# Spatial density of two sympatric species Yellow-necked Mouse *Apodemus flavicollis* and Bank Vole *Clethrionomys glareolus* in different environment

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**Abstract**

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The impact of two different environments (climax forest and glade) on spatial density of Yellow-Necked Mouse and Bank Vole was studied during the years 2011–2013. Species range (95% Kernel) of both species was calculated based on data obtained from live traps placed in trap grids. Eight levels of density probability that surround regions of constant probability density were used to define (i) activity centres of observed species and (ii) areas that tended to be of rather peripheral interest. Results suggest that glade with rich herbal-ground cover represented significantly more suitable habitat, as well as habitat richer in resources for Bank Vole in comparison to climax forest. In contrary, Yellow-necked Mouse had provably the highest spatial density in climax forest habitat where was lack of herbal-grounded cover typical for the glade. In particular, we suggest that different diet specialization may be one of elements in explaining the different spatial density.

**Keywords**

Bank Vole, climax forest, Glade, spatial activity, Yellow-necked Mouse

## Introduction

Distribution of organisms in the landscape is not coincidental and placement pattern of individuals is an important question of ecology (Krebs, 1999). Movement of small mammals in their environment is conditioned by factors as resources obtaining, (Quin et al., 2000; Lin et al., 2004), population density (Tioli et al., 2009), predators avoiding (Norrdahl and Korpimäki, 1998; Yunger, 2004; Fey et al., 2010) and intraspecific or interspecific competition (Myllymäki, 1977; Norrdahl and Korpimäki, 1993), need of social interaction and reproduction (Bujalska, 1973; Madison, 1980), as well as by structure (Diffendorfer et al., 1995; Cartar et al., 1997; Russell et al., 2007) and disturbance of habitat (Jacob and Hempel, 2003; Horváth and Herczeg, 2013).

The common methods for small mammal's investigation used captured-mark-recapture technique (CMR) in live traps grid. This investigation brings data about movement, but mainly about population density estimation. However, to get estimates of population density we need to determine the area occupied by the population members (Krebs, 2011). The kernel density estimation method (KDE) represents techniques where density estimate is derived from the proximity of observation to each evaluation point. Evaluation points may be the observations themselves, or a regular grid laid over the sample (Seaman et al., 1998). KDE is currently the most widely used home range technique, where

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observations are represented by sequential locations of individuals (Seaman and Powell, 1996). The next possible usage of KDE is analysis of species range, where observation are single locations of different individual organisms (Seaman et al., 1998). KDE produces two types of contouring. While first one – volume contours connect regions of equal probability density function, the second – density contours surround regions of constant probability density (Roger and Carr, 1998). Density contours thus can be used to determine the area occupied by the population members and offer the possibility to identify high or low density areas.

Two studied rodent species, the Yellow-necked Mouse *Apodemus flavicollis* and Bank Vole *Cletrionomys glareolus* live sympatrically, and can be found in wide spectrum of habitats. From continual forest (Mitchell-Jones et al., 1999; Horváth et al., 2012a; Klimant et al., 2015), through fragmentation habitats (Rajska-Jurgiel, 1992; Stanko et al., 1996) and linear vegetation (Stanko, 1994; Miklós and Žiak, 2002) to open non-forest habitats like arable land (Heroldová et al., 2007). While Yellow-necked Mouse is typical granivore species (Marsh et al., 2001), and preferred different types of forest ecosystems, Bank Vole is considered as species with mixed foraging strategy, granivorous but mainly herbivorous (Hansson, 1983), and preferred wetter habitats with denser undergrowth. Next difference is fact, that *Apodemus flavicollis* is able to utilize space of habitat vertically as well as horizontally (Holišová, 1974; Hlôška, 1999).

The spatial ecology of *Apodemus flavicollis* and *Cletrionomys glareolus* has been well studied throughout its high abundance and large area of distribution (Korn, 1986; Koskela et al., 1997; Stradiotto, 2009; Vukićević-Radić et al., 2006; Bujalska and Grüm, 2013).

This study use untraditional view to spatial density of two sympatric small mammals' species. The aim of this study is to analyze effect of habitat to spatial density of Yellow–necked Mouse and Bank Vole. We assume that (i) Bank Vole prefers higher vegetation level and has higher spatial density in a glade habitat; (ii) Yellow-necked Mouse prefers forest ecosystem and has higher spatial density in an original climax forest.

## Material and methods

### Study area

Báb forest near Nitra consists mainly of oak and hornbeam trees. According to geobotanical map there are mainly turkey oak forests (*Quercion confertae-cerris*) and Pannonian oak-hornbeam forests (Michalko et al., 1986). Forest community is included in the unit *Carpion betuli* and the association *Primulae veris-Carpinetum*. Forest is located in the southern part of Nitra undulating country on surface area of 66 ha. It is a high productivity area enclave surrounded from each side by cultivated land.

Study of small mammals was realized in two quadrats of Bab forest. First quadrat was situated in original part of forest – climax oak-hornbeam forest, second quadrat was situated in glade habitat in initial successive stage (Fig. 1).

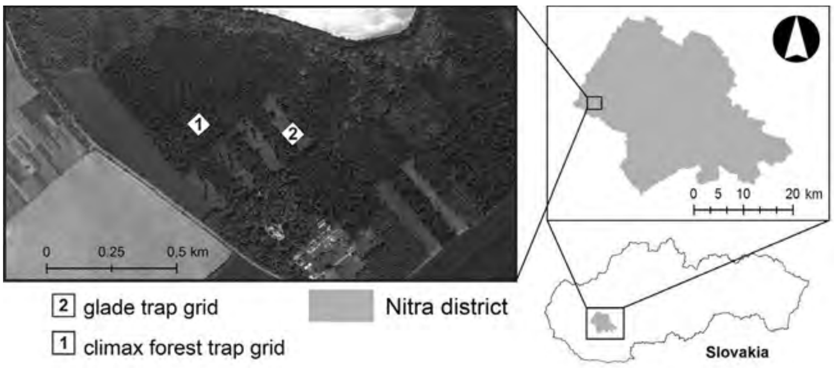


Fig. 1. Localization of two live traps grid.

### Rodent research methods

Small mammals were captured simultaneously in two trap grids by CMR – capture-mark-recapture method in course of years 2011–2013. Each trap grid consisted of 49 trapping points placed in 7 rows and 7 columns, 10 m apart from each other. Metal live traps were used with cereals and apple as a lure. 13 three-days trappings were effectuated (3,822 trap/nights). Traps were controlled two times per day. Individuals were marked with numbered ear tags. The age, sex and mass were recorded at each capture.

### Density of distribution

The annual summary of trapping data was used to calculate the probability of density occurrence. The kernel density estimation isopleths were generated with Home Range Extension (Rogers et al., 1998), module for ArcView 3.2 from CMR data. Fixed kernel estimation using reference smoothing parameter (Worton, 1995; Seaman et al., 1999) was used to calculate cumulative species range for whole study time. Analyses produce 8 isopleths, which surround regions of constant probability density. Moving inward from the outermost isopleths, which surrounds 1/8× the maximal probability density function value, each successive isopleth surrounds an additional 1/8× the maximal probability density function value. Thus, the innermost contour line encompasses 7/8× the maximal probability density function value (Roger and Carr, 1998). Analyses thus produced 8 isopleths – levels of density occurrence probability (LoDP). Level 8, represents center of species occurrence, with the highest probability of species occurrence and level 1 represents total area of species utilization – species range and patch with the lowest species occurrence within study trapping grid. ArcMap 10.0 was used for density maps visualization.

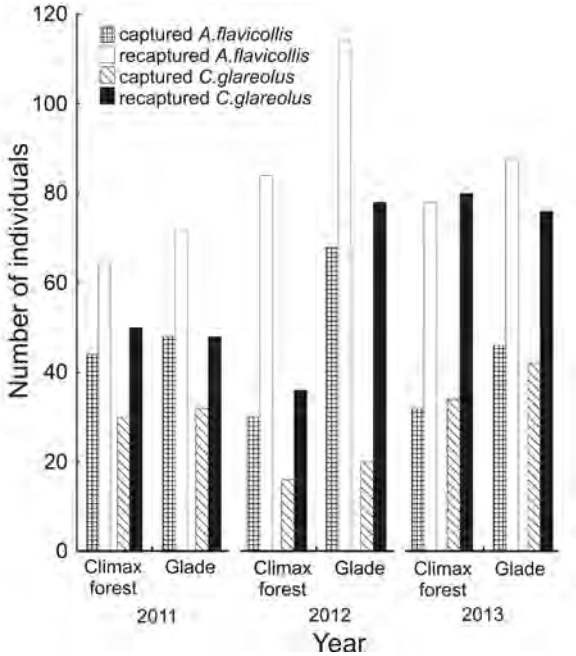


Fig. 2. Proportion of captured and recaptured *Apodemus flavicollis* and *Clethrionomys glareolus* in two habitats during the years 2011–2013.

### Statistical analyses

Total density of each trapping quadrat was calculated as average of density over all the years of monitoring, where year density was calculated as an average of all isopleths densities. STATISTICA 8.0 portable software (StatSoft Inc., 2007) was used for graphs and surface plots creation and t-test with the significance level 0.05 was used for total density differences calculations.

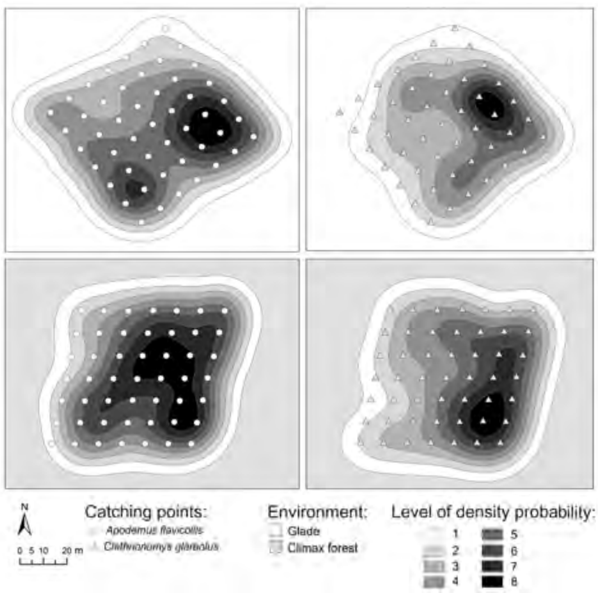


Fig. 3. Occurrence frequency of species *Apodemus flavicollis* and *Clethrionomys glareolus* within a trapping plot in forest cover and in glade.

## Results

Altogether, 711 of small mammal's individuals were caught, formed by 8 species. The Yellow-necked Mouse and Bank Vole represent dominant part of small mammals´ community in both habitats during whole three years. The year proportion of captured adults and also recaptures of individuals varied between years (Fig. 2). The Yellow-necked Mouse was more abundant species in both studied habitats during the years 2011 and 2013. In 2013 the proportion of Bank Vole in small mammals´ community increased and its abundance in both habitat was more similar to the abundance of Yellow-necked Mouse.

Results of species ranges that represent 1. LoDP (Fig. 3), and that are identical with 0.95 isopleths (i.e. 95% kernel) in this case, show that both species utilised whole area of trapping plot in both habitats. An exception was Bank Vole that did not utilise the edge part of glade quadrat. While 1. LoDP of species range suggests identical utilisation of whole monitored quadrats, in other LoDP, which surround regions of constant probability density, we can observe different density of species occurrence in particular parts of traps grid (Fig. 3). This way we can observe activity centres (8. LoDP) of particular species in monitored traps grid, i.e. areas with higher impact on species occurrence, as well as areas that tended to be of rather peripheral interest for them.

As it was expected in both habitats, spatial density of species occurrence of both species decreased with increasing size of area occurrence and changing LoDP.

The highest total values of spatial density of occurrence were observed within bank vole (mean: 3.6-04 ± 2.2E-05 SD in level 8) in glade habitat where it utilised the smallest area 162.8 m$^2$ ± 24.5 SD (Fig. 4-2). Within this species was also observed the smallest total area utilised (mean: 5,680.6 ± 197.6 SD in level 1) in monitored quadrat. The glade area utilised by bank vole had in all LoDP the highest spatial density utilised (in average 2.0 ± E-04 72.1% ± 2.6SD) (Fig. 5) in comparison to all of the other objects. On average, only 27.9% ± 2.6 SD of utilised glade area had low spatial density (<9.0 E-05).

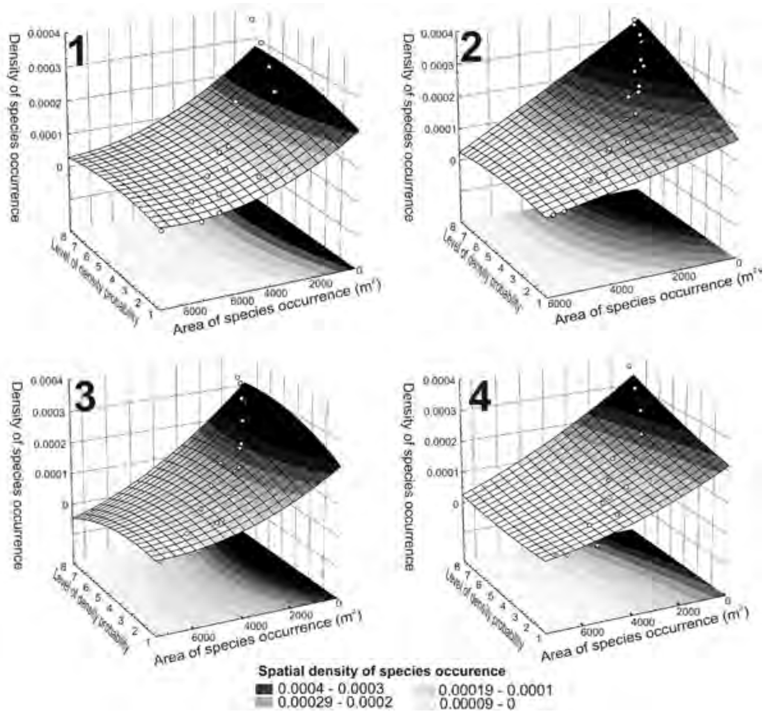


Fig 4. Relationship between spatial density of species occurrence and area of species occurrence size in particular LoDP for each species and habitat (1 – *Apodemus flavicollis* in glade, 2 – *Apodemus flavicollis* in climax forest, 3 – *Clethrionomys glareolus* in glade, 4 – *Clethrionomys glareolus* in climax forest).

In contrary, bank vole in climax forest reached the lowest spatial density (mean: 3.1E-04 ± 5.1E-05 SD in level 8), however, in the area with average surface of 378.5m$^2$ ± 279.2 SD (Fig. 4-4). The total surface utilised (level 1) by bank vole in forest habitat was higher than in glade (mean: 6,373.1 m$^2$ ± 847.7 SD), however, its total spatial density was significantly lower (Fig. 5) than the one of bank vole in glade (t-test: t = 3.59, DF = 23, n = 24, $P$ = 0.002). On average, up to 40.4% ± 4.4 SD of utilised forest area had low spatial density of species occurrence (<9.0 E-04).

Spatial density of yellow-necked mouse within activity centre in glade reached the lowest values among all areas and species observed (mean: 2.7 E-04 ± 7.68E-05 SD in level 8). Area of activity centre (level 8) was on average 217 m$^2$ ± 71.9 SD and during the period of 3 years in cumulated species range was the activity centre divided into two parts (Fig 3). Total surface area utilised in glade (level 1) was 7,463.1 m$^2$ ± 1,306.5 SD (Fig. 4-1) which represents the largest surface utilised among all of the subjects observed. In contrary, total density of occurrence in glade reached the lowest values (Fig. 5). The area with low spatial density of species (< 9.0 E-04) in this case represented up to 50.7% ± 6.0 SD.

Yellow-necked mouse average spatial density in forest activity centre (level 8) reached 2.9 E-04 ± 4.3 E-0.5SD on surface of 261.7 m$^2$ ± 66.1 SD (Fig. 4-1). Total forest habitat area utilised by species was 6,415.7 m$^2$ ± 460.9 SD. Total spatial density of yellow-necked mouse in forest (Fig. 4-2) was partially higher than in glade (t-test: t = –1.94, DF = 23, n = 24, $P$ = 0.06). Area with low occurrence density of species (<9.0 E-04) represented 40.7% ± 2.35 SD of utilised forest area..

Distribution of data (Fig. 4) suggests the lowest annual variability of spatial density and area utilised in particular LoPD of bank vole in glade.

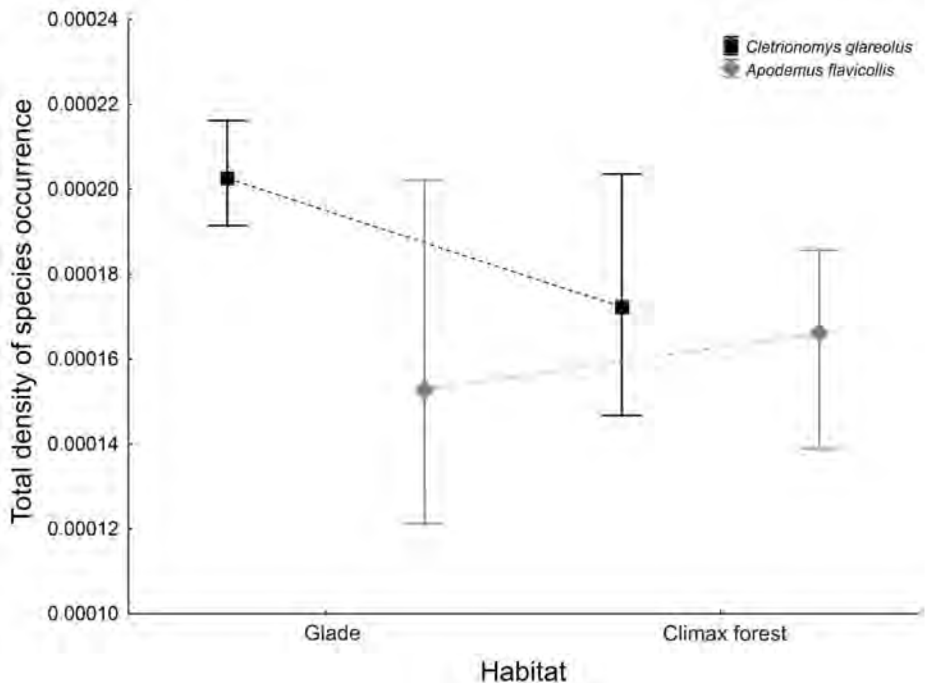


Fig. 5. Differences in total density of species occurrence for each species and habitat (mean ± min-max).

