fungal fruiting bodies called pycnidia were often apparent on older leaves (Fig. 6a), stems and in fruit lesions. Stems and branches may develop dry, brown, cracked and sunken cankers. If a canker develops at the base of a stem, it can girdle and kill the stem. In our morphological survey, the fungus in the genus *Phomopsis* was noticed as one of the most frequent fungi isolated from the infected leaves and branches of symptomatic cherry laurels and commonly identified in examined samples.

In the genus *Phomopsis* pycnidia, perithecia precede in killed tissues, often in the same stroma. Each pycnidium produces two kinds of colourless, unicellular conidia: α-conidia – ellipsoid to spindle-shaped cells that germinate readily, with dimensions 5–11 × 1.5–2.5 μm, usually with two oil droplets (guttules); and β-conidia – filamentous curved cells 15–20 × 0.7–1 μm in size that do not germinate (Sinclair and Lyon, 2005). According to Rensburg et al. (2006) α-conidia are biguttulate, fusoid with obtuse ends, (6–) 7–8 (–9) × (2–) 2.5 (–3) μm, β-and γ-conidia absent. The majority of *Phomopsis* species do not have a *Diaporthe* state and do not produce β-spores readily. In our experiments, we obtained only 1-celled, oval to ellipsoidal α-conidia, usually with two oil guttules (Figs. 6b, 6c). No β-conidia were observed. Colonies on PDA after 8 days at 25 °C were primarily white, with yellowish-gray to brownish-gray coloration, surface mycelium felty to cottony, dense and aerial. Three weeks after the cultivation, black fruiting bodies appeared on the surface of plate with mycelium (Fig. 6d).

The health state of cherry laurel trees in urban greenery may be negatively affected by various microscopic fungal pathogens. In this paper, the fungi in the Hyphomycetes and Coelomycetes classes were identified with using morphological keys. Since the morphological characteristics may not be fully reliable, the methods of molecular biology are required for detailed study and confirmation of the concerned pathogens.

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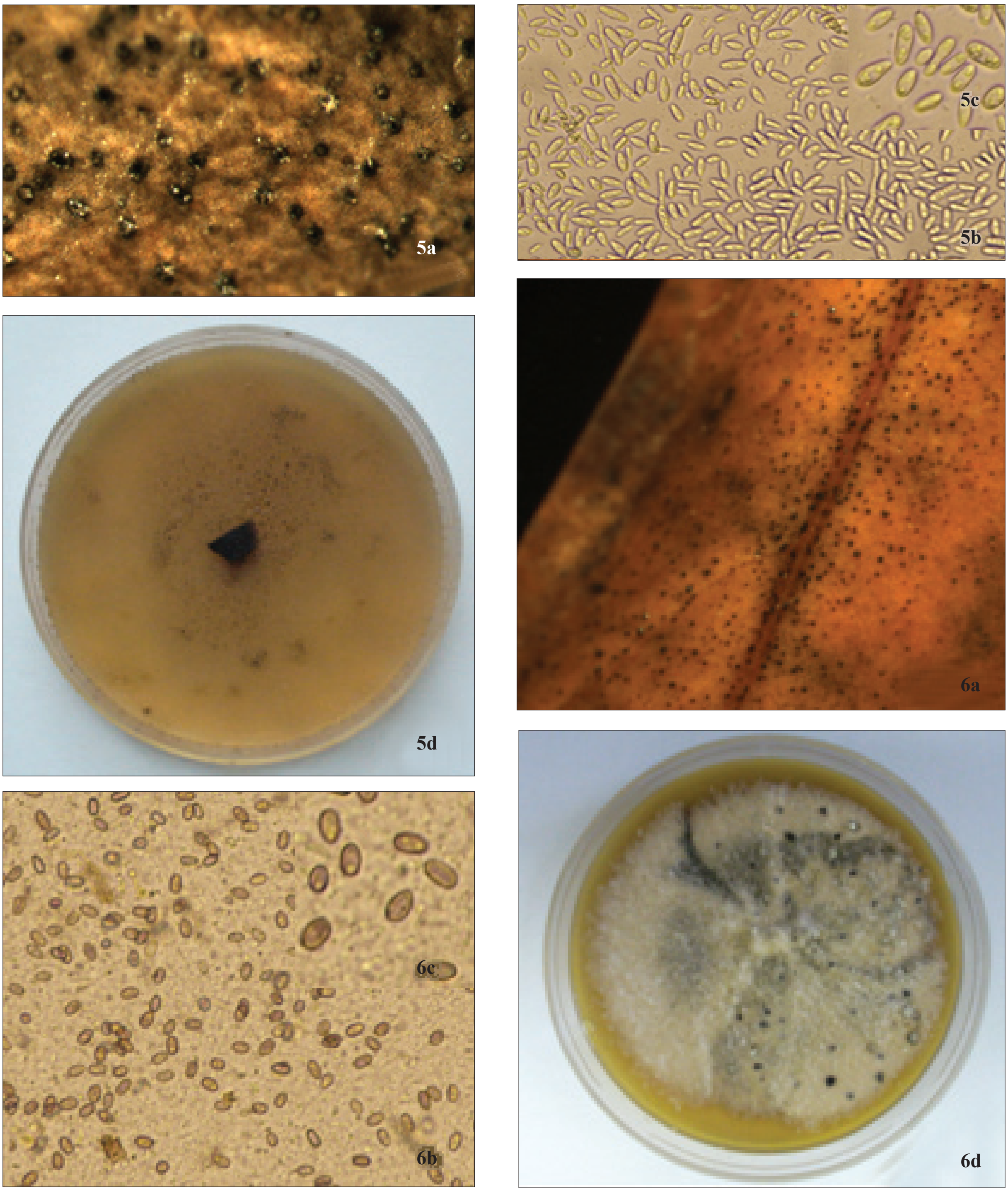

Figs 5–6. Coelomycetous fungi isolated from affected leaves and twigs of *Prunus laurocerasus*:
5. *Colletotrichum gloeosporioides*: 5a, Black acervuli on affected leaf; 5b, 1-celled conidia with obtuse apex and truncate base (400×); 5c, Conidia in detail (1,000×); 5d, Dark-gray mycelium on PDA.
6. *Phomopsis* sp.: 6a, Black pycnidia on older infected leaf; 6b, 1-celled α-conidia with two oil guttules (400×); 6c, Conidia in detail (1,000×); 6d, Culture with dense mycelium with black fruiting bodies 3 weeks after cultivation at 25 °C on PDA.

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Niektoré huby triedy Hyphomycetes a Coelomycetes izolované z napadnutých listov a konárov vavrínovca lekárskeho

Súhrn

Práca prezentuje výsledky štúdia druhovej diverzity mikroskopických patogénov, pôvodcov hubových chorôb okrasnej dreviny *Prunus laurocerasus* L. pestovanej ako súčasť urbánnej vegetácie mesta Nitra. V priebehu vegetačných období rokov 2009–2010 boli zo vzoriek listov a konárov symptomatických jedincov vavrínovca lekárskeho izolované a mikroskopicky identifikované huby triedy Hyphomycetes (*Alternaria alternata* (Fr.) Keissl., *Fusarium oxysporum* Schltdl., *Thielaviopsis basicola* (Berk. & Broome) Ferraris, *Trichothecium roseum* (Pers.) Link) a Coelomycetes (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Phomopsis* sp.). Dominantné druhy izolované z väčšiny skúmaných vzoriek zahŕňali huby: *Alternaria alternata*, *Colletotrichum gloeosporioides* a *Phomopsis* sp. Relatívne bežne bola izolovaná huba *Fusarium oxysporum*. Sporadický výskyt bol zaznamenaný pre druhy rodu *Thielaviopsis* a *Trichothecium*. Príspevok popisuje určujúce znaky anamorfných štádií (konídie, pyknidy, acervuly) skúmaných húb a ich kulturálne charakteristiky (rast hýf mycélia na živnom médiu, vzhľad kultúry). Výsledky práce naznačujú možnosť oslabenia zdravotného stavu *Prunus laurocerasus* vzájomným spolupôsobením izolovaných mikroskopických húb, ktoré sa podieľajú rozličnou mierou na poškodení hostiteľa.

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Effect of fruit size, parental trees origin and trial conditions on the growth characteristics of European chestnut seedlings

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Abstract

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In the spring of 2005, a field trial was established in forest nursery Hladomer, near community Lovce, Slovakia with the aim to find out an effect of different origin of fruits on growth characteristics of seedlings. Fruits were collected from twelve half-sib families of European chestnut, grown at an experimental plot in Horné Lefantovce. All studied families originated from old trees grown at four different localities of Slovakia (three half-sib families per locality) and exhibited above-average growth characteristics among all of 120 progenies grown at the experimental plot. Variability of growth characteristics (stem height and stem diameter) of both one- and two-year-old seedlings was significantly affected by their origin (locality of parental tree and half-sib family derived from these trees) and by different nut size used in three trial replications. In three of four studied origins, non significant differences in stem height of one-year-old seedlings between second and third replications changed to significant ones in two-year-old seedlings. These differences were assigned to different light and soil conditions on two trial blocks caused by partial shading of first and second trial blocks. Between weight of fruits and studied growth characteristics either low correlations (for data of individual fruits) or medium strong correlations (data grouped by fruit groups of different weight) were observed. Correlation between stem height means of families and stem height means of one-year-old and/or two-year-old seedlings derived from these progenies was only medium strong (r = 0.52 and 0.54) however similar inter-generation in stem diameter was very low and nonsignificant.

Key words

Castanea sativa Mill., correlation, half-sib families, seedlings, stem diameter, stem height, variability

Introduction

Although in the Slovak Republic the European chestnut is known rather as fruit tree, total area of chestnut orchards, in majority composed of old trees of seed origin, is only about 130 ha including some 30 ha of relatively young chestnut plantations established 30 to 40 years ago. Just at that time also the more intensive exploitation of chestnut in forestry begun by establishing plantations of different large area (from 1 to 20 ha) situated within indigenous forest stands or in open land. Young chestnut stands, at age up to 20 years, represent the largest proportion of chestnut plantation area in our country (about 75% of total 1,400 ha). This increased interest for chestnut resulted from favourable ecological-production characteristics observed in this species in the area of its distribution in Slovakia (high growth increment, good wood properties, intensive decomposition of litter, favourable influence on accompanying tree species, good natural regeneration). Simultaneously with chestnut plantations for forest purposes, also several experimental plots in pure and mixed chestnut stands respectively were established to study stand, production and some bio-ecological characteristics in relation to thinning interventions and species mixture. The obtained results suggested a rather good growth and production characteristics of young pure and also mixed chestnut stands (Tokár, 1985; Tokár, 1990; Tokár, 1992; Tokár, 1994; Tokár, 1999).

In addition to the mixed seed lots from old chestnut stands also seed samples from single old chestnut trees from different sites of Slovakia were used to establish experimental plots with pure chestnut stands at the experimental site Horné Lefantovce in 1965 and 1969. This method was applied in order to find a suitable source of seed for establishing future chestnut stands, with good quality timber production. Since the age of 10 years the stands of half-sib-families were periodically in five-year intervals evaluated for production and ecological parameters (basal area, stand volume, aboveground biomass, leaf area index etc.) in relation to social classes and thinning interventions. The significant differences among stands of different families in most of production characteristics observed during first evaluation were observed also in subsequent periodical evaluations (Benčaťand Tokár, 1979, 1980; Benčať and Golha, 1980; Benčať and Tokár, 1984; Tokár, 1996; Tokár and Bolvanský, 2002; Tokár, 2003).

The main objective of this work has been to test capability of the selected half-sib families of European chestnut to reproduce their above average growth potential by seed. Particularly stem height and stem diameter of one-year-old and two-year-old seedlings were studied in relation to: a) seed size, b) affiliation to different half-sib family and c) different environmental conditions at the experimental plot.

Material and methods

Study site

Experimental plot with the chestnut seedlings was established in the Nursery center Hladomer near of the village Lovce. This nursery belongs under national company Forests of Slovak Republic, enterprise Semenoles Liptovský Hrádok. It is situated at altitude 310 m a.s.l., in the south part of Tribeč Mts (48°26'41" N, 18°20'15" E). Soil in the forest nursery is clayey and in the part with our experiment has pH 7.2, content of phosphorus 233 mg, potassium 231 mg and magnesium 295 mg per 1 kg of dry soil.

Plant material

On the experimental plots in Castanetarium Lefantovce twelve half-sib families of European chestnut exhibiting above average parameters of growth characteristics were selected in order to collect fruits for growing seedlings. The selected progenies were derived from 12old mother trees grown on four different localities of Slovakia, 3 trees from each locality (Jelenec, Horné Lefantovce, Tlstý Vrch and Duchonka). At the time of fruit fall in 2004, about 150 fruits were randomly collected under trees of each progeny. Out of this amount 90 fruits were detached and divided to three groups per 30 nuts. The first group contained nuts with relatively large size and remaining two groups medium-sized and small-sized nuts respectively. Sorting was done visually. Nuts in each group were labeled with white permanent marker with numbers from 1 to 30 and individually weighted. Then the sorted fruits and remaining fruits were stored in plastic bags in refrigerator at the temperature of about 5 °C. During the storage, in one month intervals, the nuts were checked for their health condition and those damaged and spoiled by fungi and chestnut weevil larvae were discarded and replaced by healthy weighted nuts of similar size labeled with the same number as discarded nuts.

Nuts of each size-group from all 12 families were sown separately in three randomized blocks in forest nursery Hladomer. Because of space restrictions, experimental plot was of atypical, protruded rectangular shape (1.4 m × 28 m) and was congruent with the shape of beds used in this nursery. Nuts of each progeny were seeded in rows with 20 cm between rows and 15 cm within row. Number of germinated seedlings per progeny varied from 57 to 82.

In late autumn 2005 and early spring 2007, during dormancy period, in all seedlings grown from planted seed, stem height and stem diameter at the base were measured. Stem height was measured in cm with accuracy of 0.5 cm and stem diameter in mm with accuracy of 0.01 mm (by electronic sliding gauge).

Data analysis

Morphometric data of studied chestnut seedlings were subjected to computing of descriptive statistics, multifactor analysis of variance – General Linear Model, correlation analysis (Pearsons coefficients) and regression analysis. Stem height and stem diameter means for different origins were compared using Bonferroni's Multiple comparison procedure with construction of intervals for all means. Consequently in the Multiple Range Tests these intervals were used to determine which means are significantly different from which others. Computing was carried by statistic program package STATGRAPHIC PLUS 5 for Windows.

Results and discussion

Variability of fruit size

Analysis of variance proved significant effect of visual sorting fruits to three size groups (Table 1). However only first group of fruits was of significantly higher weight and remaining two fruit groups did not differed significantly. Total weight of 12 lots of fruits from different families in each group was 1,586, 1,392 and 1,367 g respectively. Particular families and different origins also significantly affected weight of fruits. While analyzing variability separately for particular origin (locality) effect of both families and fruit groups showed to be significant except of family effect in Duchonka origin (Fig. 1). Differences in progeny means of fruit weight within the particular origin were the highest in Tlstý Vrch origin. Fruits collected from progeny TV 2 were 1.7-times bigger than fruits from progeny TV 8 and were the biggest among fruits of all twelve families (Table 2). This finding is interesting in relation to the size of fruits produced by mother tree TV 2 grown at locality Tlstý Vrch. Namely these fruits used to be in average for six years two-times smaller than those produced by mother tree TV 8 (Bolvanský, 1988). The second highest difference in fruit weight among families was observed in Horné Lefantovce origin. Fruits from progeny HL A were in average 1.4-times bigger than those of HL 17. Also 1-year-old and 2-year-old seedlings grown from fruits of TV 2 and HL A progenies were higher than seedlings from fruits of progenies TV 8 and HL 17. Similar finding was reported earlier by Benčať and Tokár (1972), who pointed at the higher height of seedlings derived from fruits collected from mother tree TV 2 in spite of fact the fruits were of small size.

Variability of growth characteristics of seedlings

Variability of stem height and stem diameter of 1-year-old and 2-year-old seedlings was significantly affected by all assumed sources of variations – fruit group, origin, progeny within origin (Tables 3 and 4).

Table 1. Results of analysis of variance for weight of fruits collected from 12 progenies of four different origins and used for growing seedlings

Source	Sum of squares	Df	Mean square	F-ratio	P-value
Fruit group	64.861	2	32.431	19.96	0.0000
Origin	32.997	3	10.999	6.77	0.0002
Progeny (origin)	294.241	8	36.780	22.64	0.0000
Residual	1322.6	814	1.6248		
Total (corrected)	1723.13	827			

Table 2. Progeny and origin means and standard errors (SE) of fruit weight in fruits used for growing seedlings

Origin	Progeny	n	Fruit weight [g]	
			Mean	SE
Tlstý Vrch	TV 2	59	5.29 a	0.19
	TV 8	79	3.05 b	0.11
	TV 9	73	3.42 b	0.10
	Total	211	3.80 A	0.10
Horné Lefantovce	HL A	67	4.87 a	0.15
	HL 17	73	3.38 b	0.15
	HL 18	73	3.70 b	0.10
	Total	213	3.96 A	0.09
Duchonka	D 3	64	4.28 a	0.21
	D 5	64	3.80 a	0.20
	D 13	57	3.85 a	0.25
	Total	185	3.98 A	0.13
Jelenec	J 20	61	4.49 ab	0.17
	J 50	82	4.19 a	0.12
	J 11	76	4.65 b	0.15
	Total	219	4.43 A	0.08

Different small letters in column within an origin indicate significant differences ($P \leq 0.05$) between progeny means and different capital letters significant differences between origin means.

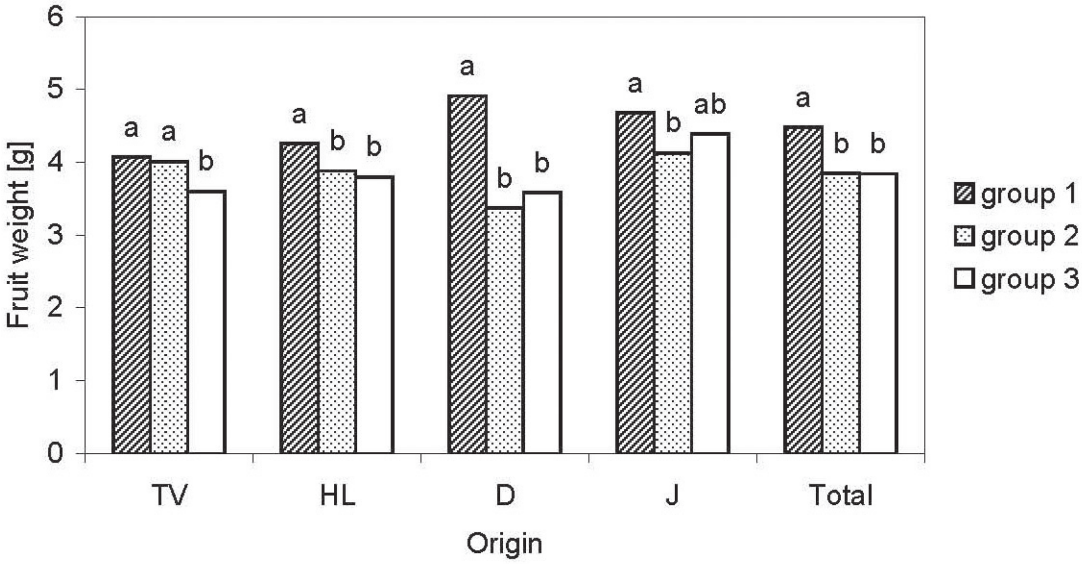

Fig. 1. Mean fruit weight of three fruit groups originated from open pollinated families of different origin (TV, Tlstý Vrch; HL, Horné Lefantovce; D, Duchonka; J, Jelenec). Different letters above columns within single origin indicate significant differences between means.

Table 3. Results of analysis of variance for stem height of 1-year-old and 2-year-old chestnut seedlings derived from 12 halfsib families of four different origins

Source	Df	1-year-old seedlings			2-year-old seedlings		
		Mean square	F-ratio	P-value	Mean square	F-ratio	P-value
Fruit group	2	1,628.4	58.94	0.0000	2,6405.00	111.17	0.0000
Origin	3	424.784	15.37	0.0000	3,949.05	16.63	0.0000
Progeny (origin)	8	219.347	7.94	0.0000	1,540.08	6.48	0.0000
Residual	814	27.6297			237.52		
Total (corrected)	827						

Table 4. Results of analysis of variance for stem diameter of 1-year-old and 2-year-old chestnut seedlings derived from 12 half-sib families of four different origins

Source	Df	1-year-old seedlings			2-year-old seedlings		
		Mean square	F-ratio	P-value	Mean square	F-ratio	P-value
Fruit group	2	11.0985	7.66	0.0005	133.729	14.35	0.0000
Origin	3	22.1547	15.28	0.0000	74.7451	8.02	0.0000
Progeny (origin)	8	9.3032	6.42	0.0000	59.0676	6.34	0.0000
Residual	814	1.4495			9.3208		
Total (corrected)	827						

Significantly the highest growth of 1-year-old and 2-year-old seedlings was observed in the block where the fruits of first fruit group were planted. 1-year-old seedlings in the second block and in the third block did not differed significantly. These differences are in accord with differences in weight among fruit groups. However the stem height in 2-year-old seedlings was significantly lower in third block than in the second one. The reason of this growth differentiation was apparently in different environmental conditions in particular blocks of experimental plot. While the first block and a part of the second block replication were afternoon under the shade of trees grown about 7 meters far of the plot margin, another part of block 2 and whole block 3 were without any shading during day. The positive effect of shading on seedling growth could be mediated indirectly through the higher soil humidity in the shaded parts.

Our results are partially in accord with those reported by Anagnostakis (2007) who observed that American chestnut (*Castanea dentata*) seedlings grown for three seasons in full sun or under a shade tent (65% shade) were larger, and fewer died from winter injury in the shade. However in another experiment started with 10 day-old germinants of American chestnut under irradiance reduced to 35, 15, and 5% of full sunlight the results after two months where rather different. The height and diameter of seedlings decreased with decreasing irradiance (et al., 2006). In both experiments the seedlings were watered on an as need basis. Cork oak (*Quercus suber* L.) seedlings grown during their first growing period under different shade levels showed increasing stem height with decreasing light, which was significantly higher only at 5% of photosynthetic active radiation. At this radiation level the collar diameter of seedlings was twice lower than at the remaining treatments (100%, 50% and 21% of lighting). It was observed that lower reduction of lighting did not significantly affect either stem height or root collar diameter (Cardillo and Bernal, 2006).

From breeding and silvicultural point of view the most important showed to be significant effect of origin and progenies within origin on growth characteristics of studied seedlings. Significantly the highest in both years and thickest in the second year were the seedlings of Horné Lefantovce origin. This was due to the very good growth performance of seedlings derived from HL A half-sib family. These seedlings were the highest and thickest among all 12 progenies studied (Table 5). On the contrary the lowest stem height and lowest stem diameter of seedlings of Duchonka origin was due to the lowest stem height of seedlings derived from D 5 half-sib family and the lowest stem diameter of seedlings coming from D 3 half-sib family.

Effect of fruit size on growth characteristics of seedlings

Correlation coefficients calculated between fruit weight and stem height and stem diameter of 1-year-old and 2-year-old seedlings based on progeny means and fruit group means pointed at medium strong correlation especially in 1-year-old seedlings (Table 6). Correlation coefficients calculated from basic data of fruit weight, stem height and stem diameter grouped by individual progenies differed markedly between families of the same origin and suggested none to medium strong correlation between fruit size and growth characteristics of seedlings (Table 7). The lowest correlation coefficients ($r = 0.0095–0.1368$) were calculated for fruits and seedlings from family TV 2, which produced the largest fruits within origin Tlstý Vrch and also among all 12

Table 5. Progeny and origin means and standard errors (SE) of stem height and stem diameter of 1-year-old and 2-year-old seedlings derived from fruits collected in 12 different families

Origin	Progeny	n	Stem height [cm] 1-year-old Mean	SE	Stem height [cm] 2-year-old Mean	SE	Stem diameter [mm] 1-year-old Mean	SE	Stem diameter [mm] 2-year-old Mean	SE
Tlstý Vrch	TV 2	59	12.47 a	0.85	37.73 a	2.21	4.50 a	0.14	11.11 a	0.41
	TV 8	79	10.23 b	0.56	35.53 a	2.00	4.13 b	0.13	10.51 a	0.32
	TV 9	73	11.78 b	0.50	37.85 a	1.70	4.53 a	0.13	11.06 a	0.33
	Total	211	11.39 C	0.37	36.95 C	1.13	4.37 B	0.08	10.87 B	0.20
Horné Lefantovce	HL A	67	17.34 a	1.09	53.66 a	2.50	5.50 a	0.17	13.90 a	0.40
	HL 17	73	10.99 c	0.64	38.82 c	2.27	4.33 b	0.14	10.72 c	0.37
	HL 18	73	13.36 b	0.89	46.44 b	2.27	4.45 b	0.17	11.99 b	0.39
	Total	213	13.80 A	0.54	46.10 A	1.49	4.74 A	0.10	12.16 A	0.24
Duchonka	D 3	64	11.00 a	0.39	38.89 a	1.81	4.00 a	0.12	10.62 a	0.33
	D 5	64	9.54 b	0.35	32.22 b	1.44	4.22 a	0.14	11.54 a	0.40
	D 13	57	11.05 a	0.51	40.25 a	2.72	4.11 a	0.13	11.24 a	0.45
	Total	185	10.51 C	0.24	37.00 BC	1.18	4.11 C	0.08	11.13 B	0.23
Jelenec	J 20	61	12.91 a	0.82	38.13 b	2.15	4.56 b	0.16	11.07 b	0.40
	J 50	82	13.48 a	0.69	44.14 a	1.61	4.94 ab	0.14	12.22 a	0.36
	J 11	76	12.25 a	0.42	39.11 b	1.80	5.01 a	0.15	12.24 a	0.35
	Total	219	12.90 B	0.37	40.72 B	1.06	4.86 A	0.09	11.91 A	0.22

Different small letters in columns within each origin indicate significant differences ($P \leq 0.05$) between progeny means and different capital letters in columns show significant differences between origin means.

families studied. Also relatively the largest fruits within origin Duchonka from the family D 3 gave emerged the seedlings, which stem height and stem diameter also did not correlate with fruit weight (r = 0.0226–0.2012). On the contrary families which produced fruits of lower size resulted to the stronger correlation between fruit weight and growth characteristics.

Correlation between basic data of fruit weight and growth characteristics of seedlings differed also when calculated by the fruit groups or by blocks. While in blocks 1 and 2 no or very low correlation was observed (0.097–0.296) in replication 3 correlation coefficients were higher (0.44–0.63) and pointed at medium strong relationship. Seedlings in the block 3 were in the first year of the same height like seedlings in the block 2 and only in the second year were lower (Fig. 2). Seedlings of both blocks were derived from the groups of fruits with the same weight so the height of seedlings in block 3 might be affected by environmental conditions or interaction progenies × environment (low soil humidity in this place). This suggests that under less favourable soil conditions growth of seedlings depends more on fruit size than in more favourable conditions. Here, apparently the real growth potential of seed can be manifested. Medium strong correlation between fruit weight and growth of 1-year-old and 2-year-old chestnut seedlings grown from these fruits was observed also in previous studies (Tokár and Benčať, 1972). In this case, strength of correlation varied significantly by year and by individual mother trees. Even in six year-old seedlings, a positive effect of fruit weight on height of the seedlings was observed (Benčať and Tokár, 1979).

Table 6. Correlation coefficients between fruit weight and growth characteristics (stem height and stem diameter) of 1-year-old and 2-years-old seedlings grown from these fruits. Entry data were progeny means, nut group means within origin and within progeny. Number of matched data is displayed in parentheses

		Stem height		Stem diameter	
		1-year	2-year	1-year	2-year
Fruit weight	Progeny means (n = 12)	0.5574*	0.3290	0.5327	0.4670
	Nut group means within origin (n = 12)	0.4087	0.5119	0.2998	0.6160*
	Nut group means within progeny (n = 36)	0.5148	0.4534	0.4489	0.5493

*significant at P = 0.05.

Table 7. Correlation coefficients between basic data of fruit weight and stem height and stem diameter of 1-year-old and 2-years-old seedlings grown from these fruits calculated by progenies, replications and in total

Name of data set / (sample size)	1-year-old seedlings		2-year-old seedlings	
	Fruit weight correlated with		Fruit weight correlated with	
	Stem height	Stem diameter	Stem height	Stem diameter
TV 2 (59)	0.1368	0.0818	0.0095	0.0805
TV 8 (79)	0.3840**	0.2561*	0.3618**	0.3042**
TV 9 (73)	0.4122**	0.1222	0.2755*	0.3478**
HLA (67)	0.2881*	0.3279**	0.3032*	0.3840**
HL 17 (73)	0.4771**	0.3715**	0.4031**	0.2842*
HL 18 (73)	0.2775*	0.2413*	0.2775*	0.2588*
D 3 (64)	0.1075	0.1075	0.2012	0.0226
D 5 (64)	0.6085**	0.450**	0.5888**	0.5490**
D 13 (57)	0.2733*	0.2575	0.4198**	0.3440**
J 20 (61)	0.3741*	0.4493**	0.2791*	0.3313**
J 50 (82)	0.3872**	0.5149**	0.2380*	0.2866**
J 11 (76)	0.409**	0.4464**	0.2799*	0.5402**
1st nut group (283)	0.2833**	0.2960**	0.2603**	0.2668**
2nd nut group (293)	0.1180*	0.1726*	0.0970	0.1662*
3rd nut group (252)	0.6251**	0.5403**	0.4448**	0.5074**
Total (828)	0.3330**	0.3279**	0.2962**	0.3252**

*and **significant at P = 0.05 and P = 0.01.

Small fruits of some trees produced more vigorous seedlings than bigger fruits of other trees. Seiwa et al. (2002) observed that stem height of one-year-old *Castanea crenata* seedlings was significantly affected by sowing depth but not by nut size. In the optimal depth (5 cm), there was little difference in the seedling height among nut-size classes although seedling biomass was dependant on nut size.

Differences in the growth of seedlings emerging from seeds of different sizes within a species have been little studied in woody plant species. Gupta et al. (1983) (studying *Leucaena leucocephala*) and Negi and Todaria (1997) (studying *Acer oblongum, Kydia calyciana, Terminalia tomentosa, Terminalia bellerica* and *Terminalia chebula*) reported faster growth in seedlings produced from large seeds. Khurana and Singh (2000) observed that seedlings from large seeds were taller and heavier, had a greater leaf area and were more tolerant of long-term extreme water stress compared to those from smaller seeds. Larger seeds have the ability to store greater amounts of carbohydrate in their endosperm or cotyledons than small seeds. This may

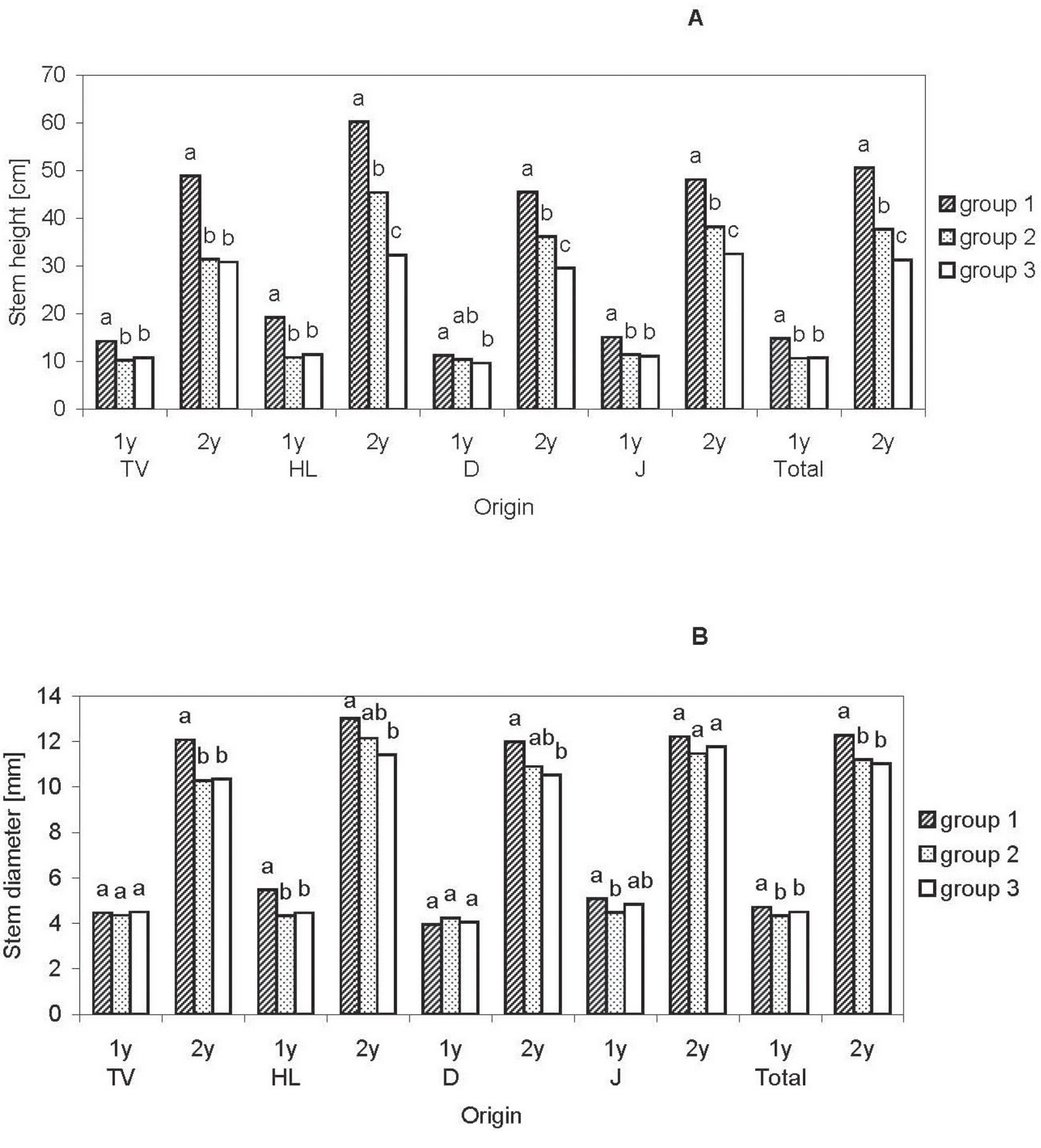

Fig. 2. Mean stem height (A) and stem diameter (B) of one-year-old (1y) and two-year-old (2y) chestnut seedlings by their origin (TV, Tlstý Vrch; HL, Horné Lefantovce; D, Duchonka; J, Jelenec) and by fruit groups. Different letters above columns within single origin and single year indicate significant differences between means.

enable early development of an enlarged resource gathering system (root or photosynthetic tissue) to produce a faster growing plant (HEWITT, 1998).

Correlations between growth characteristics of seedlings

Inter-annual correlation coefficients between stem heights and between stem diameters of one-year-old and two-year-old seedlings by individual families suggest that annual growth increments of seedlings nearly in all progenies reflected growth performance demonstrated in the first year (Table 8). Correlation coefficients for stem heights varied from 0.56 in TV 9 to 0.79 in HL 18 family and for stem diameter from 0.39 in TV 9 to 0.75 in J 20 family. Inter-annual correlations between heights and between diameters were stronger when progeny means of both growth characteristics were correlated (for heights r = 0.9147 and for diameters r = 0.8826) (Table 9). Similarly also inter-annual correlations between progeny means of stem heights of 1-year-old and stem diameter of 2-year-old seedlings and vice versa were of rather high value (0.834 and 0.702).

Inter-generation correlations between two growth characteristics of 1-year-old and 2-year-old seedlings and similar characteristics of 35-year-old seedlings belonging to 12 different progenies suggested only medium strong correlation between heights but no correlation between diameters (Table 8). However, also inter-generation correlations between heights were statistically not significant because of low number of correlated data.

In case the higher number of progenies would be involved into study the stronger inter-generation correlations could be expected. Namely evaluation of growth characteristics of F 1 progenies was apparently biased by several factors affecting to a different degree growth and vitality of trees on individual experimental plots. For instance, in some plots number of trees was during last ten years drastically reduced due to the mortality caused by fungal diseases mainly by *Phytophtora* ssp. and partially also by *Cryphonectria parasitica* (TOKÁR

Table 8. Correlation coefficients between basic data of stem heights and stem diameters of 1-year-old (1y) and 2-year-old (2y) seedlings by individual progenies

Origin	Progeny	n	Heights 1y – 2y	Diameters 1y – 2y
Tlstý Vrch	TV 2	59	0.6674**	0.6561**
	TV 8	79	0.7058**	0.5991**
	TV 9	73	0.5581**	0.3941**
Horné Lefantovce	HL A	67	0.6354**	0.6428**
	HL 17	73	0.7339**	0.6861**
	HL 18	73	0.7951**	0.7140**
Duchonka	D 3	64	0.6562**	0.4077**
	D 5	64	0.5791**	0.6274**
	D 13	57	0.6704**	0.7369**
Jelenec	J 20	61	0.7546*	0.7528**
	J 50	82	0.6229**	0.7165**
	J 11	86	0.7257**	0.6897**
	Total	828	0.6933**	0.6657**

*and **significant at P = 0.05 and P = 0.01.

Table 9. Correlation coefficients between progeny means of stem height and stem diameter of 1-year-old (1y), 2-year-old (2y) and 35-year-old (35y) seedlings. Sample size in all correlation n = 12

	Diameter 1y	Height 2y	Diameter 2y	Height 35y	Diameter 35y
Height 1y	0.8619**	**0.9149****	0.8340**	0.5244*	–0.0216
Diameter 1y		0.7020**	**0.8826****	0.3410	0.0327
Height 2y			0.7996**	0.5442*	– 0.1651
Diameter 2y				0.3782	0.1084
Height 35y					–0.2795

*and **significant at P = 0.05 and P = 0.01.

et al., 2004). This is true for progenies from Tlstý Vrch provenance, which exhibited a high mortality rate during last ten years. While having the highest performance till the age of 30 years, these progenies were overtopped in height by progenies from Duchonka provenance during consecutive five years (Tokár and Bolvanský, 2002). Another source of error in data from half-sib families could be represented by the effect of environment as this factor was not minimized by trial replications. Each family was namely planted only in one plot.

Our results suggesting similar progeny ranking in stem height and rather different ranking in stem diameter at different age of European chestnut are in accord with the study on full-sib families of black walnut at age of 11 and 21 years (Peng et al., 1992). In black walnut an early height showed to be a good indicator of later height (Mc Keand et al., 1979; Rink, 1984).

Although repeatability of growth characteristics of observed 12 progenies was not very high in some progenies with high growth performance this repeatability was very high. This is true especially for half-sib family HL A of Horné Lefantovce origin, which could be used to produce seeds for establishing chestnut plantations.

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Vplyv hmotnosti plodov, pôvodu materských stromov a podmienok pokusu na rastové charakteristiky semenáčikov gaštana jedlého

Súhrn

Na jar roku 2005 bol založený v lesnej škôlke Hladomer, neďaleko obce Lovce pokus v poľných podmienkach za účelom sledovania rastových charakteristík semenáčikov gaštana jedlého v závislosti na pôvode experimentálneho materiálu. Plody na výsadbu boli zozbierané zo 45-ročných jedincov 12 polosúrodeneckých rodín (potomstiev pochádzajúcich z voľného opelenia jednotlivých stromov) a nachádzajúcich sa na experimentálnej ploche „Castanetárium Horné Lefantovce". Materské stromy sledovaných potomstiev rástli a niektoré ešte aj rastú na lokalitách Horné Lefantovce, Jelenec, Duchonka a Tlstý vrch. Vybrané potomstvá pri posledných dvoch hodnoteniach (1995 a 2000) vykazovali nadpriemerné hodnoty rastových charakteristík spomedzi 86 potomstiev nachádzajúcich sa na experimentálnej ploche. Variabilita rastových charakteristík (výška a hrúbka kmeňa) jedno- a dvojročných semenáčikov získaných z týchto potomstiev bola štatisticky významne ovplyvnená pôvodom materských stromov potomstiev, lokalitou – provenienciou materských stromov ako aj veľkostnou kategóriou plodov. Pri semenáčikoch, ktorých rodičovské jedince pochádzali z lokalít Horné Lefantovce, Jelenec a Duchonka, sa pôvodne štatisticky nevýznamné rozdiely vo výške 1-ročných semenáčikov medzi druhým a tretím blokom pokusu zmenili na štatisticky významné pri 2-ročných semenáčikoch. Tieto rozdiely možno pričítať vplyvu rozdielnej vlhkosti pôdy na pokusných parcelách (vyššia vlhkosť na parcele druhého bloku v dôsledku čiastočného zatienenia). Medzi hmotnosťou plodov a rastovými charakteristikami semenáčikov získaných z týchto plodov bola v obidvoch rokoch pozorovaná jednak slabá závislosť ($r = 0,29$ až 0,33 pri jednotlivých plodoch) alebo slabá až stredne silná závislosť ($r = 0,30$ až 0,62 pri plodoch zoskupených do veľkostných kategórií). Najvyššie hodnoty obidvoch rastových charakteristík boli pozorované pri semenáčikoch získaných z potomstiev pôvodu Horné Lefantovce najmä z potomstva HL A. Priemerná výška kmeňa jedincov v tomto potomstve preukazne prevyšovala výšku kmeňa pri iných potomstvách zahrnutých do pozorovaní s 5-ročným intervalom. Korelácie medzi priemernou výškou kmeňa týchto potomstiev a priemernou výškou kmeňa jednoročných a dvojročných semenáčikov získaných z týchto potomstiev boli iba stredne silné ($r = 0,52$ a 0,54). Podobné medzigeneračné korelácie v priemere kmeňa boli veľmi nízke a nepreukazné. Výsledky ukázali, že pri zakladaní porastu semenom, pri gaštane jedlom je prospešné zberať plody z porastov, ktoré majú dlhodobo nadpriemerný výškový rast.