**Discussion**

Number of both species observed during the years 2011–2013 varied, which is fully in compliance with population ecology of studied species (Oksanen et al., 2000; Horváth et al., 2012a). Demographic deviations in population size of both species are typical during the year (Gliwicz, 1988), as well as in annual comparison (Pucek et al., 1993; Horváth et al., 2012a). Factors stimulating these deviations are changes in food availability (Jensen, 1982), changes of water level (Horváth et al., 2012a), over-wintering survival (Pucek et al., 1993), disturbance of habitats (Horváth et al., 2012b), etc. However, decreasing population density leads to higher spatial activity (Wolff, 1993; Mazurkiewicz and Rajska-Jurgiel, 1998). Spatial density of bank vole and yellow-necked mouse is yet as well conditioned by age, sex, or phase of reproduction activity of individuals (Koskela et al., 1997; Bujalska and Grüm, 2013), by food availability (Tioli et al., 2009; Stradiotto et al., 2009), occurrence of some predators (Jadrzejewski et al., 1993), or by other species of small mammals (Ylönen, 1990).

Structure of habitat has a significant impact on movement and density of small mammals (Diffendorfer et al., 1995; Cartar et al., 1997; Russell et al., 2007). Impact of habitat on spatial density has become noticeably evident in our study within bank vole which had highest total density in glade area and the highest spatial density on the smallest surface in activity centre (level 8). In contrary, it had provably lower total density in forest and lower density in activity centre as well, where it, however, utilised larger surface area.

For bank voles, very dense forest without undergrowth is unsuitable, because it needs a certain level of shading to survive (Wrangel, 1940). The Bank vole is considered as species with mixed foraging strategy – granivorous-florivorous (Hansson, 1983). It is a polyphagous animal that eats seeds, fruits of trees and bushes and plants (Gebczynska, 1983). Bank vole prefers more humid biotopes with more dense undergrowth (Turček, 1953a; Turček, 1953b). All these facts together with our results, and with the fact that if needed bank vole is able to move for more than 1,000 m (Szacki and Liro, 1991) suggested that the glade in successive stage with copious herbal layer is more optimal and preferable habitat for bank vole than climax forest with lack of herbal-ground cover.

Yellow-necked mouse occupied the largest glade surface but its total density was actually the lowest recorded. In contrary, this species occupied smaller area in forest, however, with partially higher total density than in glade. Larger activity centre also shows the higher preference of climax forest with higher average density compared to the glade.

However, spatial activity could be distorted by the fact that yellow-necked mouse uses the area of habitat vertically, as well as horizontally (Balát and Pelikán, 1959; Holišová, 1974; Hlôška, 1999; Puchala, 2004). Borowski (1963) observed movement to tree-tops up to 6–7 m of height, and rarely more than 20 m. Štěpánková and Vohralík (2009) point out that it is the climbing up the tree of yellow-necked mouse that is an important part of spatial activity and that this fact has to be taken into consideration also in the study of ecology of this species.

Yellow-necked mouse is characterized by greater mobility and is strictly depended on the forest environment (Montgomery and Gurnell, 1985). It is granivore species, is predominantly a seed eater (Droźdź, 1966). The most important components of its food are big seeds of the conifers (Turček, 1957; Montgomery and Gurnell, 1985; Marsh et al., 2001) representing high-calorie and digestible food with the low content of cellulose. These characteristics of species together with our results suggest that climax forest low in herbal-grounded cover is more suitable and more preferred habitat for yellow-necked mouse.

Previous research on small animals (e.g. *Apodemus flavicollis*) has demonstrated that space use varies with resources, with home ranges typically being smaller when food was abundant and population density was high (Falls et al., 2007; Merritt et al., 2001). This confirms a decrease in size of activity centers for both species in preferred habitat types (climax forest for *Apodemus flavicollis*, glade for *Clethrionomys glareolus*) that provide optimal foraging offer.

Based on our results of spatial analysis, and also according to different preferred food, we can conclude that there is no avoiding between the yellow-necked mouse and the bank vole. Yellow-necked mouse and bank vole either utilized different parts of the study area or they shared it in such a way that when there was high abundance of one species, there was an absolute minimum of the other (Rödl, 1974; Bujalska and Grüm, 1989). Horváth and Wagner (2003) studied coexistence of yellow-necked mouse and bank vole in forest habitats and they concluded that bank vole had a limiting effect on the density and spatial distribution of yellow-necked mouse. Horváth et al. (2012a) recorded also the different preference of habitats between bank vole and wood mouse. However, interspecific competition was not the subject of our research. There might be some direct and indirect competitive interactions between the two species (Andrzejewski and Olszewski, 1963; Wójcik and Wolk, 1985), but no quantitative effects of the possible competition on population dynamics of either species have been observed (Pucek et al., 1993). However, detailed study of species range overlapping and interspecific competition could be aim of next study.

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## References

Andrzejewski, R., Olszewski, J., 1963. Social behaviour and interspecific relations in Apodemus flavicollis (Melchior, 1834) and Clethrionomys glareolus (Schreber, 1780). *Acta Theriologica*, 7: 155–168.

Balát, F., Pelikán, J., 1959. On the question of the bird boxes settlement by yellow necked mouse (Apodemus flavicollis Melch.). *Zoologické Listy*, 7: 275–285.

Borowski, S., 1963. Apodemus flavicollis (Melchior, 1834) in the tops of tall trees. *Acta Theriologica*, 6: 314.

Bujalska, G., 1973. The role of spacing behaviour among females in the regulation of reproduction in the bank vole. *Journal of Reproduction and Fertility*, Suppl. 19: 465–474.

Bujalska, G., Grüm, L., 1989. Social organization of the bank vole (Clethrionomys glareolus, Schreber 1780) and its demographic consequences: a model. *Oecologia*, 80: 70– 81.

Bujalska, G., Grüm, L., 2013. Female territoriality in small rodents: Interrelations with local population persistence. *Russian Journal of Ecology*, 44: 324–331.

Cartar, R.V., Real, L.A., 1997. Habitat structure and animal movement: the behaviour of bumble bees in uniform and random spatial resource distributions. *Oecologia*, 112: 430–434.

Diffendorfer, J.E., Gaines, M.S., Holt, R.D., 1995. Habitat fragmentation and movements of three small mammals (Sigmodon, Microtus, and Peromyscus). *Ecology*, 76: 827–839.

Droźdź, A., 1966. Food habits and food supply of rodents in the beech forest. *Acta Theriologica*, 11: 363–384.

Falls, J.B., Falls, E.A., Fryxell, J.M., 2007. Fluctuations of deer mice in Ontario in relation to seed crop. *Ecological Monographs*, 77: 19–32.

Fey, K., Banks, P.B., Ylönen, H., Korpimäki, E., 2010. Behavioural responses of voles to simulated risk of predation by a native and an alien mustelid: an odour manipulation experiment. *Wildlife Research*, 37: 273–282.

Gêbczyñska, Z., 1983. Feeding habits. In Petrusewicz, K. (ed.). *Ecology of the bank vole*. Acta Theriologica, 28, Suppl. 1. Warszawa: Polish Scientific Publishers, p. 40–49.

Gliwicz, J., 1988. Seasonal dispersal in non-cyclic populations of Clethrionomys glareolus and Apodemus flavicollis. *Acta Theriologica*, 33: 263–272.

Hansson, L., 1983. Competition between rodents in successional stages of taiga forest: Microtus agrestis vs. Cletrionomys glareolus. *Oikos*, 40: 258–266.

Heroldová, M., Bryja, J., Zejda, J., Tkadlec, E., 2007. Structure and diversity of small mammal communities in agriculture landscape. *Agriculture, Ecosystems and Environment*, 120: 206–210.

Hlôška, L., 1999. Porovnanie spoločenstiev drobných cicavcov (Rodentia, Insectivora) sukcesne odlišných biocenóz lomu v Šútove-rieke a jeho okolí [Small mammals' community comparison (Rodentia, Insectivora) in different biocoenosis of Šutovo quarry and its vicinity]. *Naturae Tutela*, 5: 95–107.

Holišová, V., 1974. Movements of Cletrionomys glareolus and Apodemus flavicollis in a mixed forest. *Zoologické Listy*, 23: 303–320.

Horváth, G.F., Herczeg, R., 2013. Site occupancy response to natural and anthropogenic disturbances of root vole: Conservation problem of a vulnerable relict subspecies. *Journal for Nature Conservation*, 21: 350–358.

Horváth, G.F., Horváth, B., Sali, N., Herczeg, R., 2012b. Community-level response to different human disturbances and land use of small mammals in two marshland habitat patches in Hungary. *Archives of Biological Sciences*, 64: 613–628.

Horváth, G.F., Schäffer, D., Pogány, Á., Tóth, D., 2012a. Spatial distribution of small mammal populations in Drava floodplain forest. *Šumarski List*, 136: 141–151.

Horváth, G., Wagner, Z., 2003. Effects of densities of two coexistent small mammal populations on the survival of Apodemus flavicollis in a forest habitat. *Tiscia*, 34: 41–46.

Jacob, J., Hempel, N., 2003. Effects of farming practices on spatial behaviour of common voles. *Journal of Ethology*, 21: 45–50.

Jędrzejewski, W., Rychlik, L., Jędrzejewska, B., 1993. Responses of bank voles to odours of seven species of predators: experimental data and their relevance to natural predator-vole relationships. *Oikos*, 68: 251–257.

Jensen, T. S., 1982. Seed production and outbreaks of non-cyclic rodent populations in deciduous forests. *Oecologia*, 54: 184–192.

Klimant, P., Baláž, I., Krumpálová, Z., 2015. Communities of small mammals (Soricomorpha, Rodentia) in urbanized environment. *Biologia*, 70: 839–845.

Korn, H., 1986. Changes in home range size during growth and maturation of the wood mouse (Apodemus sylvaticus) and the bank vole (Clethrionomys glareolus). *Oecologia*, 68: 623–628.

Koskela, E., Mappes, T., Ylonen, H., 1997. Territorial behaviour and reproductive success of bank vole Clethrionomys glareolus females. *Journal of Animal Ecology*, 341–349.

Krebs, C.J., 1999. *Ecological methodology*. 2nd ed. Menlo Park, California: Benjamin Cummings. 620 p.

Krebs, C.J., 2011. Of lemmings and snowshoe hares: the ecology of northern Canada. *Proceedings of the Royal Society B: Biological Sciences*, 278: 481–489.

Lin, Y. K., Batzli, G. O., 2004. Movement of voles across habitat boundaries: effects of food and cover. *Journal of Mammalogy*, 85: 216–224.

Madison, D.M., 1980. Space use and social structure in meadow voles, Microtus pennsylvanicus. *Behavioral Ecology and Sociobiology*, 7: 65–71.

March, A.C., Poulton, S., Harris, S., 2001. The Yellow-necked Mouse Apodemus flavicollis in Britain: status and analysis of factors affecting distribution. *Mammal Review*, 31: 203–227.

Mazurkiewicz, M., Rajska-Jurgiel, E., 1998. Spatial behaviour and population dynamics of woodland rodents. *Acta Theriologica*, 43: 137–161.

Merritt, J.F., Lima, M., Bozinovic, F., 2001. Seasonal regulation in fluctuating small mammal populations: feedback structure and climate. *Oikos*, 94: 505–514.

Michalko, J. et al., 1986. SSR. *Geobotanická mapa ČSSR, Slovenská socialistická republika* [Geobotanical map of ČSSR, Slovak Socialist Republic]. Bratislava: Veda. 168 p.

Miklós, P., Žiak, D., 2002. Microhabitat selection by three small mammals' species in oak-elm forest. *Folia Zoologica*, 51: 275–288.

Mitchell-Jones, A.J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnders, P.J.H., Spitzenberger, F., Stubbe, M., Thissen, J.B.M., Vohralík, V., Zima, J., 1999. *Atlas of European mammals*. London: Academic Press. 495 p.

Montgomery, W.I., Gurnell, J., 1985. The behaviour of Apodemus. In Flowerdew, J.R., Gurnell, J., Gipps, J.M.W. (eds). *The ecology of woodland rodents bank voles and wood mice*. *The proceedings of a symposium held at the Zoological Society* of *London on 23rd and 24th of November 1984.* Symposia of the Zoological Society of London, 55. Oxford: Clarendon Press, p. 89–115.

Myllymäki, A., 1977. Intraspecific competition and home range dynamics in the field vole Microtus agrestis. *Oikos*, 29: 553–569.

Norrdahl, K., Korpimäki, E., 1993. Predation and interspecific competition in two Microtus voles. *Oikos*, 67: 149–158.

Norrdahl, K., Korpimäki, E., 1998. Does mobility or sex of voles affect risk of predation by mammalian predators? *Ecology*, 79: 226–232.

Oksanen, T., Oksanen, L., Jedrzejewski, W., Jedrzejewska, B., Korpimäki, E., Norrdahl, K., 2000. Bank vole biology: recent advances in the population biology of a model species. *Polish Journal of Ecology,* 48, Suppl.: 197–217.

Ouin, A., Paillat, G., Butet, A., Burel, F., 2000. Spatial dynamics of wood mouse (Apodemus sylvaticus) in an agricultural landscape under intensive use in the Mont Saint Michel Bay (France). *Agriculture, Ecosystems and Environment*, 78: 159–165.

Pucek, Z., Jedrzejewski, W., Jedrzejewska, B., Pucek, M., 1993. Rodent population dynamics in a primeval deciduous forest (Bialowieza National Park) in relation to weather, seed crop, and predation. *Acta Theriolologica*, 38: 199–232.

PUCHALA, P., 2004. Obsadzovanie a využitie vtáčích búdok cicavcami v NPR Šúr [Occupy and use of bird boxes by mammals in National Natural Reserve Šúr]. In ADAMEC, M., URBAN, P. (eds). *Výskum a ochrana cicavcov na Slovensku VI.* Zvolen: Štátna ochrana prírody SR, p. 101–108.

RAJSKA-JURGIEL, E., 1992. Demography of woodland rodents in fragmented habitat. *Acta Theriologica*, 37: 73–90.

RODGERS, A.R., CARR. A.P., 1998. *The home range extension for ArcView*. Thunder Bay, Ontario, Canada: Ontario Ministry of Natural Resources, Centre for Northern Forest Ecosystem Research.

ROGERS, L.M., DELAHAY, R., CHEESEMAN, C.L., LANGTON, S., SMITH, G.C., CLIFTON-HADLEY, R.S., 1998. Movement of badgers (Meles meles) in a high-density population: individual, population and disease effects. *Proceedings of the Royal Society of London*, 265: 1269–1276.

RÖDL, P., 1974. Příspěvek k poznání prostorové aktivity Apodemus flavicollis Melch. and Clethrionomys glareolus Schreb [Spatial activity of Apodemus flavicollis Melch. and Clethrionomys glareolus Schreb]. *Lynx*, 16: 46–60.

RUSSELL, R.E., SWIHART, R.K., CRAIG, B.A., 2007. The effects of matrix structure on movement decisions of meadow voles (Microtus pennsylvanicus). *Journal of Mammalogy*, 88: 573–579.

SEAMAN, D.E., GRIFFITH, B., POWELL, R.A., 1998. KERNELHR: a program for estimating animal home ranges. *Wildlife Society Bulletin*, 26: 95–100.

SEAMAN, D.E., MILLSPAUGH, J.J., KERNOHAN, B.J., BRUNDIGE, G.C., RAEDEKE, K.J., GITZEN, R.A., 1999. Effects of sample size on kernel home range estimates. *Journal of Wildlife Management*, 63: 739–747.

SEAMAN, D.E., POWELL, R.A., 1996. An evaluation of the accuracy of kernel density estimators for home range analysis. *Ecology*, 77: 2075–2085.

STANKO, M., 1994. Small mammals' communities of windbreaks and adjusted fields in East Slovak lowlands. *Folia Zoologica*, 43: 135–143.

STANKO, M., MOŠANSKÝ, L., FRIČOVÁ, J., 1996. Small mammals in fragments of Robinia pseudoacacia stands in the East Slovakian lowlands. *Folia Zoologica*, 45: 145–152.

STATSOFT INC., 2007. *Statistica, version* 8.0. www.statsoft.com.

STRADIOTTO, A., CAGNACCI, F., DELAHAY, R., TIOLI, S., NIEDER, L., RIZZOLI, A., 2009. Spatial organization of the Yellow-necked Mouse: effects of density and resource availability. *Journal of Mammalogy*, 90: 704–714.

SZACKI, J., LIRO, A., 1991. Movements of small mammals in the heterogeneous landscape. *Landscape Ecology*, 5: 219–224.

ŠTĚPÁNKOVÁ, J., VOHRALÍK, V., 2009. Vertical activity of the yellow-necked mouse (Rodentia: Muridae) at edge of a mixed forest. *Folia Zoologica*, 58: 26–35.

TIOLI, S., CAGNACCI, F., STRADIOTTO, A., RIZZOLI, A., 2009. Edge effect on density estimates of a radiotracked population of yellow-necked mice. *Journal of Wildlife Management*, 73: 184–190.

TURČEK, F.J., 1953a. Ekologická analýza populácie hraboša lesného – Clethrionomys glareolus na Poľane v r. 1952 [Ecological analyses of Bank Vole Clethrionomys glareolus population in Poľana in 1952]. *Práce Výzkumných Ústavů Lesnických ČSR,* 3: 325–374.

TURČEK, F.J., 1953b. Ekologická analýza populácie vtákov a stavcov prirodzeného lesa na Poľane (Slovensko) [Ecological analyses of birds and vertebrates population of natural forest in Poľana (Slovakia)]. *Rozpravy České Akademie Věd a Umění, Třída II*, 62 (3): 118–178.

TURČEK F.J., 1957. Konzumácia lesných semien myšou žltohrdlou (Apodemus flavicollis) a možnosti ochrany semien a semenáčkov [Consumption of forest seeds by yellow-necked mouse (Apodemus flavicollis) and possibility of seeds and seedlings protection]. *Práce Výzkumných Ústavů Lesnických ČSR,* 12: 251–303.

VUKIĆEVIĆ-RADIĆ, O., MATIĆ, R., KATARANOVSKI, D., STAMENKOVIĆ, S., 2006. Spatial organization and home range of Apodemus flavicollis and Apodemus agrarius on Mt. Avala, Serbia. *Acta Zoologica Academiae Scientiarum Hungaricae*, 52: 81–96.

WOLFF, J.O., 1993. Why are female small mammals territorial? *Oikos*, 68: 364–370.

WORTON, B.J., 1995. Using Monte Carlo simulation to evaluate kernel-based home range estimators. *Journal of Wildlife Management*, 59: 794–800.

WÓJCIK, J.M., WOLK, K., 1985. The daily activity rhythm of two competitive rodents: Clethrionomys glareolus and Apodemus flavicollis. *Acta Theriologica*, 30: 241–258.

WRANGEL, H., 1940. Beiträge zur Biologie insbesondere der Fortpflanzungsbiologie der Rotelmaus Clethrionomys glareolus und Apodemus flavicollis [Biology and reproduction of Clethrionomys glareolus and Apodemus flavicollis]. *Acta Theriologica*, 30: 241–258.