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Impact of regeneration cutting on sulphate concentration and amount in throughfall water in conditions of submontane beech forests in the Western Carpathians Mts

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Abstract

Janík, R., Bublinec, E., Dubová, M. 2011. Impact of regeneration cutting on sulphate concentration and amount in throughfall water in conditions of submontane beech forests in the Western Carpathians Mts. *Folia oecol.*, 38: 156–161.

This work summarises the values of amount and concentration of SO_4^{2-}, measured in precipitation totals on open plot and in throughfall in submontane beech forests in the Kremnické vrchy Mts, Western Carpathians Mts over a 4-year research period. The measurements were carried out in beech forest stands with stocking density modified (reduced) to various degrees in years 1989 and 2004 by regeneration cutting. The maximum concentration of SO_4^{2-} was recorded on the former clear cut: 33.34 mg l^{-1} in year 2002. The lowest value of 6.57 mg l^{-1} was obtained in year 2005 on the partial plot subjected to intensive intervention leading to the stocking density of 0.3. Higher sulphate concentration values was recorded in the autumn and winter, the lower ones in the spring and summer. No inter-annual differences in S-SO_4^{2-} content and concentration have been confirmed.

Key words

submontane beech forest, sulphate concentration, throughfall, the Western Carpathians Mts

Introduction

The cutting-edge environmental issues today are: 1. global warming, 2. ozone layer depletion coupled with greenhouse effect, 3. air pollution followed by soil acidification. They all share a common feature and primary cause: negative impact of human activities.

Research on sulphate concentration in precipitation water and in throughfall is very important, as these factors belong to the main ones involved in the process of soil acidification. Sulphur, nitrogen and solid particles cover the major part of acidification in the forest soil development.

Since the 60s to 80s of the last century, sulphur and sulphur compounds have become of a major concern worldwide – due to the rapid economic development, coal mining and rising demands from industry and consumers. The result has been a steep increase in the emitted pollutants, primarily SO_2 and nitrogen oxides. Lindberg and Lovett (1992) declare that the sulphur in form of SO_2 represents up to 60% of the total sulphur. Sulphur in atmospheric precipitation enters the soil (Stachurski and Zimka, 2000), from which it displaces basic ions and causes acidification. Acidification is a long term and cumulative process (Hruška et al., 2001) exhibiting dynamic development. The alarming degradation of life environment, especially forest soils, unveiled urgent needs to set deposition limits for forest ecosystems – as valid tools for governments in designing mitigation strategies for sulphur and nitrogen emissions in Europe and in the North America (Matzner and Meiwes, 1994).

Reduction of industry, reduction of coal mining in Poland and in the former DDR and conversion of industry across the whole "East Block" in 1990s were responded by decrease in sulphur and nitrogen emissions (Zapletal, 2006; Prechtel et al., 2001).). Evaluated with using selected indicators for sustainable development, the territory of the Slovak Republic is medium sensitive to sulphur deposition. The critical value for the SR is 10–30 kg ha^{-1} $year^{-1}$. The actual S deposition, however, is beyond these limits in about ¼ of Slovak forest soils. The total reduction in basic pollutants across the SR territory in years 1989-99 was 57.9%, representing almost 6% yearly, on average. In year 2006 was recorded an on average 50% drop in sulphur deposition (3.8–6.4 kg ha^{-1} y^{-1}) compared to year 2001.

In the neighbouring Czech Republic, the substances emitted over the period 1990–2000 dropped: SO_2 by 86% and ammonium by 53%. The decreasing trend in SO_2 emissions in W Europe (Netherlands, Germany, Sweden) since 1980 has also been recorded by Fricke and Beilke (1992) and by Prechtel et al. (2001).

Nevertheless, the danger of acidification is a still persisting ecological problem of extraordinary importance Alewel et al. (2000); mainly due to its character is long-term and due to the fact that sulphur penetrates the soil vertically and accumulates in deeper soil layers.

The aim of this work was to quantify the sulphur amount in atmospheric deposition and to support/reject the hypothesis about influence of silvicultural interventions on sulphur amount in the water fallen through tree crowns (throughfall).

Material and methods

Study area

The research plots (RP) are situated in the Kremnické vrchy Mts (48°38' N and 19°04' E), at 450–510 m a.s.l., on a slope with an inclination of 17–20°. The stand age is 80–110 years, the absolutely dominant woody species is beech, the average stand height is 28 m (Barna, 2004). The site climate is moderately warm, moderately hilly District B5 with a mean annual temperature $t_{1951–1980}$ 6.8 °C and the mean annual precipitation over the same period 778 mm (Schieber, 2006). The mean precipitation total in the growing season is 395 mm (Dubová and Bublinec, 2006). The length of growing season is 115–165 days.

As for the type, the plots have been classified into the 3-rd forest vegetation tier (fvt), fertile order B, transient order from fertile to nitrophilous (B/C), group of forest types Fagetum pauper inferiora.

As for coeno-taxonomy, the central association is Dentario bulbiferae-Fagetum, frequent is also Carici pilosae-Fagetum.

In year 1989, the stands were subjected to a series of parallel cuts differing in intensity, with the primary aim to reduce the stand stocking density. There was obtained a series of partial plots (PP) with different reduced density. In year 2004 was applied the second series of cuts, aimed at further density reduction and at creation of new ecological conditions. In such a way, there have been modelled conditions for better understanding and use of abilities of commercial stands in submontane beech forests.

Throughfall

Precipitation water as well as throughfall water was collected in plastic collectors with a defined catchment surface placed by 10 on each plot. A collector consisted of a funnel with a filter fixed on a collection container. To limit evaporation, the container was placed in a larger vessel. The water was sampled regularly, at monthly intervals and after each noticeable precipitation event.

Chemical analyses

The amount of SO_4^{2-} was determined by titration with lead nitrate in dithizon and converted by calculation to the sulphate sulphur.

Statistic methods

Statistical calculations of parameters of measure and position as well as the tests were made with using the package Statistica v 7. The normality of distribution of the data set was tested with the Shapiro-Wilk's W test. The significance of inter-annual differences was evaluated with the Student's t test. The results were also verified with the statistical package Statgraphics. Simple regression was used for assessment of the influence of precipitation totals on sulphate concentration in atmospheric precipitation and in soil solutions for the individual soil horizons.

Results and discussion

Sulphur amount and concentration on partial plot H

In year 1989 was this plot treated with a clear cut removing the entire forest cover. At the time, SO_4^{2-} concentration on this plot was the highest among the partial plots. Later, however, the plot was grown with a 12–14 year-old young stand, reaching, towards the end of the study period a mean height of 12 m, which could influence the measured values. The mean values of sulphate concentration were 17.2 mg l^{-1}. The precipitation total and throughfall amount displayed a comparatively high variability, making 61.9%. The general trend in SO_4^{2-} concentration was found decreasing (Table 1). The annual dynamics in over 2002–2004 exhibited maximum values in summer: 43.1 mg l^{-1}. The lowest values were measured in autumn: 6.9 mg l^{-1}. Since the intervention in 2004, where this partial plot was left without intervention (no parent stand to remove), maximum values of SO_4^{2-} concentration occurred in the autumn

(17.13–22.06 mg l^{-1}), minimum in the spring – 7.03 mg l^{-1}. As for the precipitation total (Table 2), higher values were measured after the intervention in 2004, with 1,003.4–1,381.7 mm (2002–2004) precipitation fallen on this plot. The sulphate sulphur deposition in year 2002 was 67.1 kg – exceeding the critical value for Slovakia set by Kalúz (2004) as 30 kg ha^{-1} y^{-1} by more than two times. Such a contradiction with the just discussed distinct drop in emitted sulphur oxides may be assigned to the nature of these substances as well as to good dispersing conditions (a 204 m high chimney) concerning the important pollution sources: the aluminium plant in Žiar nad Hronom and the fossil-fuel power station in Nováky.

Table 1. Descriptive statistics of SO_4^{2-} concentrations in the Kremnické vrchy Mts (Western Carpathians Mts) in years 2002–2006

Plot/stocking	H (0.0/0.0)	I (0.3/0.0)	S (0.5/0.3)	M (0.7/0.5)	K (0.9/1.0)
Valid	4	4	4	4	4
Mean	17.3	13.9	13.6	15.6	14.5
Minimum	11.6	6.6	9.2	9.2	11.7
Maximum	33.3	26.2	21.8	24.4	20.5
Std. deviation	10.7	8.5	5.6	6.5	4.0
Std.error	5.4	4.3	2.8	3.3	2.0
Coeff. of variation	61.9	61.2	41.2	41.7	27.7

Stocking before/after cutting in year 2004.

Table 2. The concentrations of SO_4^{2-} in the Kremnické vrchy Mts (Western Carpathians Mts) in years 2002–2006

Plot/stocking	H (0.0/0.0)	I (0.3/0.0)	S (0.5/0.3)	M (0.7/0.5)	K(0.9/1.0)
			[mg l^{-1}]		
Winter	28.90	37.10	44.40	53.50	24.90
Spring	38.23	35.08	18.75	22.60	24.72
Summer	43.10	30.82	35.81	30.30	34.22
Autumn	17.87	20.31	12.71	18.47	11.55
Vegetation period	38.02	28.42	31.86	29.57	32.14
Year 2002	33.34	26.23	21.76	21.38	20.46
Winter	–	–	–	–	–
Spring	12.96	11.71	12.75	20.04	15.52
Summer	16.20	12.20	10.01	10.68	14.69
Autumn	6.90	8.05	9.28	11.36	6.24
Vegetation period	17.16	13.31	13.01	13.93	17.98
Year 2003	11.90	11.11	11.42	16.17	12.58
Winter	10.48	–	–	–	7.60
Spring	14.10	–	–	–	14.60
Summer	10.81	11.45	6.92	9.19	10.07
Autumn	17.13	12.00	19.20	9.29	21.17
Vegetation period	12.18	6.51	8.43	9.24	12.48
Year 2005	12.33	6.57	9.19	9.21	11.71
Winter	–	–	–	–	–
Spring	7.03	8.25	19.17	11.48	9.62
Summer	19.09	20.47	15.01	11.47	20.75
Autumn	22.06	20.83	15.98	15.38	19.71
Vegetation period	12.36	12.25	11.96	11.04	12.39
Year 2006	11.57	11.78	11.84	12.42	13.38

The critical value of 14 mg l^{-1} for SO_4^{2-} (KUNCA, 2007) concentration was exceeded only once, in year 2002.

The testing did not confirm significant inter-annual differences in SO_4^{2-} concentration in the throughfall water. The results of regression analysis unveiled the strong dependence of SO_4^{2-} concentration and S-SO_4^{2-} amount on the precipitation total, with the correlation coefficient having a value of 0.85.

Sulphur amount and concentration on partial plots I, S, M ,K

The values of all the studied parameters were very similar on all the partial plots. The average SO_4^{2-} concentration ranged from 13.5 mg l^{-1} on plot S with stocking density 0.5 and 0.3 in year 1989 and 2004, respectively, to 15.5 mg l^{-1} on plot M so with the corresponding stocking density values 0.7 and 0.3. The difference compared to the former clear cut was up to 4 mg l^{-1}. The lowest variability was recorded on the control plot without intervention – 27.7%, which reflects comparatively stable and even conditions on this plot. Somewhat higher values – 41.2% were obtained on the PPs S and M. The partial plot subjected in 2004 to final shelterwood cut exhibited almost the same values as the former clear cut plot.

In the amount of deposed sulphur, the „richest" was year 2006 on all the plots, with 67.5 kg S-SO_4^{2-} on PP I and just above 45 kg on the other plots. These rather high values were also associated with high precipitation totals on the individual plots (from 1,717.3 mm on PP I to 1,035.1 mm on the control intact plot (Table 3).

The highest sulphur amounts on the individual plots were recorded in the autumn or winter. Lower values were obtained in the spring or summer – in dependence on the precipitation total over the concerned period. Occurrence of the highest sulphate sulphur amounts in atmospheric precipitation as well as in soil water show very similar patterns in the autumn and (DUBOVÁ and BUBLINEC, 2006). PICHLER et al. (2006) report maximum S-SO_4^{2-} values of precipitation in mixed forests occurring in winter months. KEISER and GUGGENBERGER (2005) carrying research on 90-year-old beech forests

Table 3. Air precipitation and throughfall in the Kremnické vrchy (Western Carpathians Mts) in years 2002–2006

Plot/stocking	H (0.0/0.0)	I (0.3/0.0)	S (0.5/0.3)	M (0.7/0.5)	K (0.9/1.0)
			[mm]		
Winter	50.0	42.0	38.0	40.0	29.6
Spring	132.5	128.4	124.8	113.5	110.7
Summer	468.9	494.4	346.9	287.4	305.3
Autumn	323.4	651.6	92.7	450.9	538.8
Vegetation period	609.3	636.4	473.3	392.1	414.0
Year 2002	974.8	1,316.4	1,102.4	891.8	984.4
Winter	–	–	–	–	–
Spring	400.0	423.2	383.1	338.5	307.6
Summer	133.6	128.5	103.8	92.3	73.9
Autumn	199.3	190.5	169.9	167.2	166.7
Vegetation period	275.6	254.9	205.2	187.0	163.9
Year 2003	732.9	742.2	656.8	598.0	548.2
Winter	26.4	28.5	–	–	170.2
Spring	284.8	217.3	–	–	241.7
Summer	598.0	261.0	467.1	454.0	454.4
Autumn	94.2	63.5	105.8	98.7	79.0
Vegetation period	950.2	529.4	539.9	520.2	751.0
Year 2005	1,003.4	570.3	572.9	552.7	945.3
Winter	–	–	–	–	–
Spring	926.5	1,228.2	677.3	711.4	663.8
Summer	190.2	265.5	186.0	164.1	144.5
Autumn	265.0	223.6	293.4	278.5	226.8
Vegetation period	1,050.0	1,387.3	846.0	804.4	757.6
Year 2006	1,381.7	1,717.3	1,156.7	1,154.0	1,035.1

in NE Bavaria recorded in autumn months occurrence of up to 53% of the total organic sulphur.

The statistical tests and regression analysis have confirmed a high influence of the precipitation total on concentration and amount of S-SO_4^{2-} on the particular PPs.

For comparison: Piirainen et al. (2004) recorded in spruce forests in N Finland an average sulphur deposition of 1.5 kg ha^{-1} after a clear cut, compared to 4.6 kg ha^{-1} before the cutting. The critical value for forest stands reported by Ågren (1992) is 3–8 kg ha^{-1} y^{-1}. Novák et al. (2007) measured 56 kg ha^{-1} y^{-1} in spruce stands in the Ore Mountains in N Bohemia at 490 m a.s.l. in 1994. This is in accordance with observations that the sulphur amount in coniferous stands is higher than in beech or generally broadleaved stands. For spruce forests in Slovenia reports Šimončič (1996) even 33 kg ha^{-1} of sulphur. Dubová and Bublinec (2006) measured in spruce stands in the Poľana Mts 65.4 kg ha^{-1} y^{-1}. The S-SO_4^{2-} amounts recorded in the atmospheric deposition in the ridge part of the Low Tatras Mts by Bublinec and Dubová (1995) were 44.5 kg ha^{-1} in the stand and 32.7 kg on the open plot.

Conclusions

Concentration and amount of SO_4^{2-} in the throughfall sampled from submontane beech forest stands is mostly dependent on the total atmospheric precipitation, on distance from the pollution source and, to a smaller extent, on intensity of the applied silvicultural treatments. We can derive the following conclusions: The highest S-SO_4^{2-} values were recorded on the former clear cut plot or on the plot subjected to intensive cut. The course of SO_4^{2-} concentration displays a general decreasing trend, depending exclusively on the precipitation amount. It follows that controlled cutting intensity can serve as a tool for controlling the sulphur deposition.

As for the annual dynamics of SO_4^{2-} concentration and amount of S-SO_4^{2-}, higher values were obtained in the autumn or winter, lower in the summer. In spite of the declared reduction of emitted sulphur oxides, there were a number of cases where the values of SO_4 concentrations were beyond the critical limits. The cause may be multiple, such as: distance from the emission source (Žiar nad Hronom, Nováky), prevailing wind direction, species composition, silvicultural methods.

Considering the sulphur amount in atmospheric precipitation and amount of sulphur deposed in soil on the particular partial plots, the studied forest stands in the Kremnické vrchy Mts, that means also the Western Carpathians Mts, can be ranked as lower-stressed with sulphur pollution not only in frame of Slovakia but also within the whole Central Europe.

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Vplyv ťažbovo-obnovného zásahu na koncentráciu a obsah síranov v podkorunových zrážkach v podmienkach podhorských bučín Západných Karpát

Súhrn

V práci vyhodnocujeme 4-ročnú periódu merania koncentrácií a množstva SO_4^{2-}, v zrážkovom úhrne na voľnej ploche a v podkorunových zrážkach podhorských bučín Kremnických vrchov patriacich do Západných Karpát. Merania sme uskutočnili v bukových porastoch, kde bol v roku 1989 a 2004 uskutočnený ťažbovo-obnovný zásah za účelom redukcie zakmenenia. Maximálna hodnota koncentrácie SO_4^{2-} bola zaznamenaná na ploche bývalého holorubu s 33,34 mg l^{-1} v roku 2002. Najnižšie hodnoty boli namerané v roku 2005 na čiastkovej ploche s intenzívnym zásahom a zakmenením 0,3 s hodnotou 6,57 mg l^{-1}. Vyššie hodnoty koncentrácií síranovej síry boli zaznamenané v jesenných resp. zimných mesiacoch, nižšie v jarných resp. letných mesiacoch. Medziročné rozdiely v obsahu S-SO_4^{2-} a koncentrácii SO_4^{2-} neboli potvrdené.

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Statistical evaluation of air pollution in a model situation of beech stands in the Western Carpathians Mts

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Abstract

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Quantity of specific airborne pollutants (H^+ and O_3) was monitored at regular intervals over vegetation periods on a series of plots with modified stocking density. The statistical testing of the obtained values resulted in finding a significant difference (99% level) in proton H^+ input between the original intact parent stand and the plot treated with heavy cut; and, after the second intervention, the original stand and the plot treated with medium cut. The lowest mean values of (H^+) were recorded on the plot with the original parent stand (1999–2006: 13 mmol H^+ day^{-1} m^{-2}). It is evident that the stands play a role of a filter significantly influencing the input of pollutants deposed inside forest ecosystems. The maximum values, especially in year 2005, reflect the effect of the second cutting intervention realised in year 2004. The differences in O_3 concentration among the particular modified plots were not found as significant as in proton load H^+. The differences in amount of proton load and amount of ground level ozone among the plots were found significant for all plots at the level of 99%. It follows that the input of emissions into forest stands depends on chemical composition of the polluted substances.

Key words

air pollution, cutting phases, European beech, ground level ozone, proton H^+

Introduction

The anthropic activities together with natural processes cause important changes, climatic included, to forest ecosystems in Central Europe. At the centre of interest are broadleaved woody plants (Greguš and Kellerová, 2002), representing about 49.7% of forest cover in Slovakia. In terms of wood production is the leading species beech *Fagus sylvatica* L., with the highest portion making 31.2% of woody plants in Slovak forests. Beech, together with spruce and fir, belongs to distinctly sensitive species. The direct effect of airborne pollutants on forests stands seems to show a general decreasing trend; on the other hand, the acidification of forest soils is still persisting – due to elements accumulated for long (Hruška et al., 2001; Walna and Kurzyca, 2006). Therefore, protection of forest ecological stability is of primary importance – by choosing and applying appropriate silvicultural and regeneration methods. The aim of our research was to find out whether and how can close-to-nature regeneration methods influence the input of polluting substances in the forest environment.

Our research aim was to quantify differences in specific emissions (ground level ozone and H^+ proton load), entering model beech ecosystems with modified stocking density. Our basic assumption was that there would be distinct differences in amounts of specific substances in the individual years of experiment between the years before and the years after the silvicultural intervention.

Material and methods

Study site

We studied specific airborne pollutants entering beech ecosystems, namely the quantitative differences in amounts of these substances among the stands growing in similar conditions but at different regeneration phases. The stocking density was modelled in such a way as to correspond to the values common in forest management practice. The original stand composition at the site was: beech (76%), fir (15%), oak (4%), and hornbeam (5%). The first felling intervention realised in February 1989 (Greguš, 1987), resulted in the following plot series: obtained by applying heavy cut, obtained by medium cut, obtained by light cut, and the intact original parent stand. In the original stand is dominant beech – covering 94.7% of the area. The stand age at the time of the first intervention was 80–90 years. The plots are situated on a west-facing slope with an inclination of 30% to 36%, very close one to other (at a distance about 100–110 m). More details concerning the plots can be found in Dubová and Bublinec (2006); Schieber (2007). The stand density in the following years was improved by Barna (2000), the second intervention adjusting the stand density was made in spring 2004 (Barna, 2008). The plot, in 1989 treated with heavy cut, was delivered of all the remaining trees in felling maturity, the density on medium intervention and light intervention plots has been reduced. The changes in stocking density associated with the two interventions as well as the development after the interventions are illustrated in Table 1.

The research plots are situated in SE part of the Kremnické vrchy Mts (φ = 48°38' N, λ = 19°04' E) at 470–510 m a.s.l. The site climate is moderate warm and moderate wet. The long term mean annual temperature is 8.2 °C, in the growing season 14.9 °C. The mean annual rainfall makes from 510 mm to 1,040 mm, in the growing season from 160 to 530 mm (Kellerová and Dubová, 2002; Janík, 2006).

As for the airborne pollutants, the research plots are neither under the direct impact of polluting substances nor extremely stressed by the transboundary transport. In the near Zvolenská basin, however, are situated three stationary energy production units, and a dense network of car and railway transport, which may cause pollution stress to the research plots when weather conditions allow to. The research on air quality was conducted on a regional scale, focussing on the ground layer in forests at an appropriate distance from the pollution source. However, the topical issue concerning regional pollution today becomes ozone – displaying in mountain and submountain areas even higher values than in urban agglomerations Tschiedel, 2001; Fleischer et al., 2005. In years 1992–1996, the area of the Western Carpathians, and so also Central Slovakia, belonged to the most stressed ones in Europe, with the critical value exceeded sometimes as much as two (Bytnerowicz et al., 2004, Kremler, 2002).

Methods

For the study of input of polluting substances in forest ecosystems is suitable the method of proton load (H^+) designed by Obr (1989), for the ground ozone (O_3) is commonly used the sorption-accumulation method (Werner, 1991).

These methods, working with passive samplers, supply the pattern of the total deposition with dry and to some extent also wet deposition. The dry deposition is running continually, meaning such phenomena as deposition of gaseous and solid particles on the plant surface.

Proton load, indicating presence of acid substances in atmosphere: gaseous SO_2, NO_x, liquid HNO_3, H_2SO_4 and solid NH_4HSO_4, neutralises alkalinity of potassium carbonate solution exposed in field. The non-neutralised residuum is determined as the difference between the non-exposed and exposed absorption solution by titration with HCl on the Tashiro indicator (Kellerová, 1999). Nitrogen oxides belong to the important ozone precursors. An increase in nitrogen oxides may cause excessive ozone formation. Proton load, as an indicator of acid substances in atmosphere can provide information about their tendencies in the region.

The Werner´s method for ozone determination uses selective reaction of indigo applied on filtering paper with the atmospheric ozone. After a seven-day exposition in the field, the sampled material is examined in the laboratory, with using spectrophotometry (Kellerová, 2002).

The quantity of specific pollutants was determined at regular intervals within the growing seasons.

Selected statistic characteristics were processed and tested with using the package Statistica v 7. The normality of distribution of the basic set was tested with the Shapiro-Wilk W-test. Significance of differences

Table 1. Stocking density in beech stands in the Kremnické vrchy Mts, modified by cutting

Phase of management process	Original parent stand	Heavy intervention	Medium intervention	Light intervention
1989 after the first intervention	0.9	0.3	0.5	0.7
2004 after the second intervention	1.0	0.0*	0.3	0.5

*The plot from which all the trees of the parent stand shelter were removed during the second intervention did not result clear as it had just been covered with a 15-year-old stand at small pole stage.

concerning the basic sets between the localities was evaluated with the Student t-test for independent variables.

Results and discussion

In year 1999, ten years after the first cutting intervention modelling the required phases of forest management process. From the viewpoint of the original parent stand, the plots obtained after heavy cut, medium cut and light cut were grown with a natural understorey at small pole stage. The original parent stand, kept intact since the beginning of the research, displayed its authentically character without understorey. The model situation was analysed statistically as relations between the stand modification and amount of specific air pollution: protons H^+ and ground level ozone, entering the stand.

Variability of proton load values in years 1999–2006 on all the modified plots ranged from 33.2% on the plot treated with medium cut (stocking density 0.5 and 0.3 after the first and the second intervention, respectively) to 54.9% on the original intact plot (stocking density 0.9 and 1.0, respectively). Smaller differences in variability of the results were recorded before the cutting in year 2004 (Table 2).

The values of standard deviation were relatively low: from 4.7 on the plot treated with heavy cut resulting in 0.3 stocking density after the first intervention, to 7.2 on the plot treated with medium cut resulting in density values 0.5 and 0.3, after the first and the second intervention, respectively.

Table 2. Descriptive statistics of proton H^+ and ground level ozone on model research plots in the Kremnické vrchy Mts before and after cutting in 2004 year

Plot	Original parent stand	Heavy intervention	Medium intervention	Light intervention
Statistics	Proton H^+ before cutting in 2004			
Mean	39.68	55.57	14.38	11.19
Minimum	20.00	10.00	5.10	4.20
Maximum	84.00	114.00	22.90	19.00
Std. dev.	15.77	25.75	5.51	6.12
Std. error	2.98	6.88	1.47	2.17
Coeff. of variation.	39.70	46.40	38.20	54.90
	Proton H^+ after cutting in 2004			
Mean	29.56	42.79	14.35	21.63
Minimum	8.00	12.00	4.20	13.00
Maximum	88.00	80.00	22.30	30.00
Std. Dev.	20.24	18.44	4.76	6.49
Std. error	3.89	4.92	1.27	2.29
Coeff. of variation.	51.10	42.90	33.30	40.10
	Ground level ozone before cutting in 2004			
Mean	40.96	48.46	14.62	21.88
Minimum	12.00	10.00	4.80	11.40
Maximum	86.00	96.00	23.40	33.90
Std. dev.	19.39	24.46	5.56	7.26
Std. error	3.73	6.78	1.54	2.57
Coeff. of variation.	47.30	50.50	38.50	33.20
	Ground level ozone before cutting in 2004			
Mean	36.00	46.71	12.69	17.05
Minimum	8.00	10.00	4.70	8.50
Maximum	85.00	90.00	21.30	25.20
Std. dev.	16.83	21.81	5.11	6.34
Std. error	3.24	5.83	1.37	2.24
Coeff. of variation.	46.70	46.70	40.20	36.80

Std. dev., Standard deviation; Std. error, Standard error; Coeff., Coefficient.

As for the values of proton load quantity, the lowest were measured on the plot treated with heavy cut: 4.2 mmol H^+ day^{-1} m^{-2} in year 2000. Also on the other partial plots were the lowest values obtained in spring 2000.

Maximum values of 33.9 mmol H^+ day^{-1} m^{-2} in year 2005 were obtained on the plot subjected to medium cut, resulting in stocking density values 0.5 and 0.3, after the first and the second intervention, respectively. Very similar situation with an amount of 30.0 mmol H^+ day^{-1} m^{-2} was in the same year on the plot treated with heavy cut. The results of the experiment show influence of the second intervention realised in year 2004. The values of the other statistic characteristics are summarised in Table (2).

The lowest average values among all the plots were recorded on the intact plot covered with the original parent stand (1999–2006: 13 mmol H^+ day^{-1} m^{-2}) – showing the evident filtering role of beech forest stand significantly influencing the amount of pollutants deposed inside the forest ecosystem.

The inter-annual differences as well as differences among the modified plots were tested by pair tests for differences between two independent sampling sets. The results show that the differences at higher significance level concerning years were observed primarily after the second cutting performed in 2004 with the aim to reduce the stocking density on the plots formerly treated with heavy, medium and light cut. A high measure of differentiation was obtained especially by comparing year 2006 with the other years.

As for the differences among the plots, at the 99% probability level were identified as significant only the differences between the original parent stand and the plot heavy cut in the first intervention and between the original stand and plot medium cut after the second intervention.

We can see that removal of trees from the stand, that means changing stocking density, has an undeniable impact on amount of atmospheric protons entering the forest stand and then the forest soil. This statement is more true more is reduced the stocking density on the plot. The significance of differences among the plots decreased with the time – in absence of tree felling and in presence of natural regeneration processes. That means that the dynamically and naturally regenerated processes started to serve their filtering role concerning the polluting substances entering the forest ecosystems, Fig. 1.

The general drop in anthropogenic emissions was supposed to draw also a drop in O_3 concentration; no unequivocal trend, however, in the area of the Western Carpathians has been observed. The measure of variability in this indicator is about 50%. The lowest value, 39.7%, was recorded on the original plot with a stocking density of 0.9, later 1.0. The highest variation coefficient values were recorded on the plot subjected to heavy or final cut of the parent stand: 51.1%, Table 1. Comparing the values before and after the cutting in year 2004, we can see relatively balanced patterns reflecting low impact of management intervention on the ozone amount. Also in this case, the variability values were lower on the original plot than on the other research plots.

The lowest value of standard deviation making 16.8 was obtained on plot medium intervention, the highest one, making 25.8, on the original intact plot.

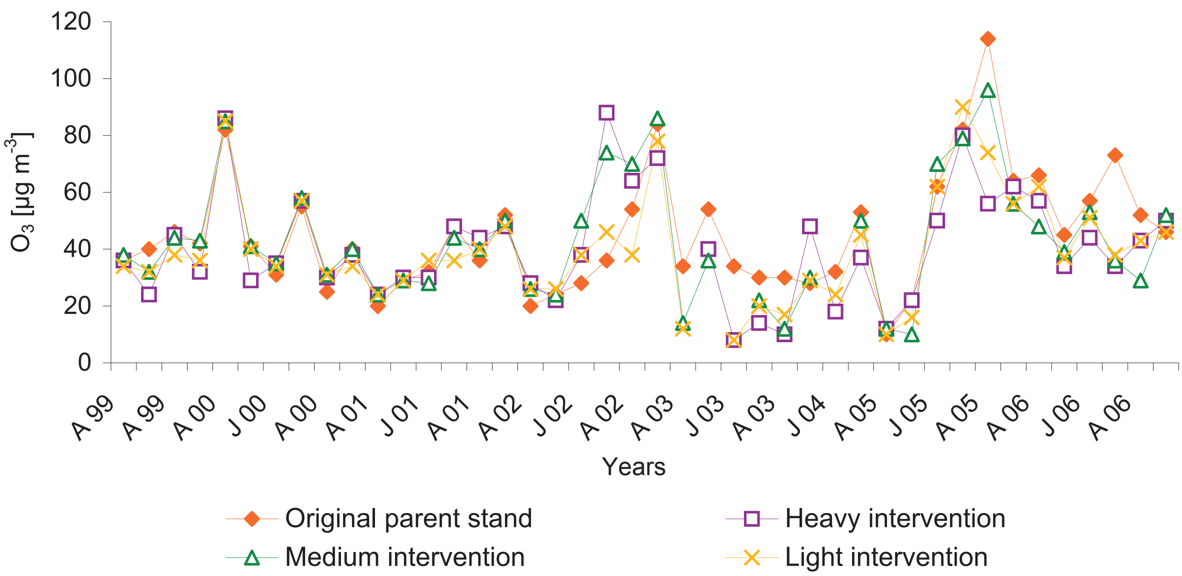

Fig. 1. Mean three-monthly values of proton load in mmol H^+ day^{-1} m^{-2} in growing seasons (S, spring; S, summer; A, autumn).

The other studied statistic characteristics are summarised in Table (2).

Low values ranging from 8.0 to 12 µg m^{-3} were measured on all partial plots, except the intact original stand (plot with a stocking density of 0.9 and 1.0 in years 1989 and 2004, respectively) on which the lowest value of this variable was about 20.0 µg m^{-3}. Very low values were observed especially in year 2005 that means the year following the second intervention. The absolute maximum, making 114.0 µg m^{-3}, was obtained in the original stand in autumn 2005. The maxima on the other experimental plots both before and after the intervention ranged between 88.0–96.0 µg m^{-3} (Fig. 2).

The differences in proton load as well as in ground level ozone were found significant among all the plots at the level of 99%. Statistical evaluation of the specific components ($O_{3,}$ H^+**)** resulted in finding that in case of these ozone substances, the reduction of stand stocking density did not influence their amount entering the forest stand to such extent as the input of protons. Certain part of atmospheric protons H^+ is intercepted by the tree crowns, the others enter the soil. As for ozone, the formation and concentration of this substance is affected by several additional factors: physical, chemical, biological and certainly some other, not recognised yet. Therefore, also the results of this experiment are to deal within a broader context, acting in synergy and representing only an isolated fragment of the state of art in ecosystems waiting for additional monitoring and study.

Conclusions

Quantitative differences in specific emissions (protons H^+ and ground level ozone) entering the modified beech stands were primarily dependent on their chemical composition – as show the statistic characteristics with differences relevant at 99% significance level.

The proton load quantity was clearly affected by removing trees from the stand, which means by stocking density reduction. This was evident, with 99% probability, namely after the first intervention for the original stand and the plot treated with heavy cut and after the second intervention between the original stand and the plot subjected to medium cut.

The stands 10–15 years after the intervention were continually and dynamically regenerated. The tree crowns and stand canopy were already serving the filtering function for substances entering the forest stands and soil from the contaminated atmosphere. This fact was backed-up also with very similar variability values.

As for the time trends, evident inter-annual differences at a higher significance level occurred especially after the second cutting intervention realised in year 2004.

The statistic evaluation resulted in finding that the modified stand density did not show as obvious influence in case of ground level ozone as in case of proton load.

The studied specific atmospheric elements exhibited different effects – depending not only on the chemical nature of the substance and on the state of ecosystem but also on a range of other factors acting in synergy.

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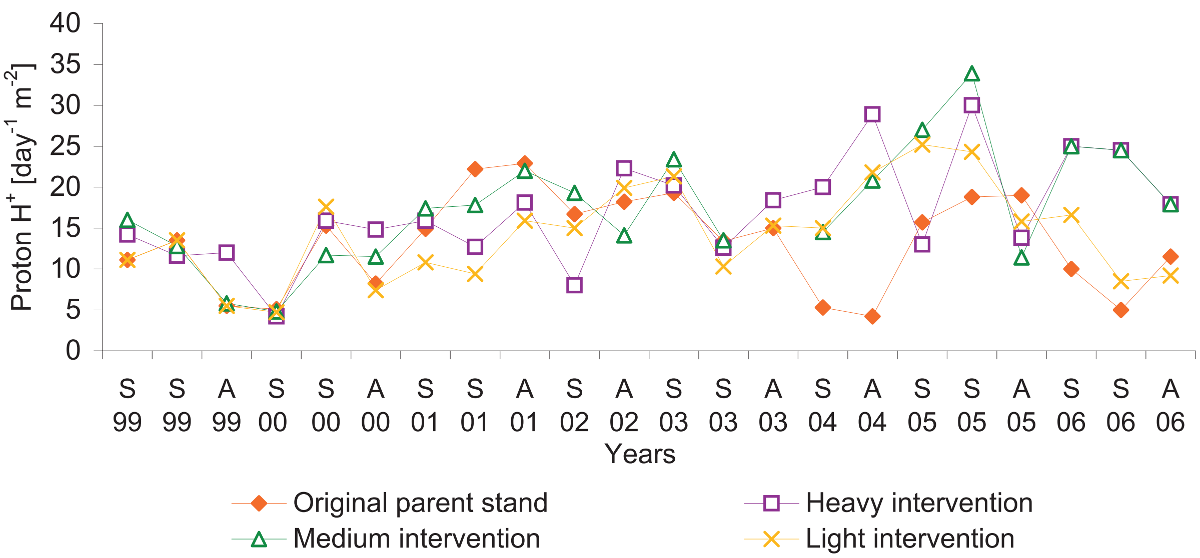

Fig. 2. Mean monthly ozone concentration values (µg m^{-3}) in growing seasons April–September.

2/0034/10, 2/0055/10, 2/0068/10). We also acknowledge D. Kúdelová for preparing the English text.

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Štatistické hodnotenie kvality znečisteného ovzdušia v modelových podmienkach podhorských bučín Západných Karpát

Súhrn

Kvantita špecifických imisných látok (H^+ a O_3) sa zisťovala v pravidelných časových intervaloch na plochách s modifikovaným zakmenením počas vegetačných období a štatisticky sa vyhodnotila.

Pri testovaní plôch sa s 99 %-nou istotou potvrdili významné diferencie v inpute protónov H^+ medzi pôvodným porastom a plochou s intenzívnym zásahom, resp. po druhom ťažbovom zásahu s plochou so stredne intenzívnym zásahom.

Najnižšie priemerné hodnoty (H^+) zo všetkých plôch boli zaznamenané na pôvodnej materskej ploche bez zásahu (1999–2006: 13 mmol H^+ deň$^{-1}$ m^{-2}). To ukazuje, že porast pôsobí ako filter a významne ovplyvňuje množstvo deponovaných znečisťujúcich látok do vnútra lesného ekosystému. Z maximálnych hodnôt dosiahnutých najmä v roku 2005 vidieť vplyv druhého ťažbového zásahu realizovaného v roku 2004.

Významnosť rozdielov koncentrácii O_3 medzi jednotlivými modifikovanými plochami a rokmi nebola potvrdená na takej vysokej úrovni ako to bolo pri protónoch H^+.

V prípade významnosti rozdielov medzi množstvom protónovej záťaže a množstvom prízemného ozónu sa táto potvrdila medzi všetkými skúmanými plochami navzájom na hladine významnosti 99 %. Z čoho vyplýva, že významným faktorom pri vstupe imisií do porastu je ich chemické zloženie.

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Reticulate phylogeny in the genus *Sorbus*: the case of *Sorbus haljamovae* Bernátová et Májovský

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Abstract

Kučerová, V., Gömöry, D. 2011. Reticulate phylogeny in the genus *Sorbu*s: the case of *Sorbus haljamovae* Bernátová et Májovský. *Folia oecol*., 38: 169–175.

Phylogenetic relationships between diploid sexual species *S. aria* and *S. chamaemespilus* and their putative alloploid hybrid *S. haljamovae* were studied on 8 localities in Central Slovakia. Six polymorphic chloroplast microsatellite markers were used to infer polymorphism levels in parental taxa and direction of hybridization. Six haplotypes were identified in the analyzed material. Haplotype 2 was found exclusively in *S. chamaemespilus*, which was found to be completely monomorphic. Haplotypes 1 and 4 were found only in *S. aria*, haplotypes 3 and 5 were shared by *S. aria* and *S. haljamovae*. A single specimen of *S. aucuparia*, used as outgroup in the phylogenetic analysis, contained a specific, highly divergent haplotype. Based on these results, we suggest that *S. haljamovae* is a polyphyletic taxon originating from at least two independent hybridization events.

Keywords

chloroplast microsatellites, hybridization, reticulate phylogeny, *Sorbus haljamovae*

Introduction

Phylogeny has for long been considered a simple branching process. In most phylogenetic models, a taxon (or, more generally, an operational taxonomical unit) can be linked only to its ancestor (usually a hypothetical taxonomical unit), but the connection between already existing taxa is not allowed. Reticulate relationships among taxa have, however, been recorded in many situations. In the plant kingdom, the most important underlying mechanism is hybridization. Almost 25% of plant species are supposed to be involved in hybridization (Mallet, 2005). Hybridization and introgression are thus rule rather than exception in the plant kingdom and the occurrence of hybrids is by far not limited to specific sites (Arnold, 2006; Cronn & Wendel; 2004; Rieseberg & Carney, 1998). The view of hybridization as a lapsus of nature is shifting towards a full recognition of its role as a creative force in plant evolution and speciation (cf. Wissemann, 2007).

In the case of alloploidy, hybridization is associated with genome duplication. Polyploidy is a common mechanism of the diversification and speciation in plants (Rieseberg, 1997). Most plant taxa have overcome a change of the genome size in their evolutionary past (Soltis et al., 2004). Many but not all alloploid taxa are apomictic, so that complicated hybrid complexes have been formed in several genera (cf. Nelson-Jones et al., 2002).

Reticulate evolution is not limited to the interspecific level. Intraspecific evolution also includes exchange of genetic material among genetically different individuals and populations (Smouse, 2000), which may represent recognized taxonomical units (subspecies, varieties) or populations, whose morphological distinctness need not justify a separate taxonomic classification, but which may be strongly differentiated at adaptive as well as neutral genes.

Alloploidy as a mechanism of speciation has widely occurred in the genus *Sorbus*, where plenty of taxa (usually stendoendemics) have been described, originating putatively from alloploid hybridization (Nelson-Jones et al., 2002). Some taxa were even suggested to be hybrids of three or more species (Bernátová and Májovský, 2003). For our study, we selected one of the pink-flowered hybridogenous taxa, namely *Sorbus haljamovae* Bernátová et Májovský, which, based on morphological assessment, is supposed to have originated from alloploid hybridization between *S. aria* and *S. chamaemespilus* (Bernátová and Májovský, 2003). It occurs mostly in subalpine or dealpine communities with the dominance of *Pinus mugo* on calcareous rocks, the occurrence is limited to high mountain ranges of Central Slovakia (Veľká Fatra, Malá Fatra, Nízke Tatry).