YLÖNEN, H., 1990. Spatial avoidance between the bank vole Clethrionomys glareolus and the harvest mouse Micromys minutus: an experimental study. *Annales Zoologici Fennici*, 27: 313–320.

YUNGER, J.A., 2004. Movement and spatial organization of small mammals following vertebrate predator exclusion. *Oecologia*, 139: 647–654.

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# Comparative analysis of different methods of staining the larvae *Haemonchus contortus*, *Mullerius* sp. (Nematoda, Strongylida) and *Strongyloides papillosus* (Nematoda, Rhabditida)

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**Abstract**

Boyko, O.O., Duda, Y.V., Pakhomov, O.Y., Brygadyrenko, V.V., 2016. Comparative analysis of different methods of staining the larvae *Haemonchus contortus*, *Mullerius* sp. (Nematoda, Strongylida) and *Strongyloides papillosus* (Nematoda, Rhabditida). *Folia Oecologica*, 43: 129–137.

We conducted an analysis of 14 methods of staining nematode larvae of the species *Haemonchus contortus* (Rudolphi**,** 1803), *Strongyloides papillosus* (Wedl, 1856) and *Mullerius* sp. It was established that alizarin red, brilliant blue, gentian violet and bromophenol blue did not colour the nematode larvae acceptably without heating. The most contrasting staining of the cuticle of *H. contortus*, *S. papillosus* and *Mullerius* sp. was achieved using brilliant blue, methylene blue, Ziehl's solution (with heating of preparations) and Lugol's solution (without change in temperature). The staining of the nuclei of the intestinal cells of *H. contortus* was the best by using Lugol's solution, while to the best reveal the morphological peculiarities of the gullet of *S. papillosus* and *H. contortus*, stains brilliant blue, methylene blue, brilliant green (with heating of preparations) and Lugol's solution (without change in temperature) have been observed. Differentiation of the nematodes of the gullet *H. contortus* and *S. papillosus* is possible through determination of the presence of the cuticle using brilliant green (with heating of preparations). The methods of differential diagnostics presented here allow near-patient testing of the larvae of the above-mentioned nematode species to be conducted without paralyzing them by formalin or spirit, which saves time in their identification.

**Key words**

identification of larvae, intestinal pulmonary parasites of ungulates, near-patient diagnostics of parasites

## Introduction

Strongylida of wild and domestic animals are common in many countries of the world, including Ukraine (Boyko and Brygadyrenko, 2016). This has great ecological significance for these parasites affect the numbers of ungulates. Therefore methods of differentiation and identification are very valuable for monitoring changes in their population density and distribution in the wild (Kuzmina, 2012; Boyko, 2015; Boyko et al., 2009; Boyko et al., 2016). Some scientists have conducted epidemiological studies trichostrongylidae infections in young cattle (Tharaldsen and Helle, 1984), other studied physiopathologie of digestion in sheep with trichostongylidoses (Dakkak, 1984), *Dictyocaulus viviparus* infection in red deer (Corrigall, 1985), fenbendazole treatment for the prophylaxis of nematodiasis (the lungworm, *Dictyocaulus viviparus*, and trichostrongylidae) in grazing calves (Downey and Cawdery, 1992). However, a wide range of questions

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has remained under researched. Particularly, the registration of the intensity of staining of juveniles stages by different dyes can simplify the diagnostic. The identification is made according to peculiarities of morphology. Especial attention is paid to the body length, caudal end of the larvae's indusium, structure of the pharynx, the number and the shape of the intestinal cells, the shape of caudal end of the larvae. *H. contortus* larvae are 730 µm long, have 16 intestinal cells of triangular shape, which are positioned in two lines, the pharynx is a thin tube. This differentiates them from larvae of Strongylida of the airways. They are smaller, intestinal cells are imperceptible, and the caudal end has a thorn (Van Wyk et al., 2004; Van Wyk and Mayhew, 2013). Often dyes are added to the preparations (Andrews and Daniela, 1974; Jones and Khalil, 1984; Ogawa and Egusa, 1986; Ashton and Wirasinha, 1973; Srisuphanunt et al., 2009; Hansen and Perry, 1994; Baker, 2008; Ogawa and Egusa, 1986). The methods of staining by cotton blue or acid fuchsin lactophenol are used for identifying nematodes at all stages of development in plant tissue and at the same time for their exact localization. Staining with bromophenol blue allows all endoparasitic and semi-endoparasitic root-knot nematodes to be identified at all stages of development in roots. Stained green and dark-blue, the larvae and eggs can be clearly seen on unstained plant tissue (Franklin and Goodey, 1949). A mixture of picric acid and aniline blue can be used for staining sections of ligneous roots. In reddish-yellow plant tissue nematodes and eggs that have obtained a blue colour are clearly seen (Gundy et al., 1959; Mamiya and Kiyohara, 1972).

Currently, only obsolete methods of differentiation for larvae of Strongylida in mammals can be found in the literature. This involves their culturing and defining of morphological peculiarities such as body length, caudal end of indusium, number and shape of intestinal cells. Therefore methods of differential staining of Strongylida larvae need to be developed. The objective of our research is to use different stains to colour the larvae of different nematodes (*Haemonchus contortus* (Rudolphi, 1803), *Strongyloides papillosus* (Wedl, 1856) and *Mullerius* sp.) and thus facilitate their differentiation and identification.

## Materials and methods

### Animals and experimental conditions

In the experiment we used larvae of the following nematode species *Haemonchus contortus* (Rudolphi, 1803), *Strongyloides papillosus* (Wedl, 1856) and *Mullerius* sp. The larvae of *H. contortus* (third stage) and *S. papillosus* (different stages) were cultivated during 8 days at a temperature of 25 °C, while those of the *Mullerius* sp. (first stage) were collected from the excrement of small cattle.

### Test chemicals

For their coloration we used 14 stains which are often used for identifying protozoa and nematode larvae (Enaida et al., 2006; Gregory et al., 2004; Houas et al., 2001; Lachheb et al., 2002; Mane, Babu, 2011; Myung Park et al., 2007; Namasivayam et al., 1998; Qamar et al., 2005; Selvakumar et al., 2004): alizarin red, brilliant blue, Turk's solution, brilliant green, methylene blue, amido black 10B, eozin, Ziehl's solution, Lugol's solution, gentian violet, Romanowsky-type, Sudan, bromophenol blue, orange G. We added 0.1 ml of solution containing live larvae (5–12 individuals) to 0.1 ml of stain solution. The larvae were distinguished from the excrement using Baermann Test (Zajac et al., 2011). We used 2 variants of the experiment:

- 0.1 ml of staining solution, 0.1 ml of preparation with live nematode larvae, temperature of 25 °C
- 0.2 ml of staining solution, 0.1 ml of preparation with live nematode larvae, temperature of 60 °C.

The following methods of preparing the stains were used:

1. 1% alizarin red (0.1 g alizarin red to 10 ml of distilled water)
2. 1% brilliant blue (0.1 g brilliant blue to 10 ml of distilled water)
3. Turk's solution (methylene blue to 3% of acetic acid)
4. 1% brilliant green (0.1 g brilliant green to 10 ml of 60% ethyl alcohol)
5. 1% methylene blue (0.1 g methylene blue to 10 ml of 60% ethyl alcohol)
6. 1% amido black 10B (0.1 g amido black 10B to 10 ml of distilled water)
7. 1% eozin (0.1 g eozin to 10 ml of distilled water)
8. Ziehl's solution (10 ml of saturated spirit solution of fuchsine, 90 ml of 5% carbolic acid)
9. Lugol's solution (5 parts of iodine, 10 parts of potassium iodide and 85 parts of water)
10. 1% gentian violet (0.1 g gentian violet to 10 ml of distilled water)
11. Romanowsky-type (0.1% azure solution and 0.1% of eosin solution)
12. 1% Sudan (0.1 g Sudan to 10 ml of distilled water)
13. 1% bromophenol blue (0.1 g bromophenol blue to 10 ml of distilled water)
14. 1% orange G (0.1 g orange G to 10 ml of distilled water).

## Results

### Staining of larvae without temperature fixation

The results of experiments without temperature fixation showed different degrees of staining of the larvae of *H. contortus*, *S. papillosus* and *Mullerius* sp. by the different stains (Table 1). All studied species of nematodes were resistant to the stains alizarin red, brilliant blue, Turk's solution, amido black 10B, eozin, gentian violet, Romanowsky-type, Sudan, bromophenol blue and orange G. Their cuticle was penetrated by Lugol's solution. *H. contortus* larvae were also stained with brilliant green, methylene blue, Ziehl's solution. For *H. contortus*, a high degree of penetration was shown by brilliant green, Ziehl's solution and Lugol's solution. When brilliant green stain was used, the larvae *H. contortus* obtained a uniform green color. This stain penetrated not only below the cuticle, but also the intestine, the cells of which were stained the most intensively (Fig. 1a).

In contrast, after staining of *H. contortus* larvae with Ziehl's solution, the larvae obtained a pink colour, and the intestinal cells were poorly visible (Fig. 1b). When Lugol's solution was used, the larvae of *H. contortus* were coloured light-brown. Their cuticle and the marked cells of the intestine did not differ in the extent of staining (Fig. 1c).

Table 1. Results of staining the nematode larvae

| Stain | Without temperature fixation | | | With temperature fixation | | |
|---|---|---|---|---|---|---|
| | *H. contortus* | *S. papillosus* | *Mullerius* sp. | *H. contortus* | *S. papillosus* | *Mullerius* sp. |
| Alizarin red | – | – | – | ++ | ++ | ++ |
| Brilliant blue | – | – | – | ++ | + | +++ |
| Turk's solution | – | – | – | – | – | – |
| Brilliant green | ++ | – | – | +++ | +++ | + |
| Methylene blue | + | – | – | +++ | +++ | +++ |
| Amido black 10B | – | – | – | – | – | – |
| Eozin | – | – | – | – | – | – |
| Ziehl's solution | ++ | – | – | +++ | +++ | +++ |
| Lugol's solution | ++ | +++ | + | +++ | +++ | +++ |
| Gentian violet | – | – | – | + | + | + |
| Romanowsky-type | – | – | – | – | – | – |
| Sudan | – | – | – | – | – | – |
| Bromophenol blue | – | – | – | ++ | +++ | ++ |
| Orange G | – | – | – | – | – | – |

Extent of staining: +++, intense; ++, average; +, insignificant; – –, not stained.

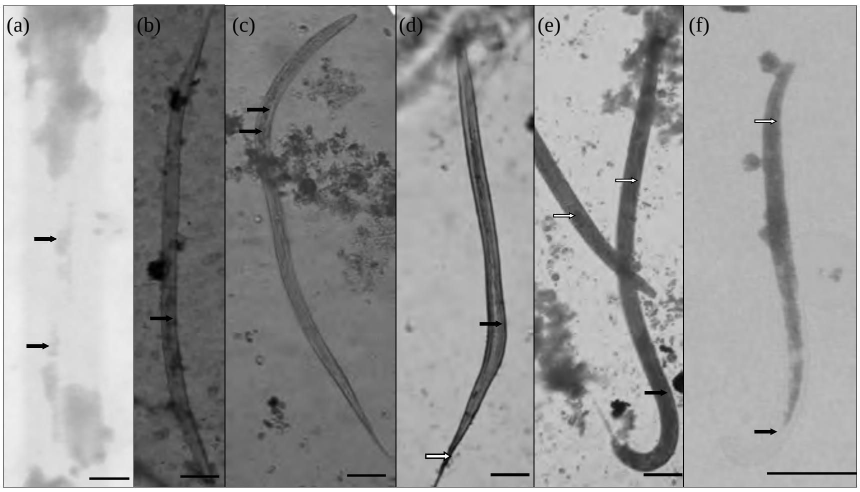


Fig. 1. Results of staining nematode larvae with brilliant green, Ziehl's solution, Lugol's solution, methylene blue, without temperature fixation: (a) – *Haemonchus contortus* (Rundolphi, 1803) stained with brilliant green (the pointer indicates cells of the intestine), (b) – *H. contortus* stained with Ziehl's solution (the pointer indicates cells of the intestine), (c) – *H. contortus* stained with Lugol's solution (the pointer indicates cells of the intestine), (d) – *H. contortus* stained with methylene blue (the black pointer indicates the intestine, the white one points to the caudal end of the cuticle), (e) – *Strongyloides papillosus* (Wedl, 1856) stained with Lugol's solution (the black pointer indicates the intestine, the white one points to the pharynx), (f) – *Mullerius* sp. stained with Lugol's solution (the black pointer indicates the caudal end, the white one points to the apical) end); bar = 10 µm (a) or 50 µm (b–f).

Methylene blue gave the larvae a paler colour. Unlike the results of the previous experiments, this stain penetrated only the cuticle and produced a violet tinge. The caudal end of *H. contortus* became dark violet. The intestinal cells of the larvae remained uncoloured.

When brilliant green was used, the larvae of *H. contortus* were viable, but became less active. Excellent results were given by methylene blue, Ziehl's solution and Lugol's solution: the larvae remained motionless, the cells of their intestines were visible.

The integuments of *S. papillosus* and *Mullerius* sp. let through only Lugol's solution. The larvae of *S. papillosus* (different stages) obtained a light-brown colour, their pharynx and the cells of their intestines were darker than the rest of their body (Fig. 1e). *Mullerius* sp. larvae were stained light-brown, their caudal ends were lighter than the intestines and apical ends (Fig. 1f).

**Staining of larvae with temperature fixation**

For staining of larvae with temperature fixation we used the same nematode species (Table 1). The colour of larvae intestine of all studied species did not change through Turk's solution, amido black 10B, eozin, Romanowsky-type and orange G.

A high extent of coloration among all studied species was shown by methylene blue, Ziehl's solution and Lugol's solution. When methylene blue was used, *S. papillosus* larvae obtained a very dark colour in their pharynx and intestine, which differed them from larvae of *Mullerius* sp. and *H. contortus*. Among larvae of *Mullerius* sp. the whole body was observed to be of a uniform bright blue colour (except the caudal end, which had a light colour, Fig. 2a, b). The larvae of *H. contortus* were also coloured to blue when Ziehl's solution and Lugol's solution were used and the preparations heated, all larvae turned bright pink (Fig. 2c) and brown.

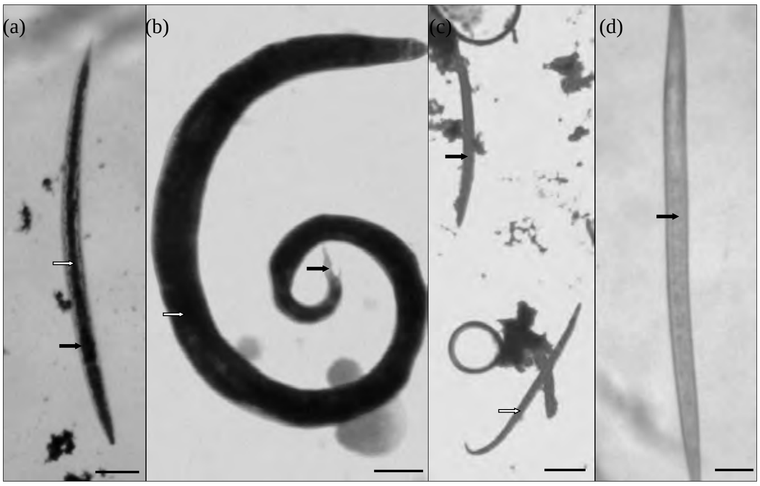


Fig. 2. Results of staining nematode larvae with methylene blue, Ziehl's solution, alizarin red with temperature fixation: (a) – *S. papillosus* stained with methylene blue (the black pointer indicates the pharynx, the white one point to the intestine), (b) – *Mullerius* sp. stained with methylene blue (the black pointer indicates the caudal end, the white one points to the intestine), (c) – *S. papillosus* and *Mullerius* sp. stained with Ziehl's solution (the black pointer indicates *S. papillosus*, the white one points to *Mullerius* sp.), (d) – *H. contortus* stained with alizarin red (the black pointer indicates the intestinal cells); bar = 10 µm (b) or 50 µm (a, c, d).

The stain alizarin red showed different results: the larvae of studied species were also coloured identically but with less intensity. They obtained a light-pink tinge (Fig. 2d). Other agents penetrated the cuticles of the larvae to different degrees. Larvae of *Mullerius* sp. were most strongly affected by staining with brilliant blue when heated, their bodies being uniformly coloured bright-blue. Larvae of *H. contortus* obtained a slightly brighter colour, but the cuticle, pharynx and intestinal cells were also uniformly coloured (Fig. 3a, b).

In the experiment with brilliant green, the brightest colours were obtained at *H. contortus* and *S. papillosus*. *S. papillosus* larvae obtained a strongly visible bright-green colour on the whole body and dark-green on the gullet and intestine. The integuments of *Mullerius* sp. larvae were characterized by a lesser penetrating capacity for this stain. Their larvae obtained a uniform light-green colour (Fig. 3c, d). The bodies of *H. contortus* larvae were uniformly coloured bright-green and the apical and caudal ends obtained a slightly brighter colour (Fig. 3e, f).

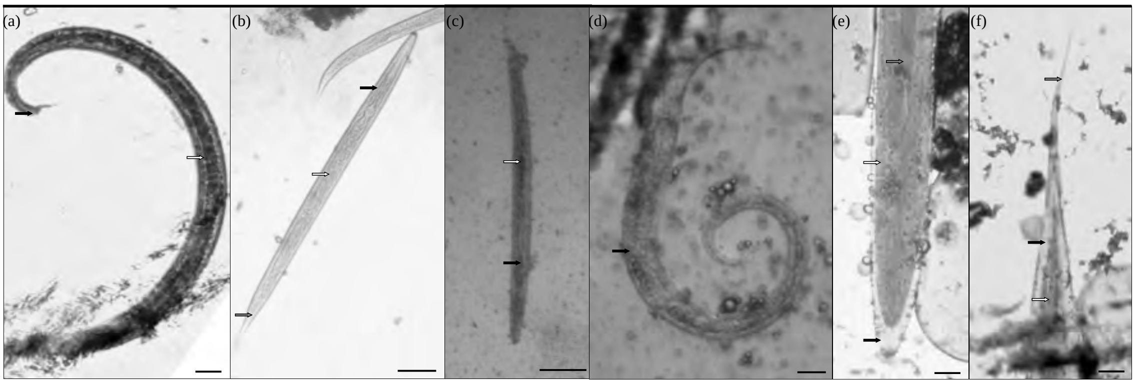


Fig. 3. Results of staining nematode larvae with brilliant blue, brilliant green with temperature fixation: (a) – *Mullerius* sp. stained with brilliant blue (the black pointer points to the caudal end, the white one indicates the intestine), (b) – *S. papillosus* stained with brilliant blue (black pointer indicates the pharynx, the white one points to the intestine, the grey one indicates the caudal end), (c) – *S. papillosus* stained with brilliant green (the black pointer indicates the pharynx, the white one points to the intestine), (d) – *Mullerius* sp. stained with brilliant green (the black pointer indicates the intestine), (e) – apical end *H. contortus* stained with brilliant green (the black pointer indicates the cuticle, the white one indicates the larva body, the grey one points to the pharynx), (f) – caudal end *H. contortus* stained with brilliant green (the black pointer points to the cuticle, the white one indicates the larva body, the grey one indicates the caudal end of the cuticle); bar = 10 µm (a, d–f) or 50 µm (b, c).