Using maternally inherited markers, we tried to identify which species in the *S. aria – S. chamaemespilus* complex are the maternal ones in the interspecific hybridization, and how the monophyletic or polyphyletic origin of the hybridogenous taxon *S. haljamovae* is reflected in its geographic distribution.

Material and methods

Samples of subalpine *Sorbus* taxa were collected on the summits of mountain ranges of Central Slovakia (Table 1) during 2009–2010. Sampling was done preferably during the flowering seasons to allow reliable determination of the collected specimens, which was done by Dr. D. Bernátová (Botanical Garden of the Comenius University, Blatnica) either directly in the field or subsequently on herbarium specimens. Sampling was completed by herbarium specimens deposited in the Botanical Garden in Blatnica. A single *S. aucuparia* specimen was also included to provide outgroup for the analysis of phylogenetic relationships among chloroplast haplotypes.

Total genomic DNA was isolated from air-dried leaves using CTAB method according to Doyle and Doyle (1987), which was modified for a smaller amount of plant material. For genotyping, six microsatellite loci (*trnT-Lpm4, trnT-Lpm3, rps16pm2, rps16pm1, rpl16pm1, trnT-Lpm1*) according to Chester et al. (2007) were used. Different size of alleles and suitable fluorescent labelling enabled multiplexing primers into one group. All PCRs were performed in 6 µl reactions in a GeneAmp® PCR System 9700 (Applied Biosystems) or iQ5 real-time PCR detection system (Bio-Rad) thermal cycler. PCR Master mix contained approximately 50 ng of DNA, 3 µl Qiagen Multiplex PCR Kit and the following concentrations of primers: 0.4 µM trnT-Lpm4, 0.4 µM trnT-Lpm3, 0.05 µM rps16pm2, 0.05 µM rps16pm1, 0.05 µM rpl16pm1, 0.4 µM trnT-Lpm1. Amplification profile consisted of an initial denaturation step at 95 °C for 15 min, followed by 32 cycles with the following profile: 30 s denaturation at 94 °C, 90 s annealing step at 50 °C and 1 min extension step at 72 °C. Final extension was 8 min at 72 °C. Amplification products were separated on a ABI 3100 DNA sequencer, and genotypes were determined using the GeneMapper software v 3.7.

Distances between haplotypes were assessed as the sums of insertions/deletions over the analyzed microsatellite loci. Although the stepwise mutation model is generally considered more appropriate for tandem-repeat loci than the infinite allele model, size differences of alleles did not indicate stepwise insertions/deletions except in locus 3, with 1 bp size differences. Therefore, we considered that the size difference is equal to the number of mutational events at locus 3, whereas a single indel was supposed to produce alleles at the remaining loci. Phylogenetic relationships among haplotypes were reconstructed using the nearest neighbour clustering.

Haplotypic diversity of taxa at individual locations was assessed by gene diversity (Nei, 1978):
$h_e = 1 - \Sigma_i p_i^2$; p_i being the frequency of the *i*th haplotype.

Geographical distribution of taxa was visualized in the ArcView 3.2 environment (ESRI, Redlands, USA).

Results and discussion

As expected, the number of alleles found at the chloroplast loci under study was generally low (Table 2). In spite of analyzing three generally recognized species

Table 1. Basic characteristics of the sampling locations

Location	Longitude	Latitude	Altitude	Forest type class[1]	Soil type (WRB)[2]
Pekarová	18.9644	48.9572	880	Fagetum delapinum	Litho-Rendzic Leptosol
Poludňové skaly	19.0839	49.2378	1,090	Fagetum delapinum	Rendzic Leptosol
Salatín	19.3628	48.9792	1,600	Mughetum calcicolum	Rendzic Leptosol
Siná	19.5719	49.0002	1,150	Fageto-Piceetum	Rendzic Leptosol
Skalná alpa	19.1919	48.9839	1,440	Mughetum calcicolum	Foli-Rendzic Leptosol
Stratenec	18.9675	49.1794	1,460	Mughetum calcicolum	Litho-Rendzic Leptosol
Čierny kameň	19.1464	48.9378	1,380	Mughetum calcicolum	Rendzic Leptosol
Haľamova kopa	18.9944	48.8986	1,070	Fageto-Piceetum	Rendzic Leptosol

[1]Hančinský (1972)
[2]Fao/Iiasa/Isric/Isscas/Jrc (2009)

belonging to different subgenera, only two to four alleles per locus were found, whereby one allele at each locus was unique for *S. aucuparia*. At the loci *trnT-Lpm4*, *rps16pm2*, *rps16pm1* and *rpl16pm1*, differences among alleles by multiples of the repeat motif length could be identified. However, larger insertions/deletions were present at the loci *trnT-Lpm3*, *rpl16pm1* and *trnT-Lpm1*. As we did not sequence the alleles, we were unable to identify the number of mutation events that could have produced variation at such loci. Therefore, for the sake of the phylogenetic analysis, we considered such alleles separated by a single insertion or deletion event.

Allele combinations resulted in six haplotypes. One, highly divergent, was found only in the *S. aucuparia* specimen, used as outgroup in the phylogenetic analysis. The other putatively diploid sexual species did not share any haplotype. All specimens of *S. chamaemespilus* (represented by the by far biggest sample size

Table 2. Haplotypes and the corresponding allele sizes at chloroplast microsatellite loci found in the studied *Sorbus* taxa

Haplotype	*trnT-Lpm4*	*trnT-Lpm3*	*rps16 pm2*	*rps1 6pm1*	*rpl16 pm1*	*trnT-Lpm1*	Taxon
1	115	164	270	100	143	272	*S. aria*
2	115	164	271	100	143	272	*S. chamaemespilus*
3	115	187	269	100	144	272	*S. aria, S. haljamovae*
4	115	187	270	100	144	272	*S. aria*
5	115	187	271	100	144	272	*S. aria, S. haljamovae*
x	121	162	268	104	112	239	*S. aucuparia*

Table 3. Sample size (*n*) and haplotypic diversity (h_e; Nei 1978) of *Sorbus* taxa at individual locations

Population	*S. chamaemespilus*		*S. haljamovae*		*S. aria*	
	h_e	*n*	h_e	*n*	h_e	*n*
Pekarová	–	–	–	–	0.653	7
Poludňové skaly	–	1	0.000	6	0.000	5
Salatín	0.000	19	–	–	–	1
Siná	0.000	8	0.000	2	0.555	15
Skalná alpa	0.000	21	0.000	10	0.500	2
Stratenec	0.000	2	–	–	0.500	6
Čierny kameň	0.000	16	–	–	–	–
Haľamova kopa	–	–	–	–	0.000	4
Total		67		18		40

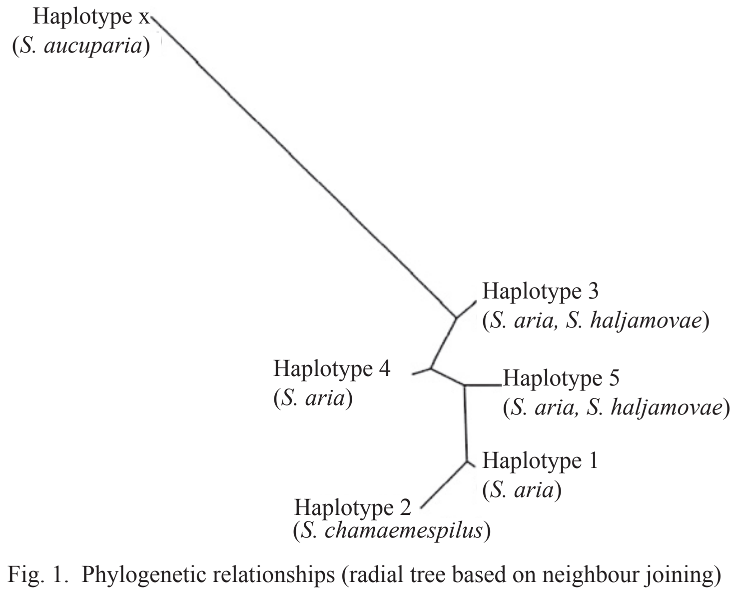

Fig. 1. Phylogenetic relationships (radial tree based on neighbour joining) among chloroplast haplotypes in the *Sorbus* complex.

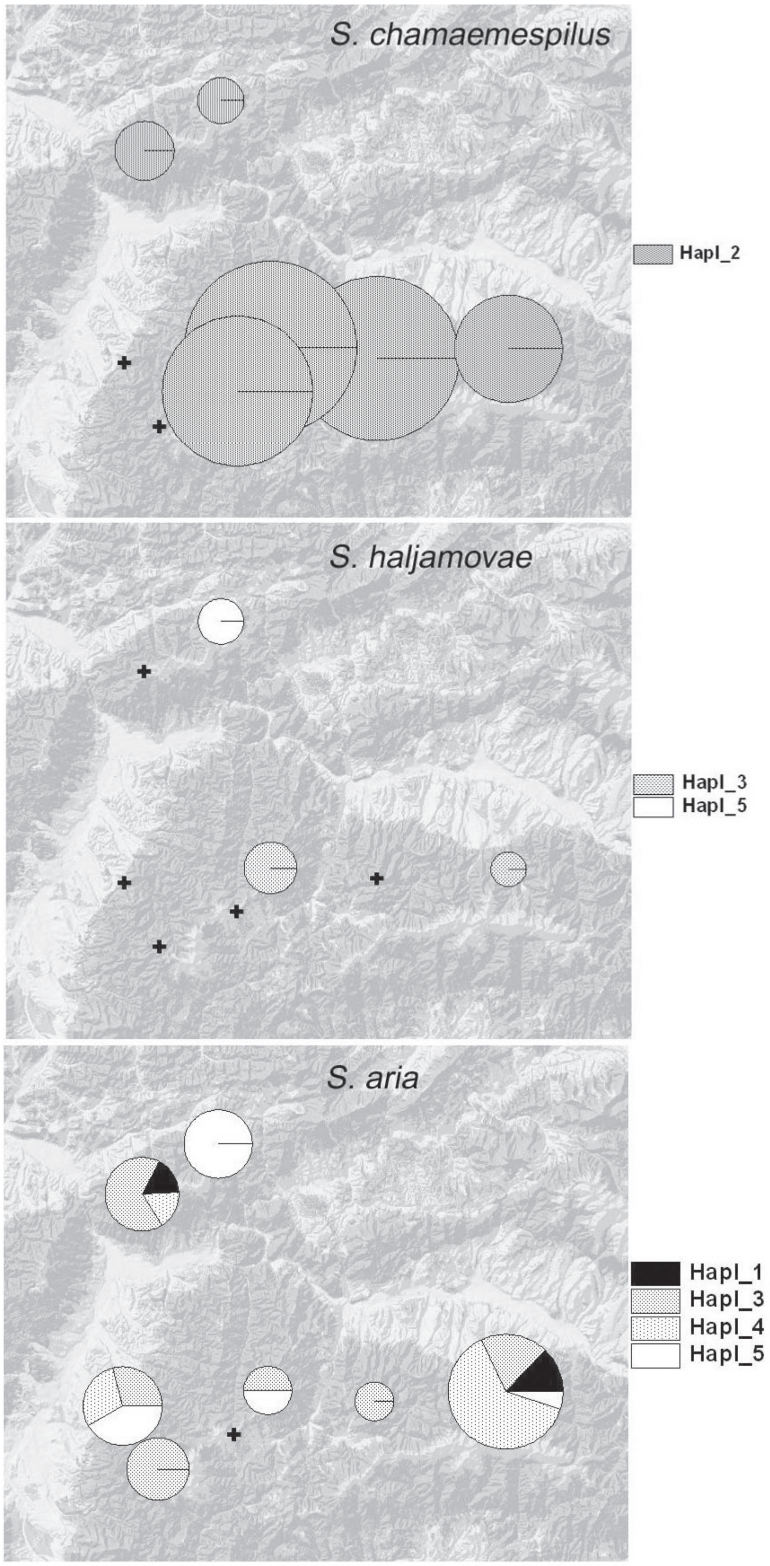

Fig. 2. Distribution of haplotypes in individual populations of *Sorbus* taxa. Symbol size corresponds to sample size, cross means absence of a taxon at the respective location.

in our collection; see Table 3) contained exclusively haplotype 2, which was not found in any other taxon. On the other hand, *S. aria* was found polymorphic, containing 4 haplotypes. Among them, haplotypes 3 and 5 were shared with *S. haljamovae* (Table 2). Phylogenetic relationships among haplotypes, as revealed by the neighbour-joining tree, do not exhibit any clear pattern related to taxonomy (Fig. 1) or geography (Fig. 2).

Central position of *S. aria* (although polymorphic) between *S. aucuparia* and *S. chamaemespilus* (Fig. 1) might reflect its ancient status, also confirmed by the fact, that *S. aria* aggregate is an obligatory participant in all known interspecific crosses in *Sorbus* (Nelson-Jones et al., 2002).

Relatively small sample sizes at many locations do not allow make any definite conclusions about the local polymorphism levels. However, most local populations were monomorphic (Fig. 2). Actually, only *S. aria* exhibited polymorphism at the local population level, with up to four haplotypes per population (Table 3).

Hypothetically, the observed patterns allow several explanations in terms of phylogeny and biogeography, nevertheless, not equally probable:

- Incomplete lineage sorting. Because we did not estimate genome size by flow cytometry, we actually cannot be sure that *S. haljamovae* really is an alloploid hybrid. It may eventually represent another diploid (sexual or apomictic) taxon sharing ancestral polymorphisms with *S. aria*.
- Homoplasy of and/or indpendent mutation events. Because microsatellite alleles represent tandem repeats of short sequence motifs and arise easily through slipping of DNA polymerase during the DNA replication, SSR loci are highly mutable and thus the probability that identical allele sizes are formed by independent insertions or deletions of sequence motifs is not negligible. Moreover, homoplasious alleles may arise by reverse mutations.
- Polyphyletic origin of *S. haljamovae*, i.e. polytopic *in situ* formation of hybrids through independent (alloploid) hybridization events. The fact that *S. haljamovae* shares maternal haplotypes with the local *S. aria* population at each location indicates that *S. aria* contributed by female gametes and *S. chamamespilus* was the father in such natural crosses.

We consider the first hypothesis not very probable. There is a general consent that there are only 5 sexual diploids in the genus *Sorbus* in Europe (Nelson-Jones et al., 2002; Robertson et al., 2010). All pink-flowered taxa or taxonomically unclassified individuals are generally supposed to have originated from hybridization of *S. chamaemespilus* with another taxon (usually *S. aria* has been suggested to contribute by a part of the hybrid genome). Morphologically, at least concerning flower, fruit and leaf traits, *S. haljamovae* represents transition between *S. chamaemespilus* and *S. aria* (Bernátová and Májovský, 2003).

The second concern is more serious. Any phylogenetic analysis is based on the assumption that similarities of traits under study is due to homology, not homoplasy. Admitting that identical allele sizes observed in different taxa result from independent mutations would ruin the relevance of this marker type for the reconstruction of phylogeny in general. However, organellar tandem repeats are commonly used for this purpose (cf. Pleines et al., 2009; Weising and Gardner 1999). In particular, cpSSRs have also successfully been used for the study of alloploid and homoploid hybridization (Angioi et al., 2010; Li et al., 2009; Zeinalabedini et al., 2010), including the genus *Sorbus* (Chester et al., 2007). We do not feel competent to judge the mutation rates of different parts of the chloroplast genome: chloroplast DNA is generally regarded as conservative, but this need not apply to the whole chloroplast sequence and microsatellites may represent mutation hotspots. Nevertheless, although the haplotype divergence within the subgenera *Aria* and *Chamaemespilus* is small, *S. aucuparia* specimen used in our study is characterized by a highly divergent haplotype and the remaining material we analyzed indicates that there is a plenty of haplotypes in the subgenus *Sorbus*, all of them equally genetically distant from those found in *S. aria* and *S. chamaemespilus* (unpublished data). If allele-size sharing by different *Sorbus* taxa is due to random processes of forming new mutations and reverting them to the original state across taxa, then such process should be in action in the whole genus, not only particular subgenera.

Conclusions

We consider polyphyletic origin of *S. haljamovae* the most plausible explanation of the observed haplotype distribution patterns. Why different crossing events produced at least two morphologically homogeneous groups (*S. haljamovae* and *S. zuzanae*) remains a question. We do not have reliable information about reproduction biology of subalpine *Sorbus* taxa, extent of apomixis (suggested by Jankun, 1993, as an important mechanism of evolution in *Sorbus*), ploidy level distribution in the populations of the putative parental and hybridogenous taxa, or back-crossability of hybrids with parental species and thus potential for introgression. No signs of backcrosses have been reported from Slovakia, but this is not a proof of the absence of introgression.

There remains thus much unknown about the formation of putatively hybridogenous taxa in subalpine *Sorbus*. In addition to fundamental scientific importance for biology, the information about reticulate phylogeny patterns is relevant for practice in the case of trees. For acquiring of forest reproductive material and designing appropriate measures of gene conservation (mainly *in situ*), the extent and direction of introducing alien genes in the gene pool of a population should be known. Another aspect of relevance for society is nature conservation. Frequently, a single alloploid apomictically propagating genotype was classified as a species. Because of rarity, such taxa frequently enjoy protection at the species level, which is not reasonable in this case. Elucidation of mechanisms of hybrid speciation and preservation of species integrity may thus be relevant for the elaboration of effective approaches to species conservation.

Acknowledgements

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Retikulátna fylogenéza v rode *Sorbus*: prípad *Sorbus haljamovae* Bernátová et Májovský

Súhrn

Sledovali sme fylogenetické vzťahy v rámci hybridného komplexu *S. aria – S. chamaemespilus*. Taxón *S. haljamovae* je považovaný za aloploidného hybrida oboch diploidných a pohlavne sa množiacich rodičovských druhov. Materiál (celkovo 135 jedincov) bol zozbieraný resp. získaný z 8 lokalít vysokých pohorí stredného Slovenska. Na identifikáciu hybridných vzťahov boli použité maternálne dedené markéry, konkrétne 6 chloroplastových

mikrosatelitov, ktoré všetky vykazovali polymorfizmus. V analyzovanom materiáli sme našli 6 haplotypových kombinácií. Haplotyp 2 bol nájdený len u *S. chamaemespilus*, populácie tohto taxónu boli monomorfné. Haplotypy 1 a 4 sa našli len u *S. aria*, haplotypy 3 and 5 sme identifikovali u *S. aria* a *S. haljamovae*. Vzorka *S. aucuparia* použitá vo fylogenetickej analýze pre zakorenenie fylogramu, vykazovala špecifický haplotyp výrazne divergentný od zostávajúcich (obr. 1). Jedine populácie *S. aria* boli polymorfné. Na lokalitách so spoločným výskytom *S. aria* a *S. haljamovae* oba taxóny vždy zdieľali chloroplastový haplotyp (obr. 2). Tieto výsledky naznačujú, že *S. haljamovae* je polyfyletický taxón, ktorý vznikol prinajmenšom dvomi nezávislými kríženiami rodičovských druhov a následne sa rozšíril na ďalšie lokality.

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Phyllophagous sawflies (Hymenoptera, Symphyta) in pine stands (*Pinus sylvestris*) in a sandy lowland, Slovakia

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Abstract
Kulfan, M., Holecová, M., Beracko, P. 2011. Phyllophagous sawflies (Hymenoptera, Symphyta) in pine stands (*Pinus sylvestris*) in a sandy lowland, Slovakia. *Folia oecol*., 38: 176–182.

In the period of 2008 and 2009, the authors studied and compared taxocoenoses of phyllophagous sawflies (Symphyta, Hymenoptera) on Scotch pine on four study plots in the Borská nížina lowland (southwestern Slovakia). In total, 6 species of sawflies belonging to two families were found. Nursery Pine Sawfly *(Gilpinia frutetorum)* with the markedly highest abundance in all study plots is the most numerous species. The highest total abundance of sawfly larvae was found on 20-year-old pine trees of forest stand wall and in close stand of young 10-year-old pines. According to the dendrogram based on Wishart's index two different groups of sawfly communities are specified: sawfly communities in dense stands in contrast with sawfly communities in open pine stands. Statistically significant difference between the values of Shannon-Wiener's diversity of sawfly communities in open and dense stands was found. The test anosim shows that there is no significant difference between study plots with regard to species composition of a sawfly community and abundance of individual species.

Key words
Borská nížina lowland, pine defoliators, sawflies, southwestern Slovakia

Introduction

Fauna of sawflies (Symphyta) in their larval stage has been insufficiently explored in Central Europe. Most of the works of the Carpathian Basin comes from studies on adult sawflies (Roller and Haris, 2008). From the Slovak territory, there exist only old faunistic data on the occurrence of economically important diprionid species (Symphyta, Diprionidae) as well as from the Borská nížina lowland in the southwestern Slovakia (Mocsáry, 1900; Ortvay, 1902; D´Agnolo, 1940). So far, little attention has been paid to Symphyta larvae on *Pinus* in Slovakia. Jamnický (1963, 1988, 1990) in his studies on biotic and abiotic factors affecting tree species growing in the upper limit of the forest and in the subalpine vegetation zone in the Tatra mountains (High Tatras, Belianske Tatras, West Tatras and Low Tatras) and some other mountains in Slovakia was the one who examined larvae of sawlies (Symphyta). Analyzing insects on two pine species – *Pinus mugo* and *P. cembra* (dwarf pine vegetation tiers) he has extended knowledge on bionomy of dominant phyllophagous species. Hrubík (1988) studied bionomy of two pests of sawflies living on *Pinus sylvestris* in urban areas. Roller (1999) found relatively rich assemblages of sawfly adults sampled by Malaise traps in 4 localities (Pernek, Jakubov, Studienka, Malacky) of Borská nížina lowland (richness of 65 to 132 species). So far, there has been no research of larval stages of sawflies on *Pinus sylvestris* in Slovakia yet.

The main goal of this paper is a comparison of Symphyta larvae on Scotch pine of different age and different structure of pine stands in four plots in the Borská nížina lowland. Study plots belong to biotop of cultivated Pine forests and semi-native pine-oak forests.

Material and methods

Research of Symphyta larvae of Scotch pine (the age of pines 5 to 100 years) was carried out at four study plots in the Záhorie Protected Landscape Area around the village Lakšárska Nová Ves (DFS grid square: 7468/69) in 2008 and 2009. The beating method of 1 m long branches of 50–200 cm above the ground was applied (200 beats = 10 samples on competent plot at each sampling). One sample refered to 20 beats. Each study plot was visited during the vegetation season from April to September at approximately monthly intervals. The larvae were preserved in 70% ethanol and identified in the laboratory. The voucher specimens of all sawfly species detected in the study are deposited in the collections of the authors.

Description of the study plots (the situation in 2008):

Plot 1: Stand of no canopied young pines (free-growing pines of age 20 years) on sand dune gradually going to the stand of about age 100 years.

Plot 2: Dense stand of young pine trees (canopied pines, close forest stand), age 5 years.

Plot 3: Forest stand wall of pines (no shielded pine trees located on the edge of forest stand, open pine stand), age 20 years.

Plot 4: Dense stand of young pine trees (canopied pines), age 10 years.

Determination of Symphyta larvae was made by means of publication Viitasaari and Varama (1987) and Viitasaari (2002).

The cluster analysis of the communities was performed using the computer program NCLAS (Podani, 1993). The clustering method complete linkage in combination with Wishart's similarity ratio was used (Wishart, 1969). Species communities were compared using Principal Component Analysis (PCA) as an indirect gradient method (Ter Braak and Šmilauer, 1998). Diversity was characterised using the Pielou's index of equitability (e), Shannon-Wiener's index of total species diversity (H') and Simpson's index of dominance (c) (Ludwig and Reynolds, 1988; Poole, 1974).

Analysis of similarities (anosim) was used to compare communities between the groups of site classes. anosim is a nonparametric procedure that evaluates whether the average similarities between samples within individual groups are closer than the average similarities of all pairs of replicates between groups (Clarke, 1993). For significance testing, the ranked similarity within and between groups is compared with the similarity that would be generated by chance. Essentially the samples are randomly assigned to groups 10,000 times, and Rank similarity (R) is calculated for each permutation. The observed value of R is then tested to determine significant difference from random distribution. For anosim was used PAST 1.95 software (Hammer et al., 2001).

Results and discussion

In the studied area six species of sawflies, belonging to two families – Diprionidae (genera *Gilpinia* and *Diprion*) and Pamphiliidae (genus *Acantholyda*), were found. Nursery Pine Sawfly (*Gilpinia frutetorum*) with the markedly highest abundance in all study plots is the most numerous species (Table 1, Figs 1–3). *Gilpinia virens* was also recorded in all plots (Table 1, Figs 1–3). The highest total abundance of larvae was found in 2008 on the plot 3 (20-year pines of forest stand wall) and in 2009 on the plot 4 (dense stand of young 10-year pines) (Figs 1–2). Larvae of web-spinning pine-sawfly (*Acantholyda hieroglyphica*) were found only on 5 year old and 20 year old pines – plots (Table 1). According to the published observations they usually prefer 2–5 or 3–4 year old pines (Pschorn-Walcher, 1982;

Table 1. Overview of sawfly species found on study plots

Species	Study plot/Abundance				Abundance together
	A	B	C	D	
Gilpinia frutetorum (Fabricius, 1793)	32	20	44	23	119
Gilpinia virens (Klug, 1812)	6	8	4	6	24
Gilpinia pallida (Klug, 1812)	0	3	3	8	14
Gilpinia variegata (Hartig, 1834)	1	4	3	5	13
Diprion similis (Hartig, 1837)	0	0	1	1	2
Acantholyda hieroglyphica (Christ, 1791)	0	2	1	0	3
Total abundance	39	37	56	43	175

A = plot 1, B = plot 2, C = plot 3, D = plot 4.

Achterberg and Aartsen, 1986). Based on the zoogeographic distribution the found species belong to a group of Eurosibirian faunal elements. Of them *G. frutetorum* and *Diprion similis* were introduced to North America and/or Canada (*G. frutetorum*) (Pschorn-Walcher, 1982). Both species have a relatively high tendency to outbreaks (Herz and Heitland, 2002). Also *G. frutetorum* may cause significant damages on pines as it showed highest abundance in studied plots (Table 1, Figs 1–3).

Dendrogram constructed on the basis of abundance similarity (Wishart's similarity ratio) identified two clusters of coenoses. The first cluster consists of sawfly community of sparse 20-year pine stand (free-growing young pines) gradually going to the stand of age of about 100 years – open pine stand (study plot 1) and

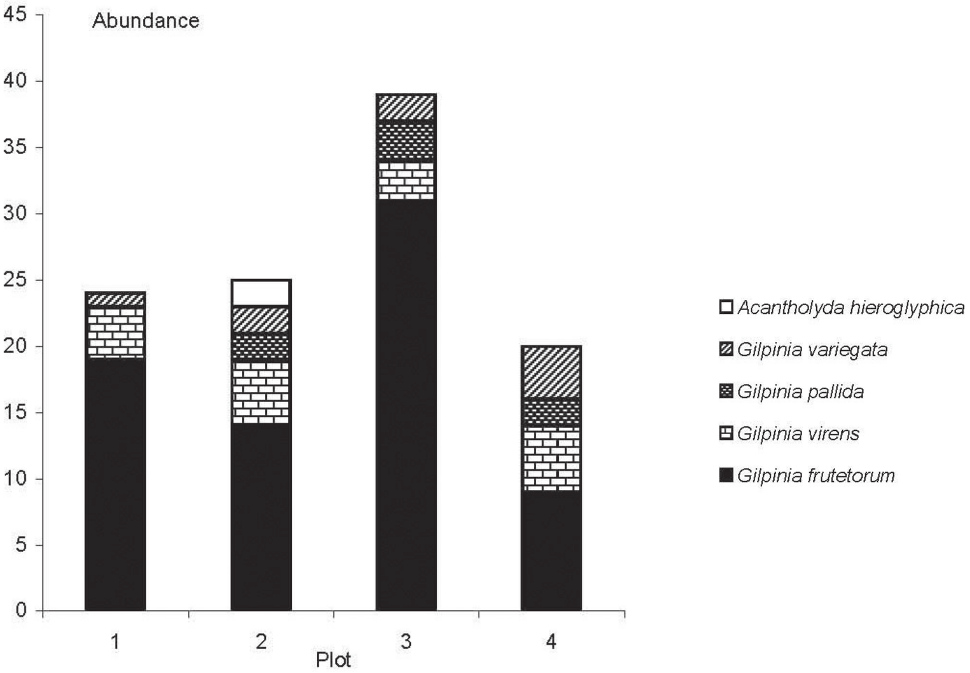

Fig. 1. Proportion of sawfly species in 2008 found on four study plots.

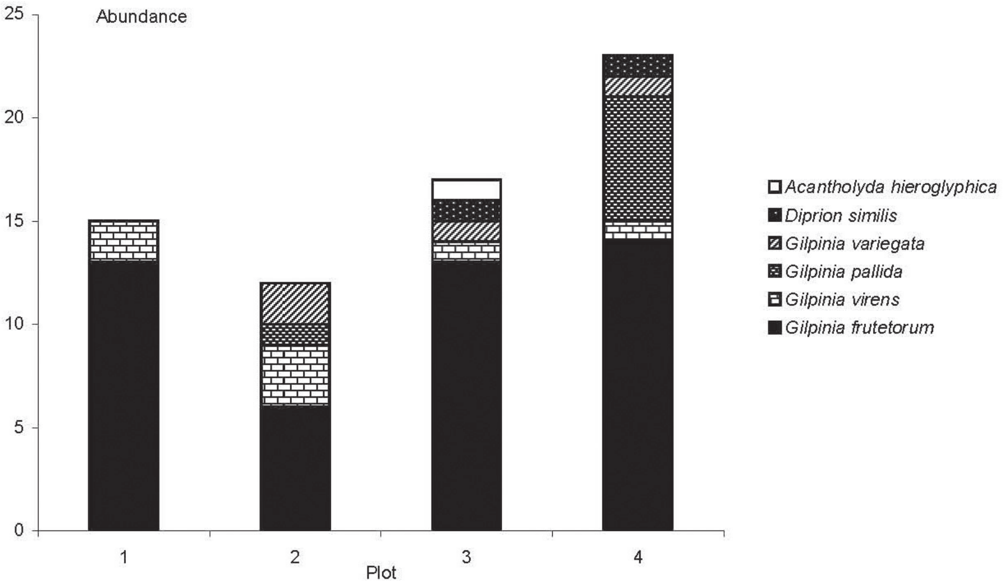

Fig. 2. Proportion of sawfly species in 2009 found on four study plots.

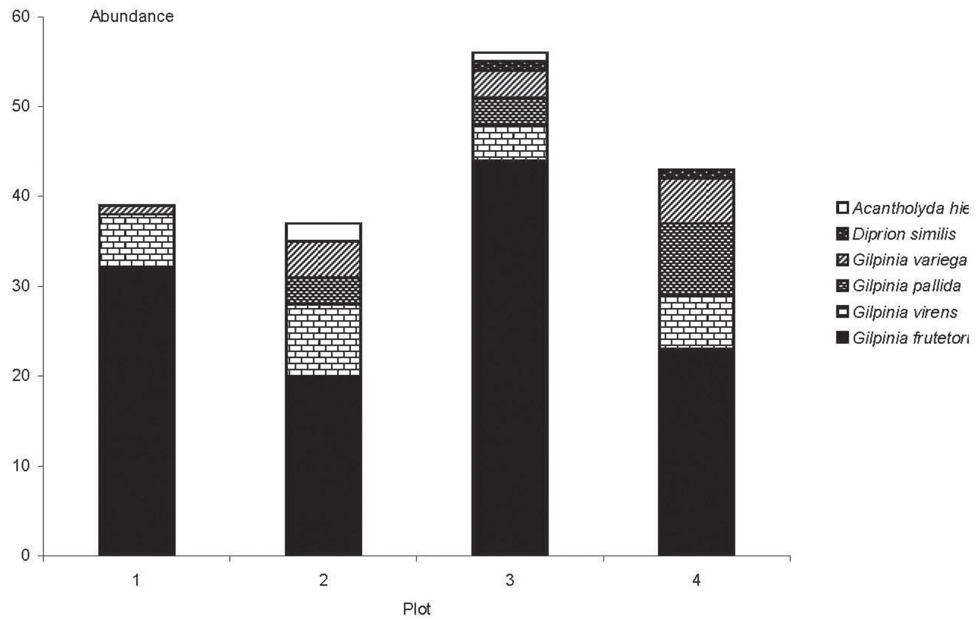

Fig. 3. Proportion of sawfly species in 2008 and 2009 found on four study plots.

sawfly community of marginal 20-year old open pine stand (forest stand wall) (study plot 3). The second cluster consists of sawfly community on young pines in a closed forest stand (communities on study plots 2 and 4). Both clusters are linked to the relatively high level of similarity. It is likely due to a relatively short distance of compared study plots. Communities on plots 1 and 3 and also on plots 2 and 4 are connected to a very high level of similarity (Fig. 4).

The distribution of the study plots and sawfly species in the space of the first two axes of Principal Component Analysis (PCA) is shown in Fig. 5. The groups of

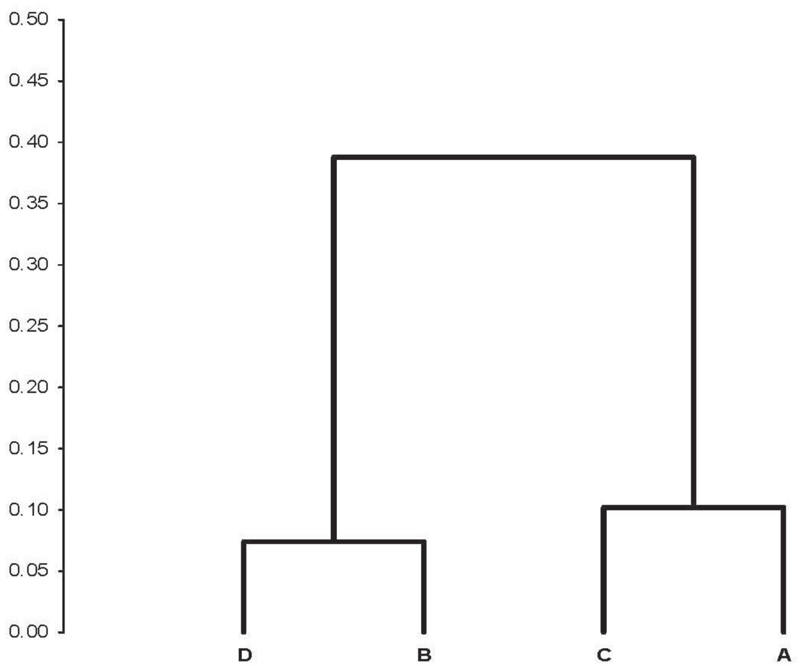

Fig. 4. Hierarchical classification of pine sawfly taxocoenoses on four study plots according to abundance similarity (Wishart similarity ratio, complete linkage) (vertical axis – dissimilarity). A = plot 1, B = plot 2, C = plot 3, D = plot 4.

species were determined on the basis of the dendrogram of abundance similarity resulting from the position of study plots and species in the ordination space of the first four PCA axes. Study plots and species were located along the first axis which has the largest information statement. On the right side of the crossing point the plots of no canopied pines (fragmented study plots) are located – plots 1 and 3 and on the left side the stands of canopied pines (closed forest stands) are present – plots 2 and 4. Euryvalent species *G. frutetorum* was more numerous in both fragmented stands – plots 1 and 3 compared with canopied stands – plots 2 and 4 (Fig. 3).

Sawfly communities in canopied stands (plots 2 and 4) have higher values of Shannon-Wiener's diversity and equitability (evenness) (Tab. 2). Statistically significant difference was found between the values of Shannon-Wiener's diversity of sawfly communities in open and closed stands (Tab. 2).

With the exception of *G. frutetorum* and *D. similis* the other species seem to be more adapted to canopied

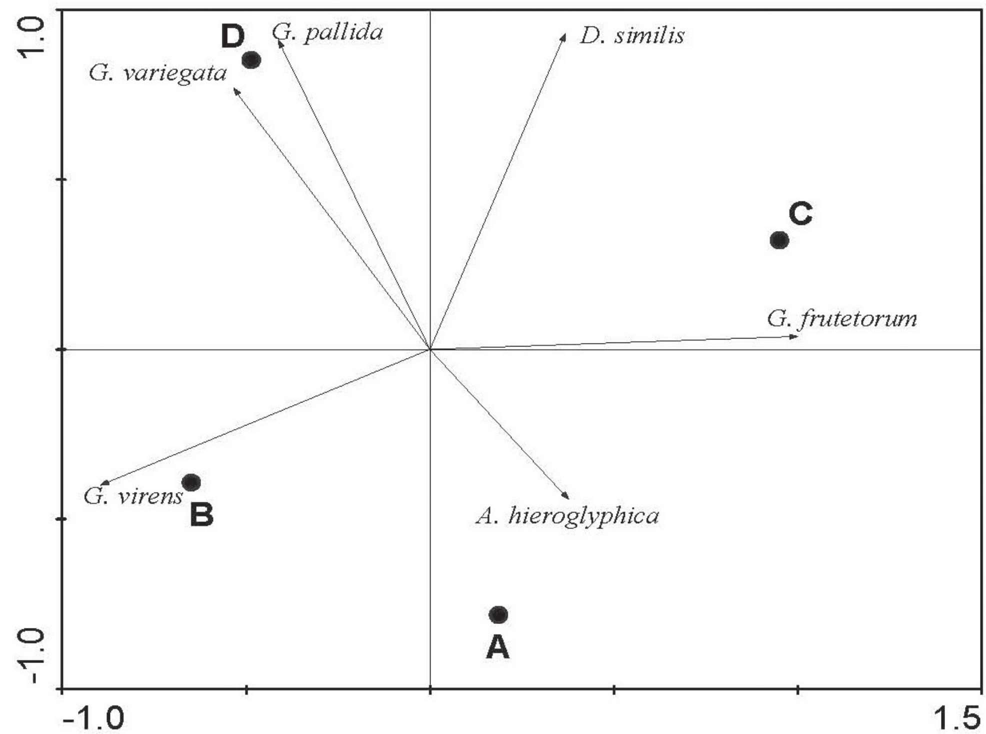

Fig. 5. PCA ordination diagram of the study plots and sawfly species score. A = plot 1, B = plot 2, C = plot 3, D = plot 4.

Table 2. Species diversity test (Poole, 1974) and basic coenological characteristics of sawfly communities on four study plots in 2008 and 2009

Study plots	Value	A	B	C	D
	spp	4	5	6	5
	e	0.467	0.786	0.494	0.783
	c	0.664	0.360	0.608	0.354
	H'	**0.648**	**1.266**	**0.885**	**1.260**
A	**0.648**	0.000	76.756	95.608	77.864
B	**1.266**	3.087**	0.000	93.626	76.820
C	**0.885**	1.099ns	1.843ns	0.000	98.212
D	**1.260**	3.229**	0.031ns	1.910ns	0.000

A = plot 1, B = plot 2, C = plot 3, D = plot 4, ssp = number of species, e = Pielou's index of evenness, c = Simpson's index of dominance, H' = Shannon-Weaver's index of species diversity. Significance levels: ** = $0.001 < P < 0.01$; ns = $0.05 < P$ (non-significant).

forest stands. The most species were found on the plot 3 which all the species were present during the research on (Table 1, Fig. 3). The lowest richness refers to the most photic stand – plot 1 (sand dune with sparse pine stand exposed to insolation) (Table 1, Fig. 3).

With regard to the Simpson's index of dominance it was confirmed that sawfly communities in the open stands (plots 1 and 3) had a „dominance concentration" to the species of *G. frutetorum* (Table 2, Fig. 3). On the other hand dominance (according to Simpson's index of dominance) of sawfly communities in canopied stands (plots 2 and 4) was spread over a larger number of species (Fig. 3).

The test ANOSIM shows that there is no significant difference between study plots with regard to species composition of sawfly community and abundance of individual species. In other words, the variability in species composition of sawfly community and abundance of individual species within each study area is not significantly different from the variability between plots (Table 3).

Table 3. Pairwise tests of ANOSIM.
Full data ANOSIM – R = –0.21, p = 0.84

p-values	A	B	C	D
A	x	1	0.664	0.660
B	1	x	1	1
C	0.664	1	x	1
D	0.660	1	1	x

A = plot 1, B = plot 2, C = plot 3, D = plot 4.

Regarding the occurrence of larvae during the season *Gilpinia frutetorum* had two abundance peaks, the first in mid May and the second in early October (records from late April to early October). The seasonal records of other, less numerous species are as follows: *G. virens* – from mid May to early October, *G. pallida* – from late April to the first third of June, *G. variegata* – from early June to early September, *Diprion similis* and *Acantholyda hieroglyphica* – from the second third of May to early September.

In conclusion we can say that the study plots are relatively poor in sawfly species. None of the found species showed significant increased abundance compared, for instance, with *Neodiprion sertifer* (Geoffroy, 1785) – pest of *Pinus mugo* from the family Diprionidae in the Giant Mountains (NEHRING, 1894; RÖHRING, 1895) or with the pests of *Pinus cembra* in Tatras – *Acantholyda erythrocephala* (Linnaeus, 1758) from the family Pamphiliidae and *Microdiprion pallipes* (Fallén, 1808) from the family Diprionidae (JAMNICKÝ, 1988).

It would be necessary to perform long-term monitoring of abundance of individual sawfly species on Scots pine to make more precise and comprehensive conclusions in Borská nížina lowland.