Similar results were received from staining larvae with bromophenol blue: the brightest colour was obtained by larvae of *H. contortus* and *S. papillosus*. The maximum extent of coloration was seen in *S. papillosus*. The cuticles of *Mullerius* sp. were also affected by this stain when the preparations were heated, though the extent of coloration was lower. Among the *Mullerius* sp. the colouring was not uniform: only the apical end of the larvae became bright-blue, the rest of the body was pale-blue (Fig. 4a–c).

In comparison with previously mentioned stains, the cuticles of the larvae of the three studied species of nematodes turned out to be resistant to gentian violet. Their bodies assumed a violet tinge. We observed that the larvae of *S. papillosus* changed colour only in the area of the pharynx and intestinal cells. The larvae of *Mullerius* sp. obtained a uniform violet tinge, and the cuticles of *H. contortus* larvae were coloured only in the area of the intestine, the caudal end did not absorb the agent (Fig. 4d–f).

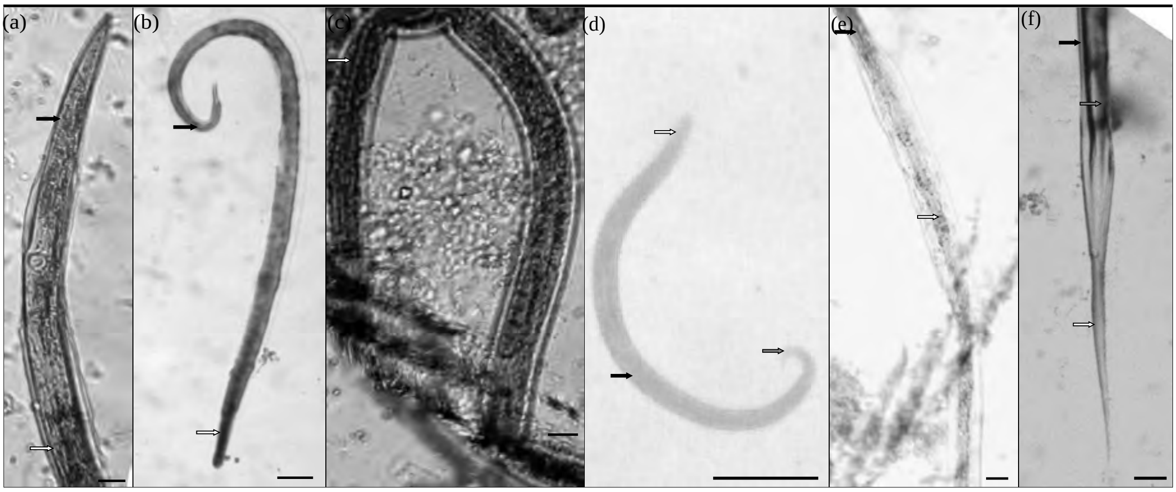


Fig. 4. Results of staining nematode larvae with bromophenol blue, gentian violet with temperature fixation: (a) – *H. contortus* stained with bromophenol blue (the black pointer indicates the apical end, the white one points to the intestine), (b) – *Mullerius* sp. stained with bromophenol blue (the black pointer indicates the caudal end, the white one indicates the apical end), (c) – *S. papillosus* stained with bromophenol blue (the white pointer indicates the apical end), (d) – *Mullerius* sp. stained with gentian violet (the black pointer indicates the intestine, the white one points to the apical end, the grey one points to the caudal end), (e) – *S. papillosus* stained with gentian violet (the black pointer indicates the pharynx, the white one points to the intestinal cells), (f) – *H. contortus* stained with gentian violet (the black pointer indicates the cuticle in the intestinal area, the white one points to the caudal end of the cuticle, the grey one points to the intestine) bar = 10 µm (a–c, e, f) or 50 µm (d).

The larvae of *S. papillosus* considerably differ in size at different stages of development. During the first hours their size corresponds to the size of nematode larvae in the windpipes of small cattle – *Mullerius* sp. For this reason, they are difficult to differentiate using microscopes of low magnification. When increasing the magnification, the number of microscopic fields also increases. Therefore identification and differentiation of larvae requires more time. For differentiation of these two species of nematode larvae Lugol's solution can be used. When using it, the preparation temperature should not be changed. Larvae of *S. papillosus* uniformly obtain a brown colour, and the larvae of *Mullerius* sp. obtain a light-brown (Table 2).

Table 2. Differential diagnostics of *S. papillosus* and *Mullerius* sp. larvae when using Lugol's solution

| Characteristics | *S. papillosus* | *Mullerius* sp. |
|---|---|---|
| Colouring of the apical end | Light-brown | Brown |
| Colouring of the pharynx | Different from body colour, dark-brown | Not different from body colour, brown |
| Colouring of the intestine | Different from body colour, dark-brown | Not different from body colour, brown |
| Colouring of the caudal end | Uniform light brown | Different from body colour, light-brown |

For differentiation of nematode larvae in the gullet, specifically representatives of Strongylida (*H. contortus*) and Rhabditida (*S. papillosus*), methylene blue, brilliant green, Ziehl's solution and Lugol's solution can be used. For identifying larvae it is enough to use only one of these agents. Staining using this method does not require heating (Table 3).

Table 3. Differential diagnostics of *H. contortus* and *S. papillosus* larvae using staining

| Characteristics | *H. contortus* | | | | *S. papillosus* | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Colouring of the apical end | Blue | Green | Pink | Brown | Not coloured | Not coloured | Not coloured | Light-brown |
| Colouring of the pharynx | Blue | Green | Pink | Brown | Not coloured | Not coloured | Not coloured | Dark-brown |
| Colouring of the intestine | Not coloured | Dark-green | Pink | Cell membrane and nucleus dark-brown | Not coloured | Not coloured | Not coloured | Dark-brown |
| Colouring of the caudal end of the cuticle | Dark-blue | Green | Pink | Brown | Not coloured | Not coloured | Not coloured | Bright-brown |
| Colouring of the cuticle | Blue | Green | Pink | Brown | – | – | – | – |

1, methylene blue; 2, brilliant green; 3, Ziehl's solution; 4, Lugol's solution.
Dash for *S. papillosus* means absence of cuticle.

Thus, larvae of *H. contortus* are coloured by first three agents shown in Table 3, while the larvae of *S. papillosus* remain uncoloured when treated by these agents. When Lugol's solution is used, larvae of *H. contortus* and *S. papillosus* do change their colour. The larval stages of *S. papillosus*, similar in size to those of *H. contortus*, are coloured bright-brown with a pronounced dark-brown hue to the pharynx and intestine. In contrast to *S. papillosus* the colour of *H. contortus* is brighter. For differentiation of *H. contortus* and *S. papillosus* brilliant blue can also be used, as *H. contortus* obtains a blue colour, and *S. papillosus* a violet colour.

## Discussion

Lugol's solution is used for identification of nematode larvae (Dancescu and Mahjoub, 1981; Devaney et al., 1992; Amarante et al., 1999; McMurtry et al., 2000). Using this solution it was possible to identify nematode larvae of *Bunostomum* sp. and *Gaigeria* sp. at the third stages (the internal structure was clearly seen). In differential diagnostics these larvae clearly differed (they quickly took on a uniform obtaining brown colour across their entire length) from others, their apical end was from the start coloured less intensively than the other parts of their body (Van Wyk and Mayhew, 2013). Lugol's solution was used for identifying *Cooperia oncophora* (Railliet, 1898), *Ostertagia ostertagi* (Stiles, 1892), *Strongyloides stercoralis* (Bavay, 1876) (DeRosa et al., 2005, 2008; Larsson et al., 2007; Areskog et al., 2014; Afzal and Steven, 2001).

One of the methods of lung strongyloidosis diagnostics (*S. stercoralis* filariform) is to stain the saliva sample with Gram. In a slide of human faeces stained with auramine O a larva of *S. stercoralis* was observed under ultra-violet light (Afzal and Steven, 2001).

Apart from differential diagnostics, the staining method was also used for defining the viability of nematodes using a solution of malachite green in the proportion 50 mg of preparation to 100 cm³ of distilled water. Dead larvae were intensively coloured green with a blue tinge, and live larvae were much paler and were not coloured at all (Hastings and Bosher, 1938; Williams et al., 1989).

The identification of species of microfilaria is performed using coloured slides or thick drops of blood. The preparations are dried out, then hemolyzed and coloured according to Romanowsky-Giemsa, Wright (the cuticle of the microfilaria obtains a pale-violet colour, and the nucleus substance of the body a dark violet colour). The Bella method is most efficient for counting microfilaria in blood, where the Romanowsky-Giemsa or the hot haemotoxilin colour filter are used (Martínez-Palomo and Martínez-Báez, 1977; Kozek et al., 1983; Tocidlowski et al., 2000; Egyed et al., 2001).

Therefore, when using Turk's solution, amido black 10B, eozin, Romanowsky-type, Sudan and orange G nematode larvae of the digestive tract and in windpipes do not become coloured without heating the preparation. No data have been found concerning the usage of these stains for identification or differentiation of larvae and eggs of nematodes.

Alizarin red is also not a typical stain in parasitology. We discovered that nematode larvae are capable of being penetrated by the agent through their cuticle. One of the main conditions of such staining is fixation of the larvae by heating the preparation to 55 °C. After using alizarin red, larvae become pale-pink.

Brilliant green is used in parasitology for better viewing of the micropreparations with endameras. No research has been conducted on the usage of this stain for identifying Strongylida larvae. Our results have shown that *H. contortus* larvae can absorb this agent through their integuments. When the temperature is raised the larvae become bright-green with a light-green cuticle, and the apical and caudal ends of the cuticle are tinged light-green, while the larvae themselves become bright-green. Larvae of the intestinal nematode *S. papillosus* and the nematode of the air passages *Mullerius* sp. can be coloured when heated.

In the literature we found no data covering the usage of brilliant blue for staining parasitic objects. According to the results of our studies, Strongylida larvae (*H. contortus* and *Mullerius* sp.), when heated with brilliant blue, unlike the larval stages of Rhabditida, obtain a uniform bright-blue colour. Their pharynx and intestine obtain a light-violet tinge. Adding brilliant green to a preparation with *H. contortus* colours them light-green, for which with no heating is required. *S. papillosus* and *Mullerius* sp. obtain a green colour only when heated.

Similar results were shown by the experiment with methylene blue. When added to an unheated preparation, it coloured the larvae of *H. contortus*. Around the world this stain is used for staining parasites which are localized in the blood system. The data covering the staining of *Dictyocaulus* larvae by methylene blue indicate that it can be used to differentiate them from other lung nematodes. *Dictyocaulus* larvae obtained a violet colour, the rest remained uncoloured. According to the results of our experiment, larvae of other species of nematodes of the air passages can also be coloured, but only after heating. Then, larvae of *Mullerius* sp. obtain a bright-blue colour. In the literature, no data covering identification of *S. papillosus* larvae using methylene blue were found either.

*H. contortus* larvae become coloured even before heating. One of the stains around the world is Lugol's solution. It is capable of staining Strongylida helminth larvae. It is often used for improving the reliability of larvae identification. According to the results of our experiments, this stain gives the larvae of *H. contortus*, *Mullerius* sp. and *S. papillosus* a brown tinge (heating of the preparation is not necessary).

Ziehl's solution and gentian violet are not used for identifying parasites. In our experiment when the preparations are heated, Ziehl's solution, colours *H. contortus*, *Mullerius* sp. and *S. papillosus* pink. Adding gentian violet to the nematode larvae of the studied species does not produce results without heating. The larvae become coloured only with an increase in temperature (only the *Mullerius* sp. larvae become fully coloured, the *S. papillosus* larvae become coloured only in their digestive system).

Bromophenol blue is often used in phytoparasitology. This stain has not proved useful in identifying animal parasites. According to our data, when bromophenol blue is used, *H. contortus*, *Mullerius* sp. and *S. papillosus* larvae become coloured only after the preparation has been heated. *H. contortus* and *S. papillosus* obtain a uniform bright-blue colour. The larvae of *Mullerius* sp. were seen to become coloured only in the area of the apical end of the body. The rest of the body became pale-blue.

## References


Afzal, A.S., Steven, L.B., 2001. Diagnosis of Strongyloides stercoralis infection. *Clinical Infectious Diseases*, 33: 1040–1047.

Amarante, A.F.T., Craig, T.M., Ramsey, W.S., El-Sayed, N.M., Desouki, A.Y., Bazer, F.W., 1999. Comparison of naturally acquired parasite burdens among Florida Native, Rambouillet and crossbreed ewes. *Veterinary Parasitology*, 85: 61–69.

Andrews, J.R.H., Daniel, M.J., 1974. A new species of Hymenolepis (Cestoda: Hymenolepididae) from the New Zealand long-tailed bat, Chalinolobus tuberculatus. *New Zealand Journal of Zoology*, 1: 333–336.

Areskog, M., Sollenberg, S., Engström, A., Samson-Himmelstjerna, G., Höglund, J., 2014. A controlled study on gastrointestinal nematodes from two Swedish cattle farms showing field evidence of ivermectin resistance. *Parasites and Vectors*, 7: 13.

Ashton, N., Wirasinha, P.A., 1973. Encephalitozoonosis (nosematosis) of the cornea. *British Journal of Ophthalmology*, 57: 669–674.

Baker, D.G., 2008. *Flynn's parasites of laboratory animals*. New York: John Wiley & Sons. 386 p.

Boyko, A.A., 2015. Helmintofauna of sheep and goats in Dnipropetrovsk region. *Visnyk of Dnipropetrovsk University. Biology, Medicine*, 6: 87–92.

Boyko, A., Brygadyrenko, V., Shendryk, L., Loza, I., 2009. Estimation of the role of antropo-zoonosis invasion agents in the counteraction to bioterrorism. In Dishovsky, C., Pivovarov, A.A. (eds). *Counteraction to chemical and biological terrorism in East European countries***.** NATO Science for Peace and Security Series A: Chemistry and Biology. Dordrecht: Springer Science, p. 309–315.

Boyko, A.A., Brygadyrenko, V.V., 2016. Influence of water infusion of medicinal plants on larvae of Strongyloides papillosus (Nematoda, Strongyloididae). *Visnyk of Dnipropetrovsk University. Biology, Ecology*, 24: 519–525.

Boyko, O.O., Zazharska, N.M., Brygadyrenko, V.V., 2016. Impact of helminthes infections on production in sheep in Ukraine. *Visnyk of Dnipropetrovsk University. Biology, Ecology*, 24: 3–7.

Corrigall, W., 1985. Dictyocaulus viviparus infection in red deer. *Transactions of the Royal Society of New Zealand*, 22: 123–126.

Dakkak, A., 1984. Physiopathologie digestive des trichostongylidoses ovenes. Note 3-L' ostentagiose a Ostertaqia circumcincta. *Revue de Médecine Vétérinaire*, 9: 21–22.

Dancescu, P., Mahjoub, M., 1981. Identification of nematode larvae by coal cultures in closed boxes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 75: 838.

DeRosa, A.A., Chirgwin, S.R., Fletcher, J., Williams, J.C., Klei, T.R., 2005. Exsheathment of Ostertagia ostertagi infective larvae following exposure to bovine rumen contents derived from low and high roughage diets. *Veterinary Parasitology*, 129: 77–81.

DeRosa, A.A., Chirgwin, S.R., Williams, J.C., Klei, T.R., 2008. Isolation and characterization of a gene encoding carbonic anhydrase from Ostertagia ostertagi and quantitative measurement of expression during in vivo exsheathment. *Veterinary Parasitology*, 154: 58–66.

Devaney, J.A., Craig, T.M., Rowe, L.D., Wade, C., Miller, D.K., 1992. Effects of low levels of lice and internal nematodes on weight gain and blood parameters in calves in Central Texas. *Journal of Economic Entomology*, 85: 144–149.

Downey, N.E., Cawdery, M.H., 1992. Controlled grazing trial of a fenbendazole slow-release bolus for the prophylaxis of nematodiasis in calves. *Irish Journal of Agricultural and Food Research*, 31: 105–114.

Egyed, Z., Sreter, T., Szell, Z., Beszteri, B., Oravecz, O., Marialigeti, K., Varga, I., 2001. Morphologic and genetic characterization of Onchocerca lupi infecting dogs. *Veterinary Parasitology*, 102: 309–319.

Enaida, H., Hisatomi, T., Hata, Y., Ueno, A., Goto, Y., Yamada, T., Kubota, T., Ishibashi, T., 2006. Brilliant blue G selectively stains the internal limiting membrane/brilliant blue G-assisted membrane peeling. *Retina*, 26: 631–636.

Franklin, M.T., Goodey, J.B., 1949. A cotton blue-lactophenol technique for mounting plant-parasitic nematodes. *Journal of Helminthology*, 23: 175–178.

Gregory, C.A., Gunn, W.G., Peister, A., Prockop, D.J., 2004. An alizarin red-based assay of mineralization by adherent cells in culture: Comparison with cetylpyridinium chloride extraction. *Analytical Biochemistry*, 329: 77–84.

Gundy, S.D., Thomason, J., Rackham, R.L., 1959. The life history of the citrus nematode Tylenchulus semipenetrans. *Nematologica*, 3: 283–294.

Hansen, J., Perry, B., 1994. *The epidemiology, diagnosis and control of helminth parasites of ruminants*. Nairobi: International Laboratory for Research on Animal Diseases. 171 p.

Hastings, R.J., Bosher, J.E., 1938. A study of the pathogenicity of the meadow nematode and associated fungus Cylindrocarpon radicicola Wr. *Canadian Journal of Forest Research*, 16: 225–229.

Houas, A., Lachheb, H., Ksibi, M., Elaloui, E., Guillard, C., Herrmann, J.-M., 2001. Photocatalytic degradation pathway of methylene blue in water. *Applied Catalysis B – Environmental*, 31: 145–157.

Jones, A., Khalil, L.F., 1984. Taenia dinniki sp. nov. (Cestoda: Taeniidae) from the striped and the spotted hyaena in East Africa. *Journal of Natural History*, 18: 803–809.

Kozek, W.J., Eberhard, M.L., Raccurt, C., 1983. Comparative morphology of Mansonella ozzardi microfilariae from Colombia and Haiti. A light microscope study. *Tropical Medicine and Parasitology*, 34: 33–37.

Kuzmina, T.A., 2012. Contamination of the environment by strongylid (Nematoda: Strongylidae) infective larvae at horse farms of various types in Ukraine. *Parasitology Research*, 110: 1665–1674.

Lachheb, H., Puzenat, E., Houas, A., Ksibi, M., Elaloui, E., Guillard, C., Herrmann, J.-M., 2002. Photocatalytic degradation of various types of dyes (Alizarin S, Crocein Orange G, Methyl Red, Congo Red, Methylene Blue) in water by UV-irradiated titania. *Applied Catalysis B – Environmental*, 39: 75–90.

Larsson, A., Dimander, S.O., Waller, A., Uggla, A., Höglund, J., 2007. A 3-year field evaluation of pasture rotation and supplementary feeding to control parasite infection in first-season grazing cattle – dynamics of pasture infectivity. *Veterinary Parasitology*, 145: 129–137.

Mamiya, Y., Kiyohara, T., 1972. Description of Bursaphelenchus lignicolus n. sp. (Nematoda: Aphelenchoididae) from pine wood and histopathology of nematode-infested trees. *Nematologica*, 18: 120–124.

Mane, V.S., Babu, P.V.V., 2011. Studies on the adsorption of Brilliant Green dye from aqueous solution onto low-cost NaOH treated saw dust. *Desalination*, 273: 321–329.

Martínez-Palomo, A., Martínez-Báez, M., 1977. Ultrastructure of the microfilaria of Onchocerca volvulus from Mexico. *Journal of Parasitology*, 63: 1007–1018.

McMurtry, L.W., Donaghy, M.J., Vlassoff, A., Douch, P.G.C., 2000. Distinguishing morphological features of the third larval stage of ovine Trichostrongylus spp. *Veterinary Parasitology*, 90: 73–81.

Myung Park, J., Seok Lee, I., Young Kanga, J., Nyol Paika, C., Kyung Choa, Y., Woo Kima, S., Choia, M.-G., Chunga, I.-S., 2007. Acute esophageal and gastric injury: Complication of Lugol's solution. *Scandinavian Journal of Gastroenterology*, 42: 135–137.

Namasivayam, C., Prabha, D., Kumutha, M., 1998. Removal of direct red and acid brilliant blue by adsorption on to banana pith. *Bioresource Technology*, 64: 77–79.

Ogawa, K., Egusa, S., 1986. Two new species of Paradeontacylix Mcintosh, 1934 (Trematoda: Sanguinicolidae) from the vascular system of a cultured marine fish, Seriola purpurascens. *Fish Pathology*, 21: 15–19.

Qamar, M., Saquib, M., Muneer, M., 2005. Photocatalytic degradation of two selected dye derivatives, chromotrope 2B and amido black 10B, in aqueous suspensions of titanium dioxide. *Dyes and Pigments*, 65: 1–9.

Selvakumar, N., Sudhamathi, S., Duraipandian, M., Frieden, T.R., Narayanan, P.R., 2004. Reduced detection by Ziehl-Neelsen method of acid-fast bacilli in sputum samples preserved in cetylpyridinium chloride solution. *International Journal of Tuberculosis and Lung Disease*, 8: 248–252.

Srisuphanunt, M., Saksirisampant, W., Karanis, P., 2009. Detection of Cryptosporidium oocysts in green mussels (Perna viridis) from shell-fish markets of Thailand. *Parasite*, 16: 235–239.

Tharaldsen, J., Helle, O., 1984. Epidemiologic investigations of trichostrongylid infections in young cattle in different parts of Norway. *Acta Veterinaria Scandinavica*, 25: 164–186.

Tocidlowski, M.E., Spelman, L.H., Sumner, P.W., Stoskopf, M.K., 2000. Hematology and serum biochemistry parameters of North American river otters (Lontra canadensis). *Journal of Zoo and Wildlife Medicine*, 31: 484–490.

Van Wyk, A., Cabaret, J., Michael, L.M., 2004. Morphological identification of nematode larvae of small ruminants and cattle simplified. *Veterinary Parasitology*, 119: 277–306.

Van Wyk, J.A., Mayhew, E., 2013. Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. *Onderstepoort Journal of Veterinary Research*, 80: 1–14.

Williams, A.B., Stirling, G.R., Hayward, A.C., Perry, J., 1989. Properties and attempted culture of Pasteuria penetrans, a bacterial parasite of root-knot nematode (Meloidogyne javanica). *Journal of Applied Bacteriology*, 67: 145–156.

Zajac, A.M., Conboy, G.A., 2011. *Veterinary clinical parasitology*. 8th ed. Willey-Blackwell. 354 pp.

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# Detailed mapping of geocomplexes in the vineyard landscape

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**Abstract**

Falťan, V., Pírová, L., Petrovič, F., 2016. Detailed mapping of geocomplexes in the vineyard landscape**.** *Folia Oecologica,* 43: 138–146.

Viticultural landscape is a valuable part of the cultural landscape – it was created by the long-term impact of human activities on the areas with suitable environmental conditions for the development of vineyards. A geoecological research within an example study of vineyard landscape was realised in choric and topical geographical dimension in the Doľany vicinity. Georelief was selected as a leading factor of regionalisation at the level of geochores. Information about soil-forming substrates and land cover on a topical dimension was specified within a detailed research of the terrain. Overall, five basic types of geochores and 58 types of geoecological complexes were earmarked. The most frequent occurrence of vineyards was on the alluvial cones and slopes of the Malé Karpaty Mts. As a part of the detailed research of the soil, we further characterized location conditions of different grape varieties in the Fosandle and Grefty localities.

**Key words**

Doľany, geoecological research, Slovakia, vineyard landscape

## Introduction

Detailed research of agricultural landscape is still an actual topic of landscape ecology. Human impact has been changing the landscape for centuries. Landscape changes are an expression of the dynamic interaction between natural and cultural forces in the environment (Antrop, 2005). These changes significantly affect the landscape's ecological stability (Lipský, 2001; Ivan et al., 2015), as well as biological (Löfvenhaft et al., 2004), environmental and aesthetic value (Nassauer, 1995; Pătru-Stupariu et al., 2015). In Central Europe, agricultural processes belong to important drivers of the land use changes (Muchová and Petrovič, 2010; Špulerová et al., 2011; Kopecká et al., 2012; Jusková and Muchová, 2013; Opršal et al., 2013; Havlíček et al., 2014; Jusková and Muchová, 2014; Sklenička et al., 2014; Grešlová et al., 2015; Lieskovský et al., 2015 ).

Viticultural landscape is a valuable part of the cultural landscape – it was created by the long-term impact of human activities on areas with suitable environmen-

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tal conditions for the development of vineyards (SUPUKA et al., 2011). Viticultural landscape has a remarkable landscape image, the importance for ecological stability and physiognomically it is a distinctive type of land cover. Environmental conditions also form a significant part of the terroir.

The term ´terroir´ is used very often globally, and it means the influence of natural factors, especially concerning geology, soils, relief and climate, suitable vine variety for the site and also the technological procedures of wine makers. Studying various parameters of terroir altogether is very rare and has become a subject of interest (VAN LEEUWEN et al., 2004). Over the past few years, geographic information systems have been used for the accurate mapping of vineyards (MATEČNÝ, 2014). In this context, modelling of relief indices for spatial differentiation of microclimatic conditions has also played an important role (MIKLÓS et al., 1991; MATEČNÝ et al., 2010). Modern complex physical geographical (geoecological) research is focused on a detailed description of formation, evolution, and operation of geosystems at a detailed topic level, with relevant landscape components being represented (MINÁR, 2003; FALŤAN et al., 2009; Čech and KUNÁKOVÁ, 2012; BARTÍK et al., 2014). Many Slovak wine producers are aware of the importance of terroir and are endeavouring to produce wines from grapes that are bound to a specific locality. Therefore more attention has been paid to parameters such as bedrocks, soils and climate in vineyards (ĎÖRĎ et al., 2010).

Over the last few years, the quality of Slovak wine has improved; as confirmed by the many gold medals won at various prestigious wine competitions around the world. Slovak wine is now comparable to fine world wines in terms of its quality and sensorial properties. On the other hand, vineyard areas are still being reduced (LAUKO et al., 2013; HANUŠIN and Štefunková, 2015). The wine region of the Malé Karpaty Mts has very good soil, climate and ecological conditions for wine production (ČERŇANSKÝ and KUHN, 2012). Yet vineyards are being abandoned in this region because of the current EU Agricultural policy (LIESKOVSKÝ et al., 2013). Viticulture participates in the conservation and appropriate usage of agricultural soils, and in the evolution of ecologically balanced land with economic benefits.

The objective of our research is to investigate geosystems (with relevant natural components of terroir) in the surroundings of the vineyard village Doľany in the wine region of the Malé Karpaty Mts. We will use the methodology of geoecological research and detailed mapping in both choric and topic geographical dimensions. Maps of choric dimension characterize wider (horizontal) relationships in the vineyard landscape, while research in topical dimension yields basic information on natural components of terroir in large scale and their spatial distribution.

## Material and methods

### Study area

The investigated area in the surroundings of Doľany (Fig. 1) occupies the foot of the slopes of the Malé Karpaty Mts with adjacent highland belonging to the Podunajská nížina lowland. It is an area with a significant history of grape growing. The investigated area covers 1,196.16 ha and is located between 188 and 472 m asl. The vineyards in Doľany, belonging to JM Vinárstvo Doľany (where detailed field research was carried out), cover an area of 7.56 ha and are located at the foot of Malé Karpaty at the boundary of cadastral areas of the boroughs Doľany and Dolné Orešany, at an altitude of 236 to 256 m asl.

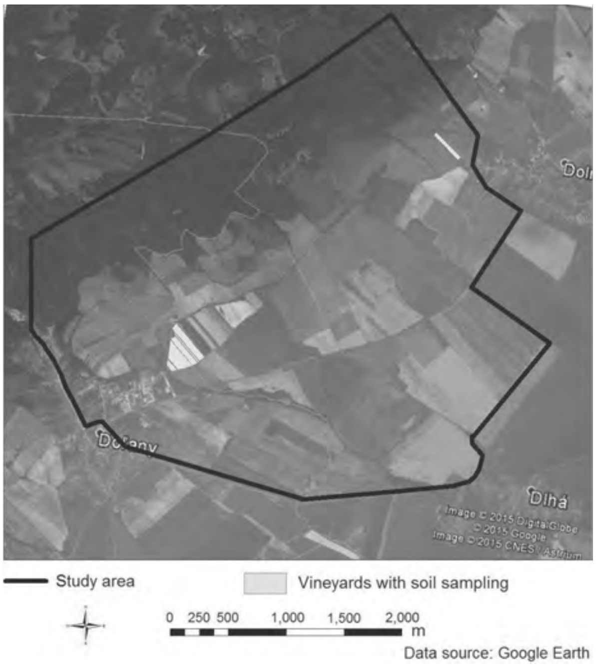


Fig. 1. Localization of study area.

Doľany, sometimes also called Ompitál by the locals, was one of the oldest German colonies under the Malé Karpaty Mts, up to the first half of the 18th century, when people started to use the Slovak language. Since 1608, Doľany has been an important wine town. Wine trade, which was dependant on the area of vineyards and the grape harvest, was especially important for the development of the town. According to the data from 1858, the vineyards occupied 98.5 ha of the total 2,206 ha belonging to the borough. White grape varieties Cirifandl and Lampart were grown there. At the end of the 19th century, vine phylloxera spread throughout the wine region, resulting in the loss of the vineyard area in the following years. In 1924, during the interwar period, the area of vineyards was only 21 ha. After

World War II, the price of wine had increased, which resulted in the expansion of viniculture. In 1950s, the collectivisation caused not only the change of the wine grape land ownership, but also influenced the level of viniculture. The co-operative viniculture shifted from growing hybrids to growing varieties such as Grüner Veltliner, Irsay Oliver, Burgundy, Silvaner. The change of situation after the fall of communism was manifested by initially good results – in 1992, the 89 ha of vineyards produced an average yield of 5.21 tons of grapes per hectare. In the following years, the wine production decreased, which was resolved again in 1996 by leasing the co-operating vineyards to private entrepreneurs (Dubovský et al., 2002).

**Geoecological research and mapping of geosystems**

The geosystems research consisted of several stages – the preparatory phase, field research and the final phase. The research process was based on the geological methodology according to the works of Minár et al. (2001) and Čech and Kunáková (2012), with emphasis on mapping in large scale.

The preparatory phase of the research consisted of collecting data and studying available materials around the observed area and its surroundings. The basemaps were studied for each relevant component of the country. The basemap of the lithology of the observed area was the Geological map of the Slovak Republic at scale of 1:50,000, available on the website of the State geological institute of Dionýz Štúr, and an additional source was a regional geological map (Polák et al., 2011). The basemap for the georelief characteristic was The Basic map of the Slovak Republic at 1:10,000. On the basis of contour lines, we have determined elementary forms of relief, to which the attributes according to the legend of the morphografic-positional types of relief have been assigned (Tremboš in Minár et al., 2001). In the case of transport slopes, attributes were extended to direct transport slope, concave transport slope and convex transport slope, from the viewpoint of the curvature contour line. The digital elevation model was created from the contour map by the tool Topo to raster with the size of the cell 10 m in the ArcGis environment. Other rasters of slope, aspect and insolation of land were derived from the digital model of relief.

Agriculturally used soils were characterized on the basis of the map service of the National Agriculture and Food Centre, through maps of evaluated soil-ecological units at a scale of 1:5,000 (Soil Portal, 2015). In forest areas, the data were derived from the WMS service of the National Forest Centre (2015). To unify the soil classification from these sources, a comparison of National Forest Centre soil systematic with the Morphogenetic classification system of soils of Slovakia was used. The information about climate characteristics was provided by the Slovak Hydrometeorological Institute for the period 1981–2010, from the stations, located nearest to examined locations. The average monthly rainfall totals were processed from the rain gauge stations in Dolné Orešany for the period 1981–2010. Similar data about air temperature in Doľany in 2014 were provided by the vine-dresser of wine-making JM Vinárstvo Doľany, which was compared with the average air temperature at the station in Slovenský Grob in 2014. Aerial scans by Google Earth from 2013 were used to characterize land cover. Particular classes of land cover were assigned on the basis of the edited methodology and CORINE land cover legend at the 4th hierarchical level at a scale of 1:50,000 according to authors Feranec and Oťaheľ (1999). Land use of the examined area was mapped at a scale of 1:10,000, therefore we set the minimal size of the mapped area to 0.1 ha. Because the work was focused on vineyard landscape, two classes of land cover evolved from the original methodology (used and unused vineyards). In the further stages of research, we concentrated on mapping habitat conditions in the earmarked categories of vineyards. Potential natural vegetation was presented according to the map of potential natural vegetation (Maglocký in *Atlas krajiny*, 2002).

In terms of methodology by authors Falťan et al. (2011), a map of geocomplexes at the level of geochores was created for the whole examined territory, meaning that relatively homogenous areas where the horizontal relationships among geotopes and their groups were examined (Mičian, 2008), with an interpretation measure of 1:10,000. In our case, as a leading factor of regionalization, georelief at the level of mezoform was selected. Furthermore, information about the bedrock, soil type and subtype, relief insolation and potential natural vegetation was examined.

The realization of detailed field geoecological research was limited by the availability of particular vineyards, subject to the various owners and unsettled owner relationships. Our research was conducted on estates belonging to the JM Vinárstvo Doľany. Particular research points and borders of the examined vineyards were localized by a GPS device. During our research, the data gathered in the preparatory phase of research were verified. By characterization of pedosphere, the soil unit and soil category were set (soil type and subtype). Thickness, granularity, soil skeleton, composition, size and form of skeleton, colour, spottiness, humidity, hardness, plasticity, structure and biological activity were set for each horizon. Moreover, samples from depths of 20–40 cm and 50–70 cm were taken from particular soil probes for chemical testing, to find the content of carbonates (titration method), nitrogen (Kjeldahl method) and pH reaction (STN ISO 10390). Concerning georelief characteristics, information about GPS location, altitude of each point, slope, orientation, meso-form of georelief, genesis, age and process demonstrations was presented. The biosphere characteristic was set through real phytocenosis, potential phytocenosis, coverage and height in particular etages. Laboratory soil analyses were realized by the accredited laboratory of the Soil Fertility and Conservation Research Institute.

In the final phase of research, we concentrated on the creation of final maps of geochors types as well as types of geoecological complexes and insolation. By processing the results from our field research, we created a map of edited soil types and subtypes over the area of the examined vineyards. The results of the soil analyses and the data provided by vine-dressers were statistically processed in MS Excel. Relief insolation maps were created from the digital relief model using an Area Solar Radiation tool every half hour, each 14th day of the year. Random settings (except for the setting of the diffuse model type) were allowed in the parameters of insolation (the chosen option was most commonly overcast). The outputs of the detailed research were processed in the ArcGIS 10 Environment.

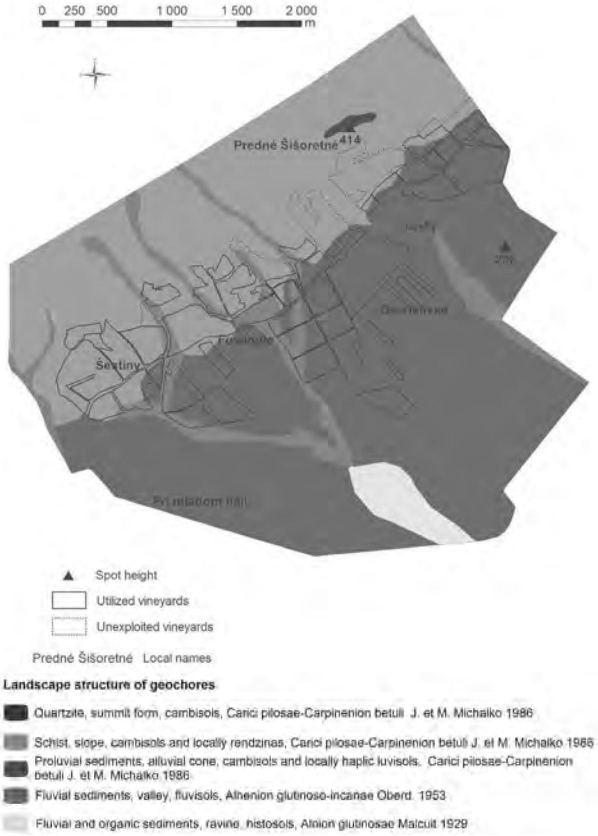


Fig. 2. Landscape structure of geochores.