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Fylofágne piliarky (Hymenoptera, Symphyta) borovicových porastov (*Pinus sylvestris*) v nížinách na viatych pieskoch, Slovensko

Súhrn

V období rokov 2008 a 2009 autori študovali a porovnávali na štyroch študijných plochách v oblasti Borskej nížiny (juhozápadné Slovensko) na borovici lesnej taxocenózy fylofágnych piliarok (Hymenoptera, Symphyta). Celkove zistili 6 druhov piliarok patriacich do 2 čeľadí. Najpočetnejším druhom je hrebenárka samotárska (*Gilpinia frutetorum*) s výrazne najvyššou početnosťou na všetkých študijných plochách. Celková najvyššia početnosť lariev piliarok sa zistila na 20-ročných boroviciach porastovej steny a v uzavretom poraste mladých 10-ročných borovíc. Podľa dendrogramu na základe Wishartovho indexu sa vyčlenili 2 odlišné skupiny spoločenstiev piliarok (spoločenstvá piliarok v uzatvorených porastoch v protiklade so spoločenstvami piliarok v otvorených borovicových porastoch). Zistil sa štatisticky preukazný rozdiel medzi hodnotami Shanonovho indexu pri spoločenstvách piliarok otvorených a uzatvorených porastov. Test ANOSIM nepotvrdil signifikantný rozdiel medzi študijnými plochami vzhľadom k druhovému zloženiu spoločenstva piliarok a abundancii jednotlivých druhov.

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Impact of deforestation and different agronomical management regimes on biodiversity of mountain ecosystem

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Abstract

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The experiment was carried out on a mountain grassland established at an altitude of 845 m in 2006–2008, after deforestation of naturally reforested pasture. The aim of research was to examine the changes in plant diversity and concentration of ergosterol in above-ground phytomass of grassland with different regimes of management. The increasing of phytodiversity on variants P (grazing), KP (grazing followed by mowing) and BM (control) during 3 years after deforestation is connected with a relatively low proportion of grasses dominated by *Agrostis capillaris*. A high percentage of legumes, particularly *Trifolium repens*, was the only presented on variant VP (grazing on burn plots). Higher number of species on variants KP and PP (grazing after reseeding with clover-grass mixture) resulted in a higher index of diversity. The values of Shannon's index of diversity (H) increased the most on variant PP over the years, where high-value species of grasses sown also influenced the grassland quality (E_{GQ}). The lowest values of H were achieved on variant VP, where the grassland quality value (E_{GQ}) was surprisedly the highest due to the dominance of *Trifolium repens* monoceonosis. On variant BM (without management) the diversity index has decreased due to reforestation (7 kinds of plants). The concentration of ergosterol in the above-ground phytomass of grassland depended on quantity of pathogenic fungi in the soil, and moisture conditions in the autumn of 2006–2008.

Key words

Cambisol, deforestation, ergosterol, forage quality, fungi, grassland diversity

Introduction

Grasslands, important ecosystems in Europe, are serving essential functions by producing feed and providing other ecosystem services (Smit et al., 2008). They are a very important part of landscape, markedly contributing to biodiversity (Isselstein et al., 2005). Central and Eastern Europe has a relatively rich biodiversity in respect to geological, historical and economic factors (Young et al., 2007), such as the grassland communities in Slovakia with the highest phytodiversity, containing up to 74 vascular plant species per 1 m^2, ranking among the communities with the highest species diversity in Europe (Novák, 2008). The processes in naturally non-forest and man-made deforested ecosystems are usually studied in tropical (Jackson and Ash, 1998; Nangendo et al., 2005) and subtropical (Fynn et al., 2004; Fairfax et al., 2009) areas, especially in savanna stands (Scholes and Archer, 1997; Reich et al., 2001), where fire plays an important role in formation of open woodland ecosystems. This type of disturbance is an inevitable factor for grassland regeneration. The local deforestation can lead to an increase of species diversity in a grassland community (Novák, 2009).

Soil texture does not influence only the contamination load by cattle, but also the quality and quantity of above-ground phytomass (Achilles et al., 2002). The traditional management of semi-natural grasslands is based on low soil loading caused by cattle (Isselstein et al., 2005). In case of a year-round cattle breeding, the load on pasture lands varies over the year. The trampling by cattle enables contact between damaged assimilatory plant organs and soil.

Soil is considered to be the refuge for almost all main taxonomic groups of fungi (Thorn, 1997). They develop in the above-ground phytomass of grassland in autumn. Fungus and blight growth can be supported by dense vegetation as well as high air humidity (Giesler et al., 1996). Fungal mycelia release mycotoxins attacking dead bodies, over seasoned and lodged plants in the humid environment (Wheeler, 1968; Opitz von Boberfeld, 1997, 2000). Their negative influence is expressed by health problems, reduction of food intake, diarrhoea, reduction of animal production efficiency, etc. (Kalač and Mika, 1997; Devegowda, 2002). Ergosterol is a prevailing steroid fungus component and an important membrane component. It also serves as a chemical indicator for quantitative determination of actual mycelia condition caused mainly by species as: *Penicillium, Aspergillus, Fusarium, Alternaria, Mucor* (Shapiro and Gealt, 1982; Yokokawa, 1987; Weete, 1989).

The aim of this study was to describe initial vegetation changes caused by deforestation of a formerly naturally reforested temperate pasture and different agronomical regimes. There were examined the following features: plant species composition, occurrence of fungi species in soil, diversity of plant species expressed by Shannon index (H) and grassland quality (E_{GQ}).

Material and methods

Study site

The experiment was conducted on a reforested pasture in the Slovenské Rudohorie Mts (48°33' N, 19°46' E) in 2006–2008. The altitude of the locality is 845 m a.s.l., the average annual precipitation reaches 927 mm and the mean annual temperature is 5.1 °C. The soil type is a Cambisol (KMm^e). The chemical characteristics of the soil determined in 2006–2008 are shown in Table 1.

The experimental area (formerly common land) was regularly grazed by sheep and cattle for centuries. In the 1950s, when the large-scale agriculture management was established, this low productive pasture was abandoned and naturally reforested. After more than 50 years a woody plant community composed of *Betula pendula*, *Corylus avellana*, *Populus tremula* and *Fagus sylvatica* was developed. Herbaceous vegetation consisted mainly of *Fragaria vesca*, *Galium odoratum*, *Cruciata laevipes, Veronica chamaedrys* and *Avenella flexuosa*. In 1993 grazing management by Charolais cattle started in this forest. The stocking rate varied from 0.30 to 0.60 cattle unit per hectare. Two grazing cycles were applied during the experiment.

The experimental area was established, and tree and shrubs were cut down and removed in May 2006. There were five treatments applied: P − grazing, KP − grazing after mowing, PP − grazing after seeding of clover-grass mixture, VP − grazing on burnt area, BM − unmanaged control (Table 2).

Table 2. Experimental variants

Variant	Applied treatment
P	Grazing
KP	Grazing followed by mowing
PP	Grazing after reseeding with clover-grass mixture
VP	Grazing on burnt plot
BM	Unmanaged control

Table 1. Chemical characteristics of the studied Cambisol

Horizon	Depth [m]	C_{ox} [%]	Hm [%]	HK/FK	pH		mmol kg^{-1}			V [%]
					H_2O	KCl	H	S	T	
Aoq	0–0.05	2.59	4.46	0.42	4.94	4.79	73.80	64.30	138.10	46.60
Bv_1	0.06–0.45	1.66	2.86	0.51	5.19	4.95	63.90	50.00	113.90	43.90
Bv_2	0.46–0.75	0.81	1.40	0.65	5.14	4.90	43.80	62.70	106.50	58.90

Aoq, humus ochric silicate horizon; Bv, cambic horizon (horizon of intrasoil weathering); Cox, organic carbon; Hm, humus; pH/H_2O, active soil reaction; pH/KCl, exchangeable soil reaction; HK/FK, ratio of humic to fulvic acids; H, hydrolytic acidity; S, sum of exchangeable basic cations; T, total sorption capacity; V, base saturation percentage.

The experiment was arranged in three randomized blocks (5 treatments × 3 replicates = 15 plots) with study plots 2 m × 2 m in size. The plots of PP variant were reseeded with grass/clover mixture (seeding rate 12.64 kg ha^{-1}) consisting of *Festuca pratensis, Phleum pratense, Poa pratensis, Dactylis glomerata, Trifolium repens* and *Lotus corniculatus* (Table 3).

Table 3. Composition of clover-grass mixture

Species – Variety	Share in mixture [%]	Quantity of seeds [kg ha^{-1}]
Festuca pratensis Huds. – Otava	19.51	3.96
Phleum pratense L. – Sobol	12.20	1.24
Poa pratensis L. – Slezanka	12.20	1.65
Dactylis glomerata L. – Rela	7.32	0.99
Trifolium repens L. – Ovčák	41.45	4.21
Lotus corniculatus L. – Polom	7.32	0.59
∑		12.64

Field activities and laboratory analyses

Phytocenological records were made on 15 permanent subplots (1 m × 1 m) placed in the central parts of the study plots (2 m × 2 m). The percentage cover (Klapp, 1965) of all vascular plant species over the area of 1 m^2 was recorded. The phytocoenoses were estimated visually before grazing in May of each year. An initial estimation was made in the spring of 2006 – to obtain baseline data for each plot. The nomenclature of plant species follows Marhold and Hindák (1998). The floristic composition of grassland expressed in percents also serves for evaluation of the grassland quality (E_{GQ}), by Novák (2004). For evaluation of floristic diversity we used the Shannon's index of diversity (H) according to Begon et al. (1997).

Herbs under protective cages (0.50 m × 0.50 m) were harvested before each grazing cycle on each study plot, with exception of the phytocenological subplots. After the harvest, the cages were replaced into another part of the study plot. The pasture lands were grazed by beef cattle of the Charolais breed. Above-ground phytomass samples were taken in 2006–2008 at the end of October and at the beginning of November (before snowing) in three repetitions. They were dried at 60 ºC, homogenised (200 g) and sent to the JLU Giessen laboratory (Germany). The concentration of ergosterol was determined after extraction by High Performance Liquid Chromatography and UV detector according to Schwadorf and Műller (1989).

Data analyses

Repeated measurements were carried out for evaluation of the species diversity and functional group data by anova. The community response was analysed with using constrained ordinations. The redundancy analysis (RDA) by Lepš and Šmilauer (2003) in the CANOCO package (Ter Braak and Šmilauer, 2002) followed by a Monte Carlo permutation test was used to evaluate trends in plant species composition, because of linear species responses and a rather homogenous species composition over the plots (Lepš and Šmilauer, 2003). A split plot design was used in the permutation type to cope with repeated measurements. We used 999 permutations in all performed analyses restricted to three split-plots, freely exchangeable whole plots, time series or linear transects at the split-plot level. Our data consisted of repeated observations of the baseline data (measurements performed before the grazing), in consequence of the interaction of treatments and year, were the most important variables. The sum of 100 subplots was used for statistical evaluation. Centring by species was applied. A standard biplot ordination diagram constructed by the CanoDraw program (Ter Braak and Šmilauer, 2002) was used to visualize the results of the CANOCO analyses.

Results and discussion

Content of ergosterol in above-ground phytomass

Labuda (2007) found the following fungal genera in a soil depth up to 0.75 m: *Penicillium* (14 species), *Trichoderma* (4 species), as well as *Mucor* and *Fusarium*. The soil contained also some other fungal species, such as *Cladosporium* (3 species), *Mortierella* (2 species) and species of genera *Absidia, Acremonium, Gelatinospora, Rhizopus, Talaromyces*, *Umbellopsis* and *Zygorrhynchus*. We assume that fungi in favourable soil conditions attack the above-ground phytomass (mainly *Penicillium* – most numerous species).

Ergosterol is a component of fungal cell membranes, and it is ussually used as an indicator of fungal biomass in soil. Its concentration in above-ground phytomass of studied plots ranged from 62 to 323 mg kg^{-1}, depending on the moisture conditions in the autumn of 2006 and the variant of experiment (Fig. 1). We have put links between the ergosterol levels found in the above-ground phytomass of grassland and the presence of pathogenic fungi in soil, as it has been already reported by Bedrna (2002) and Javoreková et al. (2008). Filamentous microscopic fungi support soil aggregates formation, but their pathogenic species can exert also negative influence on plants´ growth (Krnáčová et al., 2008). An environment favourable for fungi was created in long-lasting wet and cold weather periods in the different years. Increased occurrence of fungi in the soil was reflected in increased level of ergosterol in the above-ground phytomass. Wolf (2002) ascertained similar values in the above-ground phytomass of Festuco-Cynosuretum association (at altitude from

335 m to 460 m). We discovered fewer damaged parts of plants during the dry autumn in 2008. Ergosterol concentrations found in the above-ground phytomass in 2006 were significantly different those obtained in 2007 and 2008 (Table 4).

Floristic composition of grassland

In the studied area, fifty years after reforestation, was identified 31 herb species and 17 woody species. Due to mowing and cattle grazing the plant community Agrostis-Festucetum rubrae was dominated by *Agrostis capillaris* and *Festuca rubra* species for three years. Gremmen et al. (1998) consider *Agrostis* spp. a fast-growing grass species, propagated by means of spurs. We agree with the author's argument, but also with the opinion of Espie and Barratt (2006), that filling empty places in grasslands and creating stands by the species *Agrostis* spp. and *Festuca rubra* is influenced by environmental factors (altitude, moisture, temperature, light, high seed production, but mainly vegetative reproduction by spurs). Regarding the legumes, *Trifolium repens* species dominated. Yu et al. (2008) found out that grazing of light-demanding grassland increases the portion of white clover. Among the other herbs species, the presence of *Lysimachia nummularia* and *Fragaria vesca* was significant in the first years. These species are more frequent in wet, shady and shrubby stands. The light-demanding plants began gradually to put across themselves, especially *Taraxacum officinale*, *Leontodon autumnalis* and others (Fig. 2).

During the first three years after deforestation, the proportion of grass species in phytocoenosis was relatively low (average 26.16%). Dominance of *Agrostis capillaris* was recorded in variants P, KP and BM. The highest proportion of this species was recorded on the plot with treatment KP (17.06%). Legumes had a high proportion on variant VP (up to 80.23%). Other herbs had higher proportions than the grasses (on average 44.25%) on variants P and KP. Krajčovič (1968) and Buchgraber (2002) presented that optimal proportion of high-quality grassland for animal feeding represents 50–60% grasses, 10–30% legumes, and the rest are other herbs. The highest number of species were on variants KP and PP (41), the lowest number was on the burnt out variant VP, where we recorded only 25 species and dominance of *Trifolium repens*, because of a higher content of ash in the soil (P, K nutrients). The unmanaged variant BM was left to self-growth and formed in the opposite way as the other plots influenced by mowing and grazing. After three years, the community left without treatment was composed of 33 plant species, from them 7 were woody species. The remarkable successional development of this community independent of all treatments as well as significant differences among the treatments was detected. The woody vegetation (*Populus tremula*, *Betula pendula*, *Cerasus avium*, *Carpinus betulus*, *Rosa* spp., *Rubus* spp.) tall grasses (*Poa trivialis*, *Avenella flexuosa*, *Calamagrostis epigejos*) and tall forbs (*Viscaria vulgaris*, *Galium mollugo*) had a higher abundance on the BM variant. We note, in agreement with Luoto and Pykälä (2003) that woody

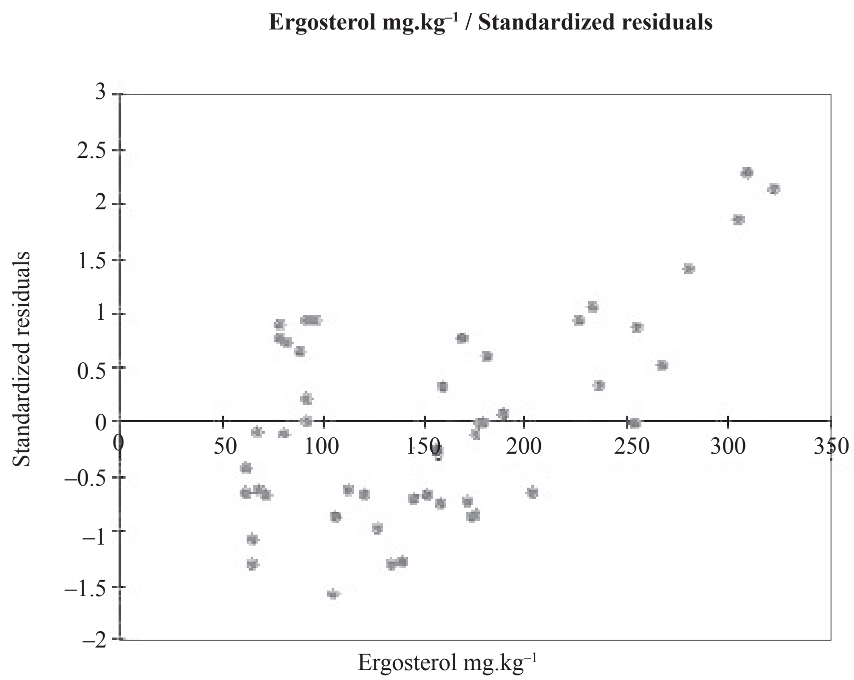

Fig. 1. Content of ergosterol in above-ground phytomass of differently treated grassland plots.

species left to self-growth are aggressive, their reproduction is fast and their succession is directed towards reforestation.

Table 4. Significance of difference in ergosterol content

Compared years	Ergosterol [mg kg^{-1}]	
	Pr. > Diff	Significant
2008 ~ 2006	<0.0001	Yes
2008 ~ 2007	0.068	No
2007 ~ 2006	<0.0001	Yes

After introduction of grazers, the plant species richness per 1 m^2 increased. The grazing had a positive effect on species richness compared to the unmanaged grassland. Due to an ash layer and excessive nutrient content in the topsoil layer, the presence of vascular plant species on the burnt plots was quite low. Only three years after abandonment of deforested sites, fast colonisation by shrub species (*Rubus idaeus*, *Rosa* spp.) and trees was re-started (especially by *Populus tremula*). Such afforestation is associated with a decline of grassland flora – as reported in many studies (Milberg, 1994; Smith and Rushton, 1994; Hanson and Fogelfors, 2000; Pykälä et al., 2005). On the other hand, any type of defoliation management can prevent reforestation with sufficient efficiency. Because of creeping stems and clonal growth of woody species, the probability of removing the majority of above-ground biomass by defoliation is low, and the *Trifolium repens* species has an advantage to quickly colonize the bare ground (Thórhallsdottir, 1990). This was also the case of VP variant.

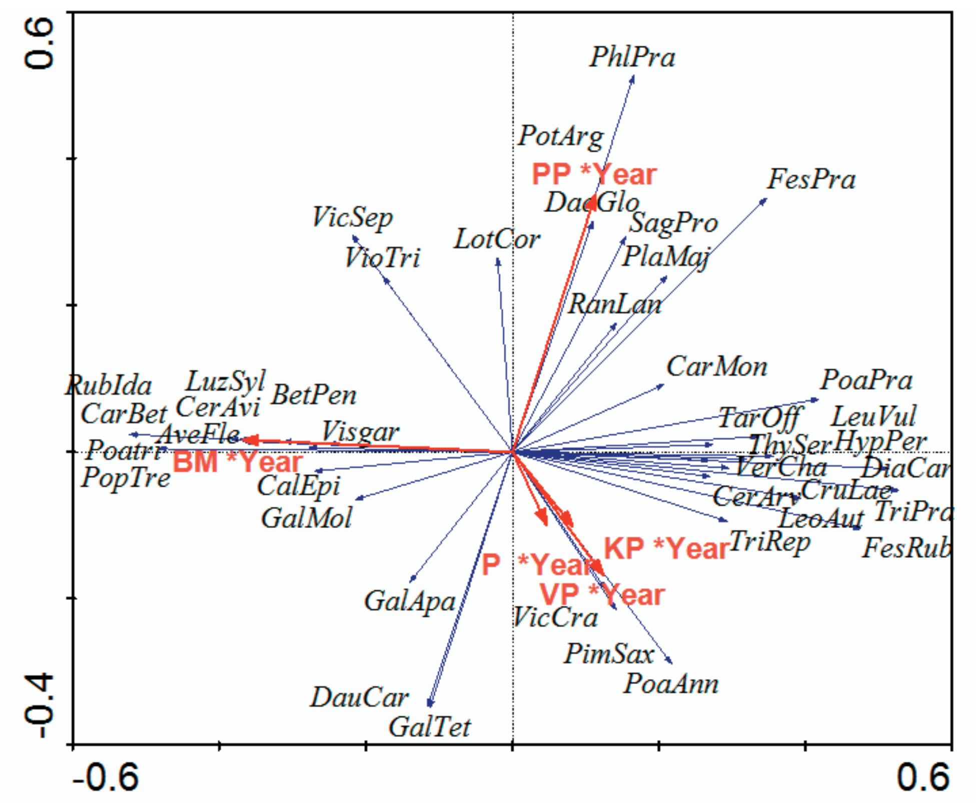

Fig. 2. Ordination diagram showing the results of RDA analysis.