## Results

The examined area contains five types of geochores, illustrated in Fig. 2. The dominant areal representation has geochore of the alluvial cones on proluvial sediments where Cambisols have formed, and potential natural vegetation is represented by Carpathian oak-hornbeam forests. More than half of the vineyards (57.7%) in the examined area are located on this geochore. A large percentage of vineyards (37.7%) is situated on the geochore of slopes that consist mainly of shist where Cambisols have formed and potentially natural vegetation is created by Carpathian oak-hornbeam forests. Some vineyards extend to both of the mentioned geochores, particularly to the valley geochore. It follows that vineyards are most often located on alluvial cones, transport and foot slopes of the Malé Karpaty Mts. The larger slope and mainly south-eastern orientation of the relief influences the incoming solar radiation power. Solar radiation is an important positioning factor. Relief insolation in the Doľany area is presented in Fig. 3, where the average insolation value is 1,082.9 kWh/m$^2$ per year. The areas with the lowest incoming solar radiation power are in particular north-eastern and eastern valleys slopes and slopes with a western exposition; on the contrary the highest values of insolation occur on the south-western oriented slopes with a larger angle. In the study area, the slopes with south-eastern orientation outweigh the cardinal points overall. The vineyards extend over an area with insolation values from 1,026.4 to 1,168.9 kWh/m$^2$ per year. High insolation values were reported at vineyards, not in use, as well as in some south oriented localities with steeper slopes on forest land.


Fig. 3. Insolation of relief.

The results of the geotopes research were processed for the areas of vineyards. Together, under the framework of 6 types of geochors, 58 types of geoecological complexes were classified by using the georelief as the leading factor of regionalization; the most commonly represented are listed in Table 1. From the viewpoint of localization of vineyards, these complexes have beneficial assorted substrate, which contains worked aluminous and sandy gravels and aluminous-chiselly down-slopes. The substrate was the basis for forming the medium to hard skeleton soils, which is important for the wine grape, mainly when taking nutrients from the soil. A very important criterion is the ease of availability to these vineyards when in need of cultivation. On the contrary, the types of geoecological complexes that have good substrate potential and high insolation of 1,125.1 to 1,175 kWh m$^{-2}$ per year (in the 5th and 6th categories of insolation), have not been used recently as vineyards, mainly because of their poor availability.

Table 1. The most frequent types of geosystems

| Code | Lithological complex | Landform | Insolation category | Soil type | Area (%) |
|---|---|---|---|---|---|
| 55 | Proluvial sediments | Cone | 2 | Cambisol | 25.86 |
| 56 | Proluvial sediments | Cone | 3 | Cambisol | 20.69 |
| 45 | Deluvial sediments | Foot slope | 3 | Cambisol | 12.07 |
| 46 | Deluvial sediments | Foot slope | 4 | Cambisol | 8.62 |
| 42 | Proluvial sediments | Foot slope | 4 | Cambisol | 6.19 |
| 51 | Fluvial sediments | Floodplain | 2 | Fluvisol | 6.90 |
| 04 | Breccias and conglomerates | Erosion-denudational slope | 4 | Rendzina | 5.17 |

The field research connected with sample collection at the examined area in Doľany was realized in vineyard localities Fosandle and Grefty (Fig. 4). The vineyard is located on an alluvial cone at an altitude between 236 and 256 m asl with a south-eastern exposition. The slope angle is from 1º to 7º. Geological bedrock is created by proluvial sediments, loamy and sandy gravels lay in the form of alluvial cones. According to the map of bonited soil-ecological units, there are Eutric Cambisols up to Luvi-Eutric Cambisols and Sagni-Haplic Luvisols in the examined vineyard. By using field research, we found an occurrence of cultivated Cambisols, Hortic Cambisols and cultivated Haplic Luvisols. At research points no. 1 and no. 2, cultivated Haplic Luvisols occur, Akp-horizon is present at a thickness of 35 cm, while no skeletons occur here, and the soils have loamy granulity. The dominant subsurface Bt-horizon contains up to 10% of skeletons and is enriched by translocated elements. Temporary B/C-horizon passes to bright substrate C-horizon (Fig. 5).

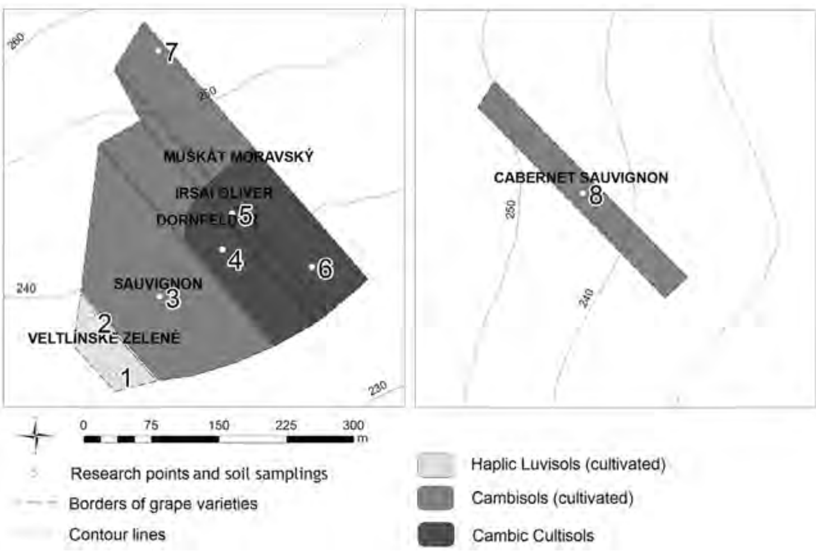


Fig. 4. Example of detailed soil map and localization of soil samples.


Fig. 5. Haplic Luvisol – soil sample no 1.

Table 2. Short description of chosen tesserae

| No | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Landform | Foot slope | | | | | | | |
| Substrate | Proluvial sediments | | | | | | | |
| Slope angle (°) | 1–2 | 1–2 | 2–4 | 3–4 | 3–4 | 4–5 | 5–6 | 4–5 |
| Soil type | Haplic Luvisol | | Cambisols (cultivated) | | | | | |
| pH | 7.09 | 7.03 | 6.72 | 7.41 | 7.01 | 7.29 | 7.37 | 7.05 |
| Carbonates (%) | under 0.01 | | | | | | | |
| Nitrogen (%) | 0.03 | 0.02 | 0.01 | 0.02 | 0.04 | 0.02 | 0.02 | 0.01 |
| Vine variety | Grüner Veltliner | | Sauvignon | Dornfelder | Irsai Oliver | Moravian Muscat | | Cabernet |

At research point nos. 4–6, Hortic Cambisols occur, with a dominant Akm-horizon, the skeletal content is from 25 to 70% and loamy granulity. Under the cultisoil horizon, the cambic Bv-horizon is located with high skeletal content (70–80%), which passes to substrate C-horizon. At research point nos. 3, 7 and 8, cultivated Cambisols occur. The cultisoil Akp-horizon has up to 30% of skeleton and loamy granulity. The dominant cambisoil Bv-horizon has a high content of skeletons (up to 70%) and loamy granulity. The sample of basic characteristics discovered in field research is listed in Table 2. In the vineyard area in Doľany, neutral soils occur – the pH value moves from 6.72 to 7.41. The content of nitrogen in the soil moves from 0.01 to 0.04%. The volume of nitrogen in the soil is low and medium. Appropriate nutrition will complement fertilization at poorer areas. The content of carbonates in the examined samples is less than 0.01%. Carbonate content in the soil is low due to site conditions, but overall the site is suitable for growing vines.

## Discussion

Landscape-ecological parameters characterising the landscape structure, function and dynamics play an important role as an indicator of sustainable development (Kozová and Pauditšová, 2001). Agricultural geosystems belong to important parts of the present landscape structure. Growing permanent crops significantly affects the character of the landscape and creates a specific type of cultural landscape. The huge changes that our society and viniculture, went through after World War II posed a threat to the traditional vineyard landscape. The significant changes are not only in the area, size and vineyard agronomical practices – or the residential pressure of large cities such as Bratislava or Nitra – but also the changing lifestyle of young generations, who do not seem to be interested in viniculture or agriculture at all (Lieskovský et al., 2013). The complex safety of the vinicultural landscape should include soil safety, in an attempt to optimize the way the landscape is used and to be able to minimize soil erosion (Dobos et al., 2014).

Intensive land use changes affect not only the country itself but also progressively limit the activities of man. Man is presently the dominant factor in the forming of landscape and adapts it to its goals. Our work focussed on the physio-geographical aspects of the terroir, presenting an important factor of the localization of vineyards. The geographical characteristic of a particular location gives the vine a specific taste, which differs from vines produced in other regions. Therefore, the geographical attitudes to the study of viniculture, with an emphasis on terroir, are nowadays actual topics (Bonfante et al., 2011; Dougherty et al., 2012). The field research and application of GIS tools is an important part of the study of vinicultural geosystems. By creating a preliminary geoecological map, we needed to choose appropriate base maps, or to create our own one by employing various methodologies (e.g. the map of elementary forms of relief). The methodology of creating the map of geosystems was influenced by the work of authors Minár et al.(2001); Čech and Kunáková (2012). These authors use the methods of the leading factor of georelief and the use of analytical maps within geoecological research and the locating of geotopes. In our research, georelief was chosen as the main factor. We filled the created areas with further important information from the viewpoint of terroir – mainly bedrock, soil type and subtype and surface relief radiation.

Similar methods are successfully applied abroad, in countries with a long-lasting experience of wine cultivation. The area analysis of terroir in Valle Tellesina (southern Italy), has been evaluated by authors Bonfante et al. (2011). The terroir was classified on the basis of soil, value of radiation, index of crop water stress and detailed climatic research. The resulting terroir units were defined according to the suitability of vine production. The problems encountered were also analysed by Carey et al. (2008) in the Stellenbosch area in The Republic of South Africa. They defined the natural terroir units on the basics of the relatively homogenous characteristics of the topography, clime, geology and soils. As the leading factor, the morphographical characteristics of surface relief (altitude, slope, orientation) and soil type were used.

Generally, the bedrock of the soil only has an indirect impact on the quality of grapes and vine. Often bedrock is almost covered by the dominancy of the soil, topographic and climatic characteristics. The majority of nutrients are gained from depths of up to 0.6 m and water from depths of up to 2 m (Huggett, 2005). The investigation of soils under impact of traditional agricultural activities is an important topic of landscape ecological research (Slámová et al., 2015). Detailed field research and sample taking of terroir is necessary. According to our research, we found differences between the data from official basis soil maps and the real field environment. At the research area in the Doľany vineyards, according to bonited soil-ecological units, are where mainly Eutric Cambisols up to Luvi-Eutric Cambisols and Stagni-Haplic Luvisols are located. According to field research, the dominant occurrence of Cambisols has been proved, however, with the significant impact of cultivation. Cultivated Cambisols have been identified in three research points; more significant transformation by cultivating has been shown in the occurrence of Hortic Cambisols in three further research points. The change has been found only in the level of subtype in the location of Stagni-Haplic Luvisols – we identified Haplic Luvisols here, probably due to the soil cultivation and drainage. Within future research, it will be important to monitor microclimatic conditions of locations during long periods, except detailed soil analyses. For that, we need stationary research and placement of meteorological stations in the vineyards.

## Conclusion

Basic relevant natural components of landscape representing physical-geographical aspect of terroir were described on the basis of detailed geosystems research in our work. Geoecological research within an example study of vineyard landscape was realised in choric and topical geographical dimension in the Doľany vicinity. Georelief was selected as a leading factor of regionalisation at the level of geochores. Information about soil-forming substrates and land cover on a topical dimension was specified within detailed research of the terrain. Overall, five basic types of geochores and 58 types of geoecological complexes were earmarked for location in the Doľany vicinity. The most frequent occurrence of vineyards was on alluvial cones and slopes of the Malé Karpaty Mts in terms of choric dimension. As part of a detailed research of soil, we further characterized location conditions of different grape varieties (Grüner Veltliner, Sauvignon, Irsai Oliver, Moravian Muscatel, Dornfelder, Cabernet Sauvignon) in the Fosandle and Grefty localities.

From the field research we found the occurrence of cultivated Cambisols, Hortic Cambisols and cultivated Haplic Luvisols with a predominantly neutral soil reaction and a nitrogen content of 0.04%. The application of our methodology provides useful information in terms of landscape and wine-growing practice. The most significant outputs include identification of different types of geocomplexes in the vineyard landscape. Combination of information about georelief, bedrock and soil cover in choric and topical dimension creates the basis for regionalisation and detailed identification of natural terroir units. With this, future research may take place focusing mainly on the study of the relationship between the physical and chemical properties of soil and microclimatic conditions of the habitat quality of the wine produced. A more detailed analysis of this issue would require the cooperation of several experts (oenologists, geochemists, etc.) from different disciplines. Analysis of natural landscape components and outputs in the form of geo-ecological maps can be used in environmental practice (landscape planning, evaluation of the carrying capacity of the landscape, evaluation of the potential or capacity of the landscape, etc.).


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### References


ANTROP, M., 2005. Why landscapes of the past are important for the future. *Landscape and Urban Planning,* 70: 21–34.

BARTÍK, M., SITKO, R., OREŇÁK, M., SLOVIK, J., ŠKVARENINA, J., 2014. Snow accumulation and ablation in disturbed mountain spruce forest in West Tatra Mts. *Biologia, Bratislava*, 69, 11: 1492–1501.

BONFRANTE, A., BASILE, A., LANGELLA, G., MANNA, P., TERRIBILE, F., 2011. A physically oriented approach to analysis and mapping of terroirs. *Geoderma*, 167–168: 103–117.

CAREY, V. A., SAAYMAN, D., ARCHER, E., BARBEAU, G., WALLACE, M., 2008. Viticultural terroirs in Stellenbosch, South Africa. I. The identification of natural terroir units. *Journal International des Sciences de la Vigne et du Vin*, 42 (4): 169–183.

ČECH, V., KUNÁKOVÁ, L., 2012. Geologický výskum a geologická databáza (na príklade katastrálneho územia obce Kolinovce) [Geoecological research and geoecological database (casestudy: cadastral area of village Kolinovce)]. *Geografický Časopis*, 64: 29–46.

ČERŇANSKÝ, S., KHUN, M., 2012. Geochemické prostredie Malokarpatskej vinohradníckej oblasti – výber vinohradníckych rajónov [Geochemical environs of the Malé Karpaty wineyard region – selection of vineyard rayons]. In JURKOVIČ, Ľ., SLANINKA, I., ĎURŽA, O. (eds.). *Geochémia 2012*. Bratislava: ŠGÚDŠ, SAGH & Univerzita Komenského v Bratislave, p. 16–17.

DOBOS, A., NAGY, R., MOLEK, Á., 2014. Land use changes in a historic wine region and their connections with optimal land-use: a case study of Nagy-Eged Hill, northern Hungary. *Carpathian Journal of Earth and Environmental Sciences*, 9 (2): 219–230.

DOUGHERTY, P.H., 2012. *The geography of wine: regions, terroir and techniques*. New York: Springer. 255 p.

ĎÖRĎ, L., LOŽEK, O., HRONSKÝ, Š., 2010. *Vplyv klimatických faktorov na kvalitu hrozna avína v modrokamenskom vinohradníckom rajóne* [Impact of climatic factors on grape and vine quality in the Modrý Kameň vineyard rayon]. Nitra: Slovenská poľnohospodárska univerzita. 103 p.

DUBOVSKÝ, J., PROSPECHOVÁ, P., TURCSÁNY, J., 2002. *Doľany*. Doľany: Obecný úrad, 214 p.

FALŤAN, V., BÁNOVSKÝ, M., BLAŽEK, M., 2011. Evaluation of land cover changes afterextraordinary windstorm by using the land cover metrics: a case study on the High Tatras foothill. *Geografie*, 166 (2): 156–171.

FALŤAN, V., KATINA, S., BÁNOVSKÝ, M., PAZÚROVÁ, Z., 2009. The influence of site conditions on the impact of windstorms on forests: the case study of the High Tatras foothills (Slovakia) in 2004. *Moravian Geographical Reports,* 17 (3): 44–52.

FERANEC, J., OŤAHEĽ, J., 1999. Mapovanie krajinnej pokrývky metódou CORINE v mierke 1 : 50 000: návrh legendy pre krajiny programu Phare [Mapping of land cover at scale 1 : 50000: draft of the nomenclanture for the Phare countries]. *Geografický Časopis*, 51: 19–44.

GREŠLOVÁ, P., GINGRICH, S., KRAUSMANN, F., CHROMÝ, P., JANČÁK, V., 2015. Social metabolism of Czech agriculture in the period 1830–2010. *Acta Universitatis Carolinae, Geographica,* 50: 23–35.

HANUŠIN, J., ŠTEFUNKOVÁ, D., 2015. Changes in vine-growing landscape diversity in the hinterland of Svätý Jur town in 1896–2011. *Geografický Časopis,* 67: 243–259.

HAVLÍČEK, M., PAVELKOVÁ, R., FRAGJER, J., SKOKANOVÁ, H., 2014. The long-term development of water bodies in the context of land use: The case of the Kyjovka and Trkmanka river basins (Czech republic). *Moravian Geographical Records*, 22 (4): 39–50.

HUGGETT, J. M., 2005. Geology and wine: a review. *Proceedings of the Geologists' Association*, 117: 239–247.

IVAN, P., JARABICOVÁ, M., MUCHOVÁ, Z., 2015. Assessment of changes in landuse by coefficient of the ecological stability. *International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management*, *SGEM*, Book 5, Vol. 1: 73–80.

JUSKOVÁ, K., MUCHOVÁ, Z., 2013. Land consolidation as an instrument for land ownership defragmentation in the Czech Republic and Slovakia. In *MendelNet 2013. Proceedings of International PhD Students Conference, November 20th and 21th, Brno.* Brno: Mendelova univerzita, p. 444–448.

JUSKOVÁ, K., MUCHOVÁ, Z., 2014. Options and trends of land consolidation in the Czech and Slovak republics, with regard to common historical development of ownership and usage rights. *International*

*Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management, SGEM*, Book 5, Vol. 2: 471–478.

Kopecká, M., Vatseva, R., Feranec, J., Oťahel, J., Stoimenov, A., Nováček, J., Dimitrov, V. 2012. Selected changes of arable land in Slovakia and Bulgaria during the period 1990–2006. *Moravian Geographical Reports*, 20 (1): 43–54.

Kozová, M., Pauditšová, E., 2001. Evaluation of the landscape structure and its changes for Local agenda 21. *Ekológia (Bratislava),* 20, Suppl. 3: 36–44.

Lauko, V., Tolmáči, L., Križan, F., Gurňák, D., Cákoci, R., 2013. *Geografia Slovenskej republiky: humánna geografia* [Geography of the Slovak Republic. Human geography]. Bratislava : Geografika. 289 p.

Lieskovský, J., Bezák, P., Špulerová, J., Lieskovský, T., Koleda, P., Dobrovodská, M., Bürgi, M., Gimmi, U., 2015. The abandonment of traditional agricultural landscape in Slovakia – Analysis of extent and driving forces. *Journal of Rural Studies*, 37: 75–84.

Lieskovský, J., Kanka, R., Bezák, P., Štefunková, D., Petrovič, F., Dobrovodská, M., 2013. Driving forces behind vineyard abandonment in Slovakia following the move to a market-oriented economy. *Land Use Policy*, 32: 356–365.

Lipský, Z., 2001. *Monitoring changes in the cultural landscape*. Praha: ČZU Praha.

Löfvenhaft, K., Runborg, S., Sjögren-Gulve, P., 2004. Biotope patterns and amphibian distribution as assessment tools in urban landscape planning. *Landscape and Urban Planning*, 68: 403–427.

Maglocký, Š., 2002. Potenciálna prirodzená vegetácia 1 : 500 000 [Potential natural vegetation 1 : 500 000]. In *Atlas krajiny SR.* Bratislava: MŽP, Banská Bystrica: SAŽP, 2002, p. 114.

Matečný, I., 2014. Spatial identification of vineyards – a prerequisite for maintainting them as a part of the cultural landscape heritage. *Acta Environmentalica Universitatis Comenianae*, 22 (2): 26–41.

Matečný, I., Jenčo, M., Matečná, G., 2010. Sklon reliéfu ako súčasť kritérií pre plnenie environmentálne šetrných postupov v poľnohospodárstve Slovenskej republiky [Relief slope angle as a part of criterions for environmentally friendly processes in agriculture of the Slovak Republic]. *Acta Geographica Universitatis Comenianae*, 54 (1): 33–52.

Mičian, Ľ., 2008. *Všeobecná geoekológia* [General geoecology]. Bratislava: Geografika. 88 p.

Miklós, L., Matečný, I., Kozová, M., 1991. Interpretation of the morphometric relief indices for spatial differentiation of microclimatic conditions. *Ekológia (Bratislava)*, 10: 223–246.

Minár, J., 2003. Detailed physical-geographical (geoecological) research and mapping in the landscape ecology. *Ekológia (Bratislava),* 22: 141–149

Minár, J., Barka, I., Bonk, R., et al. 2001. *Geoekologický (komplexný fyzickogeografický výskum a mapovanie vo veľkých mierkach* [Geoecological (complex physiogeographical) research and mapping in large scales]. Bratislava: Geografika. 210 p.

Muchová, Z., Petrovič, F., 2010. Changes in the landscape due to land consolidations. *Ekológia (Bratislava),* 29: 140–157.

National Forest Centre, 2015. *Map server.* National Forest Centre [cit. 2015-12-10]. http://lvu.nlcsk.org

Nassauer, J., 1995. Culture and changing landscape structure. *Landscape Ecology,* 10: 229–237.

Opršal, Z., Šarapatka, B., Kladivo, P., 2013. Land-use changes and their relationships to selected landscape parameters in three cadastral areas in Moravia (Czech Republic). *Moravian Geographical Reports,* 21 (1): 41–50.

Pătru-Stupariu, I., Stupariu, M. S., Tudor, C.A., Grădinaru, S.R., Gavrilidis, A., Kienast, F., Hersperger, A.M., 2015. Landscape fragmentation in Romania's Southern Carpathians: Testing a European assessment with local data. *Landscape and Urban Planning*, 143: 1–8.

Polák, M., Plašienka, D., Kohút, M., Putiš, M., Bezák, V., Filo, I., Broska, I., 2011. *Geologická mapa regiónu Malých Karpát v mierke 1 : 50 000* [Geological map of the Malé Karpaty Region in scale 1:50,000]. Bratislava: MŽP SR, Štátny geologický ústav Dionýza Štúra.

Sklenička, P., Janovska, V., Salek, M., Vlasak, J., Molnarova, K., 2014. The farmland rental paradox: extreme land ownership fragmentation as a new form of land degradation. *Land Use Policy,* 38: 587–593.

Slámová, M., Jakubec, B., Hreško, J., Belaček, B., Gallay, I., 2015. Modification of the potential production capabilities of agricultural terrace soils due to historical cultivation in the Budina cadastral area, Slovakia. *Moravian Geographical Reports,* 23 (2): 47–55.

Soil Portal, 2015. *Evaluated soil-ecological units.* National Agriculture and Food Centre [cit. 2015-11-12]. http://www.podnemapy.sk/bpej

Špulerová, J., Dobrovodská, M., Lieskovský, J., Bača, A., Halabuk, A., Kohút, F., Mojses, M., Kenderessy, P., Piscová, V., Barančok, P., Gerhátová, K., Krajčí, J., Boltižiar, M., 2011. Inventory and classification of historical structures of the agricultural landscape in Slovakia. *Ekológia (Bratislava),* 30: 157–170.

Supuka, J., Verešová, M., Šinka, K., 2011. Development of vineyards landscape structure with regard to historical and cultural values. *Ekológia (Bratislava),* 30: 229–238.

Van Leeuwen, C., Friant, P., Chone, X., Tregoat, O., Koundouras, S., Dubordieu, D., 2004. Influence of climate, soil, and cultivar in terroir. *American Journal of Enology and Viticulture,* 55: 207–217.

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# Assemblages of ground living spiders (Araneae) in peatland habitats, surrounding dry pine forest and meadows

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**Abstract**

Gajdoš, P., Majzlan, O., Igondová, E., 2016. Assemblages of ground living spiders (Araneae) in peatland habitats, surrounding dry pine forest and meadows. *Folia Oecologica*, 43: 147–155.

This research was conducted to study assemblages of ground living spider of a peatland and their surrounding habitats (margin of dry pine forest and meadows) in the Šuja peat bog (in northern part of Slovakia) in 2013. The aim of study was to classify assemblages of ground living spider into different habitat types, compare their composition and analyse the relation of species occurrence at study sites. Spiders were sampled between April and October 2013 at 8 study sites using pitfall traps. 1,974 individuals belonging to 100 species and to 21 families were captured in total. Diversity, equitability, species composition, preference for humidity, inclusion in the group of their ecological valence and habitat association were used to characterize ground living spider communities at each study site. In order to evaluate the relationship among the communities of the spiders at the sampling sites we used principal component analysis. Based on their ecological characteristics, spiders formed five groups of species associated with semi dry to mesophilous open meadow habitats, semi dry woodland habitats, mesophilous unshaded habitats, mesophilous partly shaded habitats without herbal vegetation cover and hygrophilous habitats.

**Key words**

Araneae, diversity, ground living spiders, meadows, peatlands, pine forest margin

## Introduction

Peatlands comprise over 50% of the world's wetlands. They have generally been regarded as wasteland rather than as any special, or even recognisable, part of the natural world. Recently, perceptions of peatlands have begun to change dramatically, and they are now increasingly acknowledged as a habitat type of global significance. Apart from their biological diversity, they provide goods and services to people, they play an important role in water regulation, they store carbon, and they are of value for education and research. Central European peatlands play an important role in the global and international conservation of species (Bragg et al., 2003). Peatlands are very rare, threatened and often relict ecosystems. In general, there is a lack of knowledge about ecosystem functioning and management planning and not all protected areas are protected effectively (Stanová, 2000). Therefore peatlands and other wetland fragments are mainly surrounded by drier habitats such as forests or meadows (Igondová and Majzlan, 2015).

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Spiders, an important component of peatland fauna, are used as bio-indicators of environmental quality (Buchar, 1983, 1991; Buchar and Růžička, 2002; Růžička and Boháč, 1994; Růžička, 1986, 1987; Scott et al., 2006) and for evaluation of biota changes in relation to land management and the succession (Maelfait et al., 1990; Maelfait, 1996; Maelfait et al., 2002; Buchholz, 2016).

Presented study deals with description of community of ground dwelling spiders in Šujské rašelinisko territory. The equitability and similarity in peatland habitats, surrounding dry pine forest and meadows near Šujské rašelinisko territory and classification the studied plots according to their importance from the point of view of nature conservation were assessed.

From the arachnological point of view no study was carried out on study area. Only a few studies dealt with insect fauna in this territory. Bitušík (1998) studied chironomid flies (*Chironomidae*), Badík (1994) and Šácha and Racko (2014) analysed community of dragonflies (Odonata). Majzlan et al. (2004), Majzlan and Igondová (2013) and Igondová and Majzlan (2015) provided the first data about beetles (Coleoptera) in Šujské rašelinisko locality.

## Study area and sampling sites

The Nature Reserve Šujské rašelinisko is located near the village Šuja along Rajčanka River in the cadastre territory of the village Rajecká Lesná (10.8 ha). It belongs to the Malá Fatra National Park (north Slovakia) and its conservation is subject to 4th level of nature protection. This Reserve is also part of Special Area of Conservation (SAC) which belongs to the NATURA 2000 network (13.48 ha). The area is located in altitude 470 m asl. The objects of conservation for this SAC are following habitats listed in Annex I of the Habitats Directive, namely the natural dystrophic lakes and ponds, the *Molinia* meadows on calcareous, peaty or clayey-silt-laden soils (*Molinion caeruleae*), the hydrophilous tall herb fringe communities of plains and of the montane to alpine levels and the alkaline fens. During the 1970s, the territory was destroyed by a peat exploitation mainly in the north-western part of territory (Igondová and Majzlan, 2015).

In 2010 the surrounding of the protected area was disturbed by building proposal of large parking place (ITV Rajec, 2010). Finally, the proposal was rejected and the area seems to be revitalised now. Nevertheless, studied area is still a valued wetland in Slovakia, but threatened by increasing succession. *Pinus nigra* is spreading on the north-western part from the surrounding dry pine forest and more willow shrubs spread to the centre of the peat bog from the east part of Rajčianka riverside. The exploited area is being gradually overgrown by autochthonous vegetation and partly also by ruderal vegetation. The small island situated in the centre of Šuja peat bog is shrubby with *Rubus* sp. and *Urtica* sp. in canopy and undergrowth. This island is influenced by the decline of groundwater level. The edges of neighbouring roads are surrounded by *Salix* sp. and *Alnus glutinosa* in canopy and a dense stand of reeds (*Phragmites* sp.) in the north-western and eastern parts of the territory (Igondová and Majzlan, 2015).

Eight sampling sites of peatland habitats, surrounding dry pine forest and meadows were established and they are the same as for carabid study (Igondová and Majzlan, 2015) (Fig 1):

1 (49°03'40.4"N, 18°36'57.2"E), dry pine forest (for) – forest edge at slope of Strážov hills on the limestone ground with *Pinus* sp.

2 (49°03'37.7"N, 18°36'58.6"E), osier (osi) – waterlogged area at mild depression with *Salix repens* and *Salix purpurea*

3 (49°03'37.6"N, 18°37'02.1"E), playground (plg) – meadow, mown area, flat surface, without trees, slightly dry, with frequent appearance of molehills

4 (49°03'40.3"N, 18°37'05.2"E), peat (pet) – peat bench, unexploited part of peatland, with *Rubus idaeus*

5 (49°03'42.6"N, 18°37'05.8"E), gravel bars (gra) – gravel bench in the middle of the reservation with *Betula* sp. and *Pinus* sp.

6 (49°03'44.6"N, 18°37'05.8"E), reed (ree) – damp areas around slightly flowing water with *Phragmites australis*

7 (49°03'46.3"N, 18°37'09.8"E), overgrow (ovg) – on the peat bench with *Salix* sp. and *Rubus* sp.

8 (49°03'36.9"N, 18°37'18.4"E), field-meadow (fim) – edge of the meadow and field by the Rajčianka riverside, ruderal, overgrown with vegetation.

## Material and methods

Ground living spiders were pitfall-trapped at eight sampling sites bi-weekly during the season 2013 (on following dates: 3 May, 17 May, 2 June, 16 June, 1 July, 15 July, 29 July, 12 August, 27 August, 14 September, 27 September, 12 October and 1 November 2013). Pitfall traps were installed on 14 April 2013 and exposed for 201 days. Covered traps (500 ml in size, 10 cm in diameter, half-filled with 4% formalin solution) were used to collect samples. Five traps were placed in lines in each habitat. The humidity preference was classified using an eight degree semiquantitative scale (1 – very dry, 2 – dry to very dry, 3 – slightly dry to semi-humid, 4 – unspecific, 5 – semi-humid (mesohydrophilous), 6 – semi-humid to humid, 7 – humid to very humid, 8 – very humid (Buchar and Růžička, 2002).

The Shannon-Wiener index (H') (Shannon and Weaver, 1949; Spellerberg and Fedor, 2003) was used to consider both abundance and evenness of species in the spider community. As an equitability index we used Pielou's Evenness Index or Equitability (E)

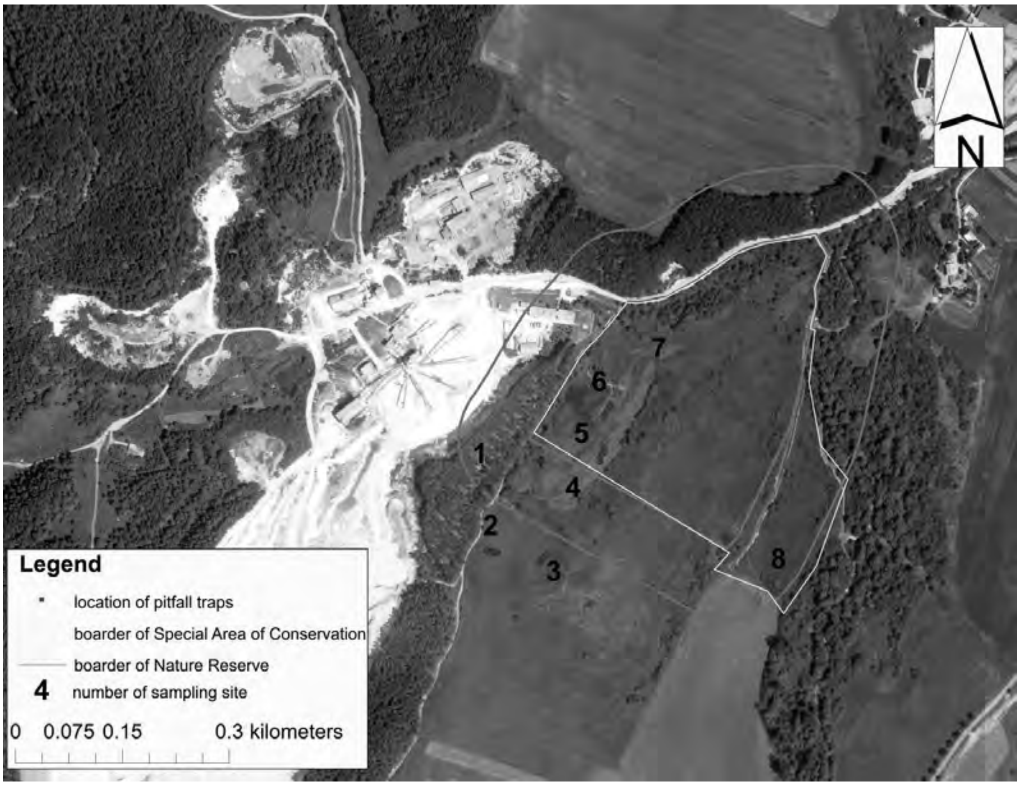


Fig. 1. Distribution of sampling sites of Šujské rašelinisko territory.

(Pielou, 1966). Analyses were carried out using the PAST program. The ground living spiders were divided into four groups: 1 (climax), 2 (climax and seminatural), 3 (climax, seminatural and disturbed or artificial), 4 (disturbed or artificial), according to the range of their ecological valence and their association with the originality of habitats (Buchar and Růžička, 2002). Principal component analysis (PCA) was conducted to evaluate the relationship among the communities of the ground living spiders at the sampling sites using the CANOCO software program (TerBraak and Šmilauer, 1998).

Though smaller distances between species points and sampling site points represent higher correlation between the values. The nomenclature and systematic order of spiders follow last version of the World Spider Catalog (World Spider Catalog, 2015).

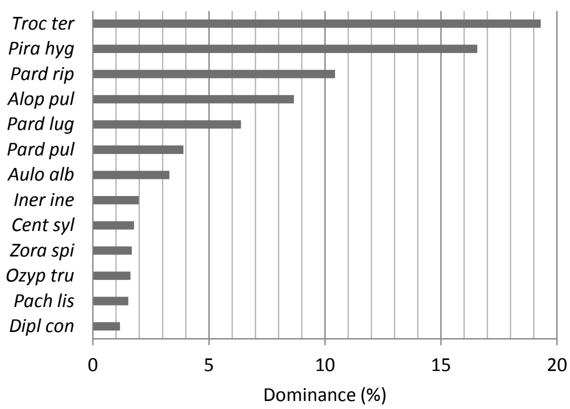


Fig. 2. Species with dominance higher than 1%.

## Results and discussion

A total of 1,974 specimens belonging to 100 species were documented (Table 1). Species *Trochosa terricola, Piratula hygrophila and Pardosa riparia* representing 46% of captured species were with the highest dominancy at studied sites. *Trochosa terricola* occurred abundantly at all sampling sites. Species *Piratula hygrophila* preferred wet habitats and *Pardosa riparia* was associated with mesophilous open habitat (Fig. 2).

Community of ground dwelling spiders at forest margin sampling site included eudominant species (>10%) *Trochosa terricola* (19.7%) and *Inermocoelotes inermis* (15.8%), further domimant species (5–10%), *Pardosa lugubris* (6.4%), *Zodarion germanicum* (6.4%), *Ceratinella brevis* (5.6%) and *Tapinocyba insecta* (5.1%). These species prefer shaded or semi shaded habitats (silvicol species) and semi or slightly dry habitats.

The osier sampling site is characterized by balanced equitability values with *Piratula hygrophila* (54.2%) as a eudominant species. This species prefers

Table 1. Abundance of ground living spiders (Araneae) sampled

| Species | Abbr | for | osi | plg | pet | gra | ree | ovg | fim | RL | Hum | O-h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mimetidae | | | | | | | | | | | | |
| *Ero furcata* (Villers, 1789) | *Ero fur* | | | 1 | 2 | | | 1 | 3 | | 5 | 2 |
| Theridiidae | | | | | | | | | | | | |
| *Robertus neglectus* (O.P.-Cambridge, 1871) | *Robe neg* | | 2 | | | | | | 1 | NT | 5 | 2 |
| Linyphiidae | | | | | | | | | | | | |
| *Agyneta affinis* (Kulczyński, 1898) | *Agyn aff* | | | 2 | 1 | | | | | | 4 | 2(1) |
| *Agyneta rurestris* (C.L.Koch, 1836) | *Agyn rur* | | | 1 | | | | | | | 6 | 3 |
| *Araeoncus crassiceps* (Westring, 1861) | *Arae cra* | | | | 1 | | | | | EN | 7 | 2(1) |
| *Bathyphantes gracilis* (Blackwall, 1841) | *Bath gra* | | | | | | | | 2 | | 5 | 3 |
| *Bathyphantes nigrinus* (Westring, 1851) | *Bath nig* | | | | | | | 1 | | | 6 | 2 |
| *Bathyphantes parvulus* (Westring, 1851) | *Bath par* | 1 | | | | | | | | | 5 | 2 |
| *Centromerus brevipalpus* (Menge, 1866) | *Cent bre* | 2 | | | | | | | | NT | 5 | 2 |
| *Centromerus sylvaticus* (Blackwall, 1841) | *Cent syl* | 2 | 7 | 2 | 3 | 1 | 14 | 5 | 1 | | 6 | 3 |
| *Ceratinella brevis* (Wider, 1834) | *Cera bre* | 13 | 1 | | | | 1 | | | | 6 | 2 |
| *Cnephalocotes obscurus* (Blackwall, 1834) | *Cnep obs* | | | | | | | 1 | | | 6 | 2 |
| *Dicymbium brevisetosum* Locket, 1962 | *Dicy bre* | | | 5 | | | | 1 | 4 | | 6 | 3 |
| *Diplostyla concolor* (Wider, 1834) | *Dipl con* | 3 | 1 | | 10 | | 1 | 7 | 1 | | 7 | 2 |
| *Dismodicus bifrons* (Blackwall, 1841) | *Dism bif* | | 1 | | 1 | | | | | | 5 | 2 |
| *Entelecara erythropus* (Westring, 1851) | *Ente ery* | | | | | | | | 1 | LC | 5 | 2 |
| *Gonatium paradoxum* (L.Koch, 1869) | *Gona par* | | 3 | 1 | | | | | | | 7 | 2 |
| *Gongylidiellum latebricola* (O.P.-Cambridge, 1871) | *Gong lat* | | 1 | 1 | 1 | | | | | | 8 | 2 |
| *Gongylidium rufipes* (Linnaeus, 1758) | *Gong ruf* | | | | 1 | | | 1 | | | 7 | 2 |
| *Mansuphantes mansuetus* (Thorell, 1875) | *Mans man* | 9 | | 1 | 1 | | | | | | 6 | 3 |
| *Micrargus herbigradus* (Blackwall, 1854) | *Micr her* | | 1 | | | | | | | | 6 | 2 |
| *Micrargus subaequalis* (Westring, 1851) | *Micr sub* | | | 2 | | 2 | | | | | 3 | 2 |
| *Neriene clathrata* (Sundevall, 1830) | *Neri cla* | 2 | | 1 | 1 | | | | 1 | | 6 | 2 |
| *Palliduphantes alutacius* (Simon, 1884) | *Pall alu* | | | | | | | | 1 | | 5 | 2 |
| *Pocadicnemis juncea* Locket & Millidge, 1953 | *Poca jun* | | 2 | 2 | 1 | 1 | 7 | 4 | | | 7 | 2 |
| *Tapinocyba insecta* (L.Koch, 1869) | *Tapi ins* | 12 | 2 | 1 | | | | | | | 5 | 2 |
| *Tapinopa longidens (*Wider, 1834) | *Tapi lon* | | | | | | | 1 | | | 5 | 2 |
| *Tenuiphantes cristatus* (Menge, 1866) | *Tenu cri* | 1 | | | 1 | | | | | | 6 | 2 |
| *Tenuiphantes flavipes (*Blackwall, 1854) | *Tenu fla* | 3 | | | | | | | | | 3 | 2 |
| *Tenuiphantes mengei* (Kulczyński, 1887) | *Tenu men* | | 1 | 2 | 5 | 1 | 1 | | | | 4 | 2 |
| *Walckenaeria acuminata* Blackwall, 1833 | *Walc acu* | | | | 1 | | 1 | | | LC | 6 | 2 |
| *Walckenaeria atrotibialis* (O.P.-Cambridge, 1878) | *Walc atr* | 2 | 1 | | | | | | | | 4 | 2 |
| *Walckenaeria dysderoides* (Wider, 1834) | *Walc dys* | 1 | | 3 | | | | | | | 3 | 2 |
| *Walckenaeria furcillata* (Menge, 1869) | *Walc fur* | 2 | | | 1 | 1 | | | | | 3 | 2 |
| *Walckenaeria mitrata* (Menge, 1868) | *Walc mit* | 1 | | | | | | | | | 5 | 2 |
| *Walckenaeria obtusa* Blackwall, 1836 | *Walc obt* | | | | | | | | 1 | | 6 | 2 |
| Tetragnathidae | | | | | | | | | | | | |
| *Pachygnatha degeeri* Sundevall, 1830 | *Pach deg* | | | 8 | 1 | | | | | | 4 | 3 |
| *Pachygnatha listeri* Sundevall, 1830 | *Pach lis* | | 7 | 3 | 2 | | 1 | 10 | 7 | | 7 | 2 |
| Araneidae | | | | | | | | | | | | |

Table 1. Abundance of ground living spiders (Araneae) sampled – continued

| Species | Abbr | for | osi | plg | pet | gra | ree | ovg | fim | RL | Hum | O-h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Araneus quadratus* Clerck, 1757 | *Aran qua* | | | | | | | | 1 | | 6 | 2 |
| *Argiope bruennichi* (Scopoli, 1772) | *Argi bru* | | 1 | | | | | | | | 4 | 3 |
| *Cercidia prominens* (Westring, 1851) | *Cerc pro* | | 1 | | | | | | | | 4 | 2(1) |
| Lycosidae | | | | | | | | | | | | |
| *Alopecosa cuneata (Clerck, 1757)* | *Alop cun* | | | 3 | | | | | | | 4(1) | 3 |
| *Alopecosa pulverulenta* (Clerck, 1757) | *Alop pul* | | 16 | 93 | 14 | 6 | 3 | 31 | 8 | | 4 | 3 |
| *Arctosa maculata* (Hahn, 1822) | *Arct mac* | | 1 | | | 2 | | | | | 8 | 1 |
| *Aulonia albimana* (Walckenaer, 1805) | *Aulo alb* | 7 | 16 | 4 | 2 | 28 | 3 | 2 | 3 | | 3 | 2 |
| *Pardosa amentata (*Clerck, 1757) | *Pard ame* | | | | 3 | | | 2 | 12 | | 7 | 3 |
| *Pardosa lugubris* (Walckenaer, 1802) | *Pard lug* | 15 | 1 | 8 | 13 | 2 | | 78 | 9 | | 3 | 3 |
| *Pardosa paludicola* (Clerck, 1757) | *Pard pal* | | | | | | | 6 | | | 7 | 3 |
| *Pardosa palustris* (Linnaeus, 1758) | *Pard pal* | | | 4 | 1 | | | | 7 | | 4 | 3 |
| *Pardosa pullata* (Clerck, 1757) | *Pard pul* | | 6 | 35 | 5 | 4 | | 2 | 25 | | 3 | 3 |
| *Pardosa riparia* (C.L.Koch, 1833) | *Pard rip* | | 9 | 140 | 15 | 3 | 1 | 12 | 26 | | 4 | 2 |
| *Piratula hygrophila* (Thorell, 1872) | *Pira hyg* | | 182 | 1 | 47 | | 18 | 13 | 66 | | 8 | 2 |
| *Piratula latitans* (Blackwall, 1841) | *Pira lat* | | | 1 | | 5 | 2 | 5 | 1 | | 8 | 2 |
| *Trochosa ruricola* (De Geer, 1778) | *Troc rur* | | | 1 | | | 1 | 1 | 2 | | 6 | 3 |
| *Trochosa terricola* Thorell, 1856 | *Troc ter* | 46 | 27 | 117 | 76 | 27 | 18 | 52 | 18 | | 4 | 3 |
| Pisauridae | | | | | | | | | | | | |
| *Pisaura mirabilis* (Clerck, 1757) | *Pisa mir* | | 2 | | | | | | | | 3 | 3 |
| Miturgidae | | | | | | | | | | | | |
| *Zora nemoralis* (Blackwall, 1861) | *Zora nem* | 3 | | | | | | | | | 3 | 2 |
| *Zora spinimana* (Sundevall, 1833) | *Zora spi* | 15 | 8 | | 1 | 3 | 1 | 2 | 3 | | 4 | 3 |
| Agelenidae | | | | | | | | | | | | |
| *Agelena labyrinthica* (Clerck, 1757) | *Agel lab* | 1 | | | | | | | | | 3 | 2 |
| *Coelotes atropos* (Walckenaer, 1830) | *Coel atr* | | | | | 1 | | | | | 5 | 1 |
| *Coelotes terrestris* (Wider, 1834) | *Coel ter* | 7 | | | | | 1 | | | | 4 | 2 |
| *Inermocoelotes inermis* (L.Koch, 1855) | *Iner ine* | 37 | 1 | 1 | | | | | | | 5 | 2 |
| *Tegenaria campestris* (C.L.Koch, 1834) | *Tege cam* | 1 | | | | | | | | | 4 | 2 |
| Cybaeidae | | | | | | | | | | | | |
| *Cybaeus angustiarum* L.Koch, 1868 | *Cyba ang* | | | | | | | | 4 | | 6 | 2(1) |
| Hahniidae | | | | | | | | | | | | |
| *Antistea elegans (Blackwall, 1841)* | *Anti ele* | | 14 | | | | 4 | | | | 7 | 2(1) |
| Dictynidae | | | | | | | | | | | | |
| *Cicurina cicur* (Fabricius, 1793) | *Cicu cic* | | | 2 | 1 | 1 | | 1 | | | 5 | 3 |
| Titanoecidae | | | | | | | | | | | | |
| *Titanoeca quadriguttata* (Hahn, 1833) | *Tita qua* | 1 | | | | | | | | | 2(1) | 2(1) |
| Liocranidae | | | | | | | | | | | | |
| *Agroeca brunnea* (Blackwall, 1833) | *Agro bru* | | | | | 1 | 5 | | | | 4 | 2 |
| Phrurolithidae | | | | | | | | | | | | |
| *Phrurolithus festivus* (C.L.Koch, 1835) | *Phru fes* | 3 | | 1 | 2 | 3 | | 6 | | | 3 | 2 |
| Clubionidae | | | | | | | | | | | | |
| *Clubiona reclusa* O.P.-Cambridge, 1863 | *Club rec* | | | | | | 1 | | | | 6 | 2 |
| *Clubiona subtilis* L.Koch, 1867 | *Club sub* | | | | | | | 1 | | | 7 | 2 |
| Zodariidae | | | | | | | | | | | | |

Table 1. Abundance of ground living spiders (Araneae) sampled – continued

| Species | Abbr | for | osi | plg | pet | gra | ree | ovg | fim | RL | Hum | O-h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Zodarion germanicum* (C.L.Koch, 1837) | *Zoda ger* | 15 | | 2 | | | | | | | 2 | 2(1) |
| Gnaphosidae | | | | | | | | | | | | |
| *Drassodes cupreus* (Blackwall, 1834) | *Dras cup* | | | | | 4 | | 1 | | | 2 | 2(1) |
| *Drassodes lapidosus* (Walckenaer, 1802) | *Dras lap* | 1 | | | | | | | | | 2 | 2 |
| *Drassodes pubescens* (Thorell, 1856) | *Dras pub* | 1 | 1 | | 1 | 3 | | | | | 2 | 2 |
| *Drassyllus lutetianus* (L.Koch, 1866) | *Dras lut* | | | | 1 | | | | | | 7 | 3 |
| *Drassyllus pusillus* (C.L.Koch, 1833) | *Dras pus* | | | 3 | | | | | 1 | | 3 | 3 |
| *Micaria formicaria* (Sundevall, 1831) | *Mica for* | | | 1 | | | | | | | 2 | 2 |
| *Micaria pulicaria* (Sundevall, 1831) | *Mica pul* | | | 3 | 1 | | | | | | 5 | 2 |
| *Scotophaeus scutulatus* (L.Koch, 1866) | *Scot scu* | | | 1 | | | | | | | 4 | 3 |
| *Zelotes apricorum* (L.Koch, 1876) | *Zelo apr* | 12 | | | | | | 1 | | | 4 | 2 |
| *Zelotes erebeus* (Thorell, 1871) | *Zelo ere* | 1 | | | | | | | | | 3 | 1 |
| *Zelotes exiguus* (Müller & Schenkel, 1895) | *Zelo exi* | | | | | 3 | | | | CR | 2 | 1 |
| *Zelotes latreillei* (Simon, 1878) | *Zelo lat* | | | 2 | 2 | 2 | 3 | 1 | 1 | | 4 | 3 |
| *Zelotes petrensis* (C.L.Koch, 1839) | *Zelo pet* | 1 | | 1 | | 4 | | | | | 3 | 2 |
| Philodromidae | | | | | | | | | | | | |
| *Philodromus collinus* C.L.Koch, 1835 | *Phil col* | | | 1 | | | | | | | 5 | 2 |
| Thomisidae | | | | | | | | | | | | |
| *Coriarachne depressa* (C.L.Koch, 1837) | *Cori dep* | 3 | | | | | | | | | 3 | 2 |
| *Ozyptila atomaria (*Panzer, 1801) | *Ozyp ato* | 1 | 1 | 6 | | 1 | | | | | 3 | 2 |
| *Ozyptila trux* (Blackwall, 1846) | *Ozyp tru* | | 16 | 4 | 2 | | | 2 | 8 | | 7 | 3 |
| *Xysticus bifasciatus* C.L.Koch, 1837 | *Xyst bif* | | | 3 | | | | | | | 4 | 3 |
| *Xysticus cristatus* (Clerck, 1757) | *Xyst cri* | | | 5 | | | | | 6 | | 4 | 3 |
| *Xysticus ulmi* (Hahn, 1832) | *Xyst ulm* | | | 1 | 1 | | | | | | 6 | 2 |
| Salticidae | | | | | | | | | | | | |
| *Euophrys frontalis* (Walckenaer, 1802) | *Euop fro* | 3 | 1 | 1 | | 6 | 1 | 3 | | | 3 | 2 |
| *Evarcha arcuata* (Clerck, 1757) | *Evar arc* | | 1 | 1 | 1 | | | 2 | | | 5 | 2 |
| *Evarcha falcata (Clerck, 1757)* | *Evar fal* | 5 | 1 | | 1 | | | | | | 3 | 2 |
| *Heliophanus flavipes* (Hahn, 1832) | *Heli fla* | | | | | | | 1 | | | 2 | 1 |
| *Myrmarachne formicaria* (De Geer, 1778) | *Myrm for* | | | | | | 2 | | | | 4 | 1 |
| *Neon reticulatus (*Blackwall, 1853) | *Neon ret* | 1 | | | 2 | 1 | 1 | 5 | 1 | | 5 | 2 |
| *Synageles venator* (Lucas, 1836) | *Syna ven* | | | | | | 1 | | | | 4 | 3 |
| *Talavera aperta* Miller, 1971 | *Tala ape* | | | 1 | | | | | | DD | 2 | 2(1) |

forest (for); osier (osi); playground (plg); peat (pet); gravel bars (gra); reed (ree); overgrow (ovg); field-meadow (fim); Red list (RL); demand on humidity (Hum); association with the originality of habitats (O–h).

wet places from lowlands to mountains and are also present at other study sites.

The higher quantity refers to the playground sampling site and the community contained three eudominant species *Pardosa riparia* (28.99%), *Trochosa terricola* (24.22%) and *Alopecosa pulverulenta* (19.3%). These species are mesophilous, inhabiting unshaded non-forest habitats, mainly meadows.

The peat sampling site with high value of equitability had two eudominant species *Trochosa terricola* (33.63%), *Piratula hygrophila* (20.8%). *Trochosa terricola* is eurytopic species concerning humidity and light demands, inhabiting edge of all forests and also open habitats from lowlands to uplands.

The gravel bars sampling site with low number of species included two eudominant species *Aulonia albimana* (24.1%), *Trochosa terricola* (23.3%). These species prefer open or partly shaded habitats, often on rock steppes.

The reed sampling site was characterized by eudominant species *Piratula hygrophila* (19.57%), *Trochosa terricola* (19.57%) and *Centromerus sylvaticus*

(15.22%). *Piratula hygrophila* is very abundant in shaded wetlands. Other two species occur in a wide range of open and forest habitats.

At the overgrow sampling site were dominant *Pardosa lugubris* (29.77%) and *Trochosa terricola* (19.85%). *Pardosa lugubris* appears along woodland edges and sunny places within woods.

Finally at the field-meadow sampling site dominant species in spider community includes *Piratula hygrophila* (29.33%), *Pardosa riparia* (11.56%), and *Pardosa pullata* (11.11%). *Pardosa pullata* and *P. riparia* prefer open or partly shaded habitats, wet and dry meadows, heathland, edges of forests, forest clearings, orchards.

The results showed that the number of species registered in the studied sites was the lowest on the gravel bars (26) and reed (24) which is similar to carabid communities (Igondová and Majzlan, 2015). The highest number of individuals (483) refers to the playground sampling site, however the equitability reaches very law value (0.59) which is similar for carabid communities evaluation (Igondová and Majzlan, 2015). Similarly as for carabids, the lowest numbers of individuals were registered on gravel bars (116) and reed (92) sampling sites. The forest, gravel bars and reed sampling sites show a highest value of equitability in ground living spider communities (0.80) suggesting higher level of ecological stability than in other sites. The highest diversity index for spiders was on forest sampling area (2.87) where carabid communities had one of the lowest values (2.06). The lowest values of diversity and equitability were at osier sampling site (Table 2).

Table 2. Diversity and equitability

| | for | osi | plg | pet | gra | ree | ovg | fim |
|---|---|---|---|---|---|---|---|---|
| Number of species | 36 | 33 | 46 | 38 | 26 | 24 | 33 | 30 |
| Number of individuals | 234 | 336 | 483 | 226 | 116 | 92 | 262 | 225 |
| Number of threatened species / potentionally threatened species | 1/1 | 0/2 | 0/2 | 1/2 | 1/1 | 0/2 | 0/1 | 0/2 |
| Diversity (Dsw) spiders | 2.86 | 1.98 | 2.24 | 2.43 | 2.59 | 2.56 | 2.46 | 2.58 |
| Max value of Diversity spiders (Hmax) | 3.58 | 3.50 | 3.83 | 3.63 | 3.26 | 3.18 | 3.50 | 3.40 |
| Diversity (Dsw) carabids | 2.062 | 2.399 | 2.628 | 2.694 | 2.201 | 1.903 | 2.737 | 2.705 |
| Equitability (Esw) spiders | 0.80 | 0.57 | 0.59 | 0.67 | 0.80 | 0.80 | 0.70 | 0.76 |
| Equitability (Esw) carabids | 0.7613 | 0.7879 | 0.7279 | 0.8 | 0.9557 | 0.8265 | 0.8304 | 0.8118 |

forest (for); osier (osi); playground (plg); peat (pet); gravel bars (gra); reed (ree); overgrow (ovg); field-meadow (fim).

**Principal component analysis (PCA)**

The PCA ordination diagram of the ground living spider communities at 8 sampling sites is shown in Figure 3. Eigenvalues of the two first axes are $\lambda1 = 0.35$ and $\lambda2 = 0.20$. The first canonical axes account for 35% of the total variance of the species data. The axis x correlates with light conditions