Explanations:
P, grazing; KP, grazing followed by mowing; PP, grazing after reseeding; VP, grazing on burning; BM, unmanaged control; RubIda, *Rubus idaeus*; CarBet, *Carpinus betulus*; Poatr, *Poa trivialis*; PopTre, *Populus tremula*; LuzSyl, *Luzula sylvatica*; CerAvi, *Cerasus avium*; veFlex, *Avenella flexuosa*; BetPen, *Betula pendula*; Visgar, *Viscaria vulgaris*; CalEpi, *Calamgrostis epigejos*; GalMol; *Galium mollugo*; GalApa, *Galium aparine*; DauCar, *Daucus carota*; GalTet; *Galeopsis tetrahit*; PoaAnn, *Poa annua*; PimSax, *Pimpinella saxifraga*; VicCra, *Vicia cracca*; FesRub, *Festuca rubra*, TriRep, *Trifolium repens*; LeoAut, *Leontodon autumnalis*; TriPra, *Trifolium pratense*; CruLae, *Cruciata leavipes*; CerArv, *Cerastium arvense*; DiaCar, *Dianthus cartusianorum*; VerCha, *Veronica chamaedris*; HypPer, *Hypericum perforatum*; ThySer, *Thymus serphyllum*; LeuVul, *Leucanthemum vulgare*; PoaPra, *Poa pratensis*; TarOff, *Taraxacum officinale*; CarMon, *Carex montana*; RanLan, *Ranunculus lanuginosus*; PlaMaj, *Plantago major*; SagPro, *Sagina procumbens*; FesPra, *Festuca pratense*; DacGlo, *Dactylis glomerata*; PotArg, *Potentilla argentea*; PhlPra, *Phleum pratense*; LotCor, *Lotus corniculatus*; VioTri, *Viola tricolor*; VicSep, *Vicia sepium*.

The successional development of grasses was similar, with exception of variant BM, the shrubs and trees on which suppressed their growth in the second year. However, the behaviour of dominant grasses was different. *Festuca rubra* showed a similar progress in all applied variants of treatments except VP variant. This species is known as a very adaptable under different management, and, therefore, it can well perform in various types of grasslands (Honsová et al., 2007; Mašková et al., 2009; Kesting et al., 2009; Lanta et al., 2009). The cover of the *Agrostis capillaris* species was supported by grazing or cutting/grazing management, on the other hand, it was reduced by reseeding a productive grassland mixture, excessive nutrient supply in soil after phytomass burning as well as by competitive abilities of shrubs on the unmanaged plots. This short grass species (Grime, 1987) is known as an indicator of young grasslands (Waesch and Becker, 2009), being common especially in low productive temperate grasslands (Hellström et al., 2003; Louault et al., 2005; Pavlů et al., 2007; Mašková et al., 2009).

Ecological values in above-ground phytomass

The increase of Shannon's index of diversity (H) and value of grassland quality (E_{GQ}) was recorded on plots of P, KP and PP variants over three years (Table 5). The value of Shannon's diversity index (H) was the highest in case of PP variant (2.68). This was connected with relatively high species richness. The lowest diversity index was obtained on VP variant, where the E_{GQ} value was the highest due to the dominance of high-quality legume monoceonosis of *Trifolium repens* species. The values of diversity index over the years increased, except to the BM variant where the decrease was recorded due to reforestation. On variant PP with sown high-quality species (*Phleum pratense*, *Festuca pratensis*, *Dactylis glomerata*, *Poa pratensis*, *Trifolium repens*) was recorded a high value of E_{GQ} (58.92). Bullock et al. (2007) note that it is not possible to generalize that only sowing of a fodder plant mixture can increase the diversity of the species community. We agree with the opinion of Begon et al. (1997) that the species diversity significantly affects not only the frequency, but also the cover of species occurring in the community and also with Isselstein et al. (2005) that use of grasslands can preserve the plant species richness of the grassland. It resists the high competitive stress from invasive species and woody vegetation from the neighbouring areas.

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Table 5. Shannon index of diversity (H) and evaluation of grassland quality (E_{GQ})

Year	P		KP		PP		VP		BM	
	H	E_{GQ}	H	E_{GQ}	H	E_{GQ}	H	E_{GQ}	H	E_{GQ}
2006	1.69	24.19	1.70	23.92	1.49	41.57	0.53	26.15	2.16	29.87
2007	2.14	39.03	2.29	36.57	2.44	58.92	0.75	74.14	1.92	19.34
2008	2.31	44.17	2.42	48.17	2.68	71.11	0.81	72.73	1.13	13.77

P, grazing; KP, grazing followed by mowing; PP, grazing after reseeding; VP, grazing after burning; BM, unmanaged control.

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Vplyv odlesnenia a rôznych agronomických opatrení na biodiverzitu horského ekosystému

Súhrn

Experiment bol realizovaný na odlesnenom horskom pasienku v 845 m n. m v rokoch 2006–2008. Na variantoch s pasením (P), kosením a následným pasením (KP) a bez manažmentu (BM) bol 3 roky po odlesnení pomerne nízky podiel tráv s dominanciou druhu *Agrostis capillaris*. Vysoké zastúpenie leguminóz, predovšetkým druhu *Trifolium repens*, bolo len na spálenisku (variant VP). Vyšší počet druhov na variantoch využívaných kosením a pasením (KP) a pasením po príseve ďatelinovo-trávnej miešanky (PP) sa odrazil vo vyššom indexe diverzity. Hodnoty hydrolytickej kyslosti sa v priebehu rokov najviac zvyšovali na variante PP, kde vysiate vysokohodnotné druhy ovplyvnili aj kvalitu pastvy (E_{GQ}). Najnižšie hodnoty H boli na variante VP, aj keď hodnota E_{GQ} bola najvyššia v dôsledku dominancie monocenózy druhu *Trifolium repens*. Na variante BM ponechanom na samovývoj (bez manažmentu) poklesli hodnoty vplyvom prirodzenej obnovy drevín (7 druhov drevín). Koncentrácia ergosterolu v nadzemnej fytomase trávneho porastu súvisela s existenciou patogénnych húb v pôde a závisela najmä od vlhkostných podmienok v jesennom období 2006–2008.

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Chronicle

The 70-th anniversary of Prof. Ing. Pavol Hrubík, DrSc.

On March 16, 2011, we celebrated the important 70-th anniversary of our colleague Prof. Ing. Pavol Hrubík, DrSc. Pavol Hrubík was born in the village of Senné, district Veľký Krtíš. Having finished the Secondary School of Forestry in Banská Štiavnica, he continued with his studies in this area at the University College of Forestry and Wood Technology in Zvolen (since 1991, the Technical University in Zvolen). His primary interest, focussed on entomology, dendrology and forest protection, has been persisting lifelong as a hobby and occupation, at the same time. His first working place (from July 1, 1964) was the Forest Enterprise Nitra, Forest District Horné Lefantovce where he performed as an operating worker. His first working place was also the place of his first contact with research workers (studying European chestnut) from the Arboretum Mlyňany of the Slovak Academy of Sciences (SAS), who redirected his interest from practice to research activities. Having accomplished one year of practice (September 1, 1965), P. Hrubík started his research work at the Arboretum Mlyňany SAS. There he continued, as a member of the group focussing on protection of woody plants, with study of his favourite subjects. The Arboretum Mlyňany – Institute of Dendrobiology SAS was his working place up to the year 1993. After delimitation of this institution and after one year spent at the Institute of Forest Ecology SAS, Branch of Woody Plants Biology (Nitra), P. Hrubík was working (from September 1, 1995 to August 31, 2009) as a university teacher at the Slovak University of Agriculture, Faculty of Horticulture and Landscape Engineering in Nitra. At present, he is working in the Botanic garden of the Slovak University of Agriculture in Nitra. His research subject, from the very beginning, has been the inventory and monitoring of biotic pests in the Arboretum Mlyňany SAS, later he switched to the study of biology and ecology of insect pests on the introduced woody plants.

In 1967, he started his PhD (CSc.) study in the Branch 41-03-9 „Agricultural and Forestry Phytopathology and Plant Protection“ as an external student. He had accomplished his PhD thesis entitled “Caterpillars of some Lepidoptera as potential pests on introduced woody plants “ by May 1972; however, he had to wait for approval of the defence until September 1983. In 1980s, his research area involved also another frequently discussed issue: animal pests on woody plants in urban greenery in varying ecological conditions across Slovakia. His research results represent an important contribution to more detailed and complex study of introduced and native woody plants in urban greenery, with the focus on qualitative and quantitative structure and biological-ecological analyses. In frame of his activities at the Arboretum Mlyňany SAS, he attended and coordinated two dendrological study trips in North Korea (1983, 1985). In years 1990–1993 he was the vice-director of the Arboretum and the Chairman of the Scientific Board (1990–1992).

In 1995 (1. 9. 1995), having accomplished 30 years of scientific work, P. Hrubík left the “pure science” for the just established Faculty of Horticulture and Landscape Engineering of the Slovak University of Agriculture in Nitra, which enabled him to couple his research and pedagogical activities in dendrology and plant protection. He implemented his outstanding knowledge in teaching the subject Orchard Dendrology – Coniferous, Evergreen and Broadleaf Woody Plants. In February 1996, he was habilitated as an Associate Professor for the scientific branch “Horticulture“; in 1997 he successfully defended his doctoral dissertation thesis under the title “Harmful entomofauna on alien woody plants in Slovakia“, and gained the scientific degree “DrSc.“ – doctor of agricultural-forestry sciences, scientific branch 41-97-9 “Plant Protection“, SAS Bratislava; together with scientific evaluation degree I. – senior scientific worker. In year 1999, he was inaugurated as a full Professor for the scientific branch 41-42-9 “Horticulture“.

During his long practice, P. Hrubík was noticeably contributing to the knowledge of insect fauna of domestic and exotic woody plants in public greenery and in major dendrological subjects in Slovakia. He reported several first findings of insect pests on introduced woody plants in Slovakia – at first on-his-own, later, at the Slovak University of Agriculture in Nitra, together with his colleague Ing. Ján Kollár, PhD. His rich scientific results were published in 12 monographs, 173 original scientific works in Slovakia, 25 original scientific works abroad, and many research papers presented at national and foreign conferences. A large number of his works (133) was published in scientific journals. As a teacher at the Faculty of Horticulture and Landscape Engineering of the Slovak University of Agriculture in Nitra, he was the tutor of numerous bachelor, diploma and dissertation theses. He acted as the coordinator of the study branch Garden and Landscape Architecture, co-coordinator of the study programme Biotechnique of Park and Landscape Arrangements, and the coordinator of the study subjects Broad-leaved Woody Plants in Garden Design, Coniferous and Evergreen Woody Plants in Garden Design, Assessment of Biotic Parameters, Woody Plants in Garden Architecture and Protection of Ornamental Plants. All the subjects he was lecturing provided printed study texts. P. Hrubík implemented his rich professional experience as a member of scientific boards (Faculty of Horticulture and Landscape engineering of Slovak University of Agriculture in Nitra and Institute of Forest Ecology SAS in Zvolen); as a member of the Advisory Committee of the PLA (Protected Landscape Area) Ponitrie; a member of the editorial boards of the journal Folia oecologica and the international journal Plant Protection Science in Prague; a member of the Accreditation Commission SAS; a member of commission for defence of doctoral (DrSc.) dissertation theses for the scientific branches Horticulture and Plant Protection; a member of the joint commission for the scientific branch Garden and Landscape Architecture at the Faculty of Horticulture and Landscape Engineering of the Slovak University of Agriculture in Nitra, and the Faculty of Horticulture of the Mendel University in Brno.

He was working on, co-leading and leading several grant projects of the VEGA and KEGA agencies; he participated in solving the multilateral COST project "Multidisciplinary Chestnut Research in Europe" and the COST project "Urban Forests". At the Faculty of Horticulture and Landscape Engineering of the Slovak University of Agriculture in Nitra in years 1995–2009, he acted as the Head of the Department of Garden and Park Design (1995–1998), later as the Head of the Department of Green Biotechnology (1998–2001), the Chair of the Academic Senate of the Faculty, the Vice-Dean and the Deputy-Dean.

Prof. Ing. Pavel Hrubík, DrSc., with his determined persistent efforts and high professionalism, has enlarged the knowledge in several scientific areas (entomology, dendrology, plant protection). Equally important is his long-year experience in pedagogical practice – with implementing the knowledge gained in the research work in years 1995–2005 at the Slovak Academy of Sciences: Arboretum Mlyňany – Institute of Dendrobiology and Institute of Forest Ecology SAS in Zvolen, Branch for Woody Plants Ecology in Nitra. He is still maintaining his cooperation with these institutions. P. Hrubík is a member of research collectives solving grant projects of the national grant agencies VEGA and APVV.

His outstanding activities in area of science communication were awarded in 1985 by the Presidium SAS with the Prize SAS for Science Communication. For his lifelong professional and pedagogical activities, the Society for Agricultural, Forestry, Food and Veterinary Sciences SAS Bratislava awarded him with the Juraj Fándly medal, and the Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering, with a Memorial medal.

Appraisal which is not possible to express with medals or awards is the appraisal and support from his wife, offering him an optimum family environment allowing him to occupy with his lifelong hobby – introduced and native woody plants and their pests. P. Hrubík and his wife have got two daughters: Vierka and Katka, and now they are enjoying good health and interesting working activities together with their families.

For the years that will follow, we wish to our colleague, celebrating his anniversary, good health and many new incentives for his activities focussed on building and protection of the life environment.

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The 70-th anniversary of doc. Ing. Ferdinand Tokár, DrSc.

On 30. 5. 2011, we celebrated the important 70-th anniversary of our colleague doc. Ing. Ferdinand Tokár, DrSc. F. Tokár was born in the village of Veľké Chrášťany, district Zlaté Moravce. Having finished his gymnasium studies in Zlaté Moravce, he continued at the former University College of Forestry and Wood Technology (today Technical University) in Zvolen. His first working place, occupied immediately after the graduation, the Forest Enterprise Partizánske (1963-1964), offered him an opportunity to implement the accumulated knowledge in silvicultural practice. In years 1965–1993, F. Tokár worked as a scientific worker at the Arboretum Mlyňany – Institute of Dendrobiology of the Slovak Academy of Sciences. From 1. 1. 1994 up to the retirement, he acted as a senior research worker at the Institute of Forest Ecology of the Slovak Academy of Sciences (SAS) in Zvolen, Branch for Woody Plants Biology in Nitra – as the Head of this Branch.

The main focus of his scientific work was put on ecology and production in exotic woody plants. This was the topic of his PhD (at that time CSc., 1974) and later Doctor (DrSc 1998) dissertation thesis, both defended at the Faculty of Forestry at the University in Zvolen. At the same University, he presented his habilitation work and obtained the title of doc. (Associate Professor, 1998) in the study branch Silviculture 41-07-9. He was working with the following model species: European chestnut (*Castanea sativa* MILL.), red oak (*Quercus rubra* L.), black nut (*Juglans nigra* L.) and black pine (*Pinus nigra* ARNOLD). F. Tokár has been acknowledged for an important and valuable input to improvement of the structure, production and tending (phyto-technique) of forest stands and to research on biomass of selected exotic woody plants grown in varied stand types (monocultures and mixed stands differentiated by the species composition). For this purpose, F. Tokár had established 27 permanent research plots in SW Slovakia and provided systematic evaluation of the data assembled on these plots. His summarised works represent a novel scientific knowledge concerning ecological demands and production of exotic woody plants in Slovakia. This knowledge has been applied with success in forest management practice (cooperation with the Forest Enterprises Topoľčianky, Levice, Palárikovo), fruit farming (established 103 ha of fruit orchards with European chestnut in the surroundings of Modrý Kameň), expert opinion practice (selected Department´s bodies). The gained knowledge means a noticeable contribution in area of production ecology and silviculture: concerning these commercially important exotic woody plants in our country.

Doc. Ing. Ferdinand Tokár, CSc. is the author of 4 monographs, 131 original scientific works, and 59 works in area of science communication. He has elaborated 86 papers published in proceedings from symposia and conferences in Slovakia and in abroad. He was the coordinator or a vice-coordinator of one international project (COPERNICUS), 14 final reports and 6 VEGA projects.

During his scientific and research work at the SAS, F. Tokár was serving several leading functions: at the Arboretum Mlyňany – Institute of Dendrobiology SAS as the Head of the Branch of Woody Plant System and Ecology; and in years 1990–1993 as the Chair of the Scientific Board of this institution.

In years 1990–1993, he was a member of the Board of Scientific Workers SAS. After the delimitation of the Institute of Dendrobiology SAS, F. Tokár was performing as the Vice-Director of the Institute of Forest Ecology SAS and the Head of the Branch of Woody Plants Biology in Nitra.

In years 1994–2004, he was a member of the Scientific Board of the Institute of Forest Ecology SAS, and a member of the Certification Commission of the Institute of Forest Ecology SAS. In years 1997–2002, he was a member of the VEGA Commission of the Ministry of Education and SAS SR for Agricultural, Forestry and Veterinary Sciences. He was a member of the Commission for Defence of PhD dissertations in the branches 15-23-9 Forest Ecology, 41-07-9 Silviculture and doctoral dissertations in the branch 41-07-9 Silviculture. In the years 1994–2004, he was the Editor-in-Chief of the scientific journals Folia oecologica and Acta dendrobiologica. In 1995–1998, he was a member of the SAS Commission of Presidium SAS for Communication and Press; in years 1996–2000, a member of the Scientific Collegium for Agricultural, Forestry and Veterinary Sciences SAS; and since 1. 7. 2004, a member of the Scientific Collegium of SAS for Biological and Ecological Sciences. He was actively participating in several scientific societies (Botanical Society SAS, Ecological Society SAS, Society for Agricultural, Forestry, Food and Veterinary Sciences SAS).

The results and efforts of F. Tokár were awarded by the Presidium SAS in 1996 with the "Honorary Silver Medal of SAS for Merits in Biological Sciences" and in 2009 with the "Honorary Medal for Merits in Biological Sciences". The life-long work of doc. Ing. Ferdinand Tokár, DrSc. was appreciated by the Society for Agricultural, Forestry, Food and Veterinary Sciences SAS in Bratislava by awarding the Juraj Fándly medal in year 2011.

We wish our colleague doc. Ing. Ferdinand Tokár DrSc. firm health, optimism, good cheer and vigour in communicating the fruits of his work to the coming generation.

In name of all colleagues

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Folia oecologica is a journal devoted to publishing original scientific papers from the fields of ecology of forest ecosystems, communities and populations of plants, fungi and animals associated with forest environment and also the ecology of woody plants growing in both forest and non-forest environment, human settlements included.

The journal also publishes short communications, methodological and survey papers in the area, book reviews, personalia and information about scientific events. The manuscripts are submitted to reviewers for evaluation of their significance.

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In the papers, it is necessary to use SI symbols. Non-integer numbers should be provided with a decimal point, (e.g. 1.7), not a comma (1,7), the thousands (with exception of years) are separated with a comma: 5,600. The variables in mathematical formulae and expressions should be written in italics, the symbols for functions and constants in the normal font, the matrices in bold capitals, the vectors in bold small letters. Latin names of genera, species, subspecies and varieties are written in italics, the name of the author of the description (or his abbreviation) normally: e.g. *Lymantria dispar* (Linnaeus, 1758), *Lymantria dispar* (L.), *Abies cephalonica* Loud. var. *graeca* (Fraas) Liu. The names of cultivars are written normally, e.g. *Olea europea* L. cv. Chalkidikis. All the tables and figures must be referred to in the text: Table 1, Tables 2–4, Figs 2–4. The authors are asked to indicate placing of the tables and figures on the text margins.

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The following form of citation should be used:

Work in a periodical

Sharov, A.A., Liebhold, A.M., Ravlin, F.W. 1995. Prediction of gypsy moth (Lepidoptera: Lymantriidae) mating success from pheromone trap counts. *Envir. Ent*., 24: 1239–1244.

Eiberle, K., Nigg, H. 1984. Zur Ermittlung und Beurteilung der Verbissbelastung. *Forstwiss*. *Cbl*., 103: 97–110.

Book

Szujecki, A. 1983. *Ekologia owadów leśnych* [Ecology of forest insects]. Warszawa: Państwowe Wydawnictwo Naukowe. 604 p.

Miller, J.R., Miller, T.A. (eds) 1986. *Insect-plant interactions*. New-York: Springer-Verlag. 342 p.

Work published in a book or in a proceedings

Basset, Y., Springate, N.D., Aberlenc, H.P., Delvare, G. 1997. A rewiew of methods for sampling arthropods in tree canopies. In Stork, N.E., Adis, J., Didham, R.K. (eds). *Canopy arthropods*. London: Chapman & Hall, p. 27–52.

Ciberej, J., Kováč, G., Bilá, A. 1999. Faktory ovplyvňujúce početný stav kamzíka vrchovského v TANAP-e [Factors influencing game populations in chamois (Rupicapra rupicapra L.) in the High Tatra National Park]. In Koreň, M. (ed.). *Päťdesiat rokov starostlivosti o lesy TANAP-u. Zborník referátov z konferencie*. Poprad: Marmota Press, p. 111–116.

Dissertation

Chromová, L. 2002. *Pôdne a vegetačné zmeny lesných spoločenstiev okolia obce Brusno (Veporské vrchy)* [Changes in soils and vegetation of forest communities of the Brusno village (the Veporské Mts.)]. PhD thesis. Bratislava: Comenius University, Faculty of Natural Sciences. 122 p.

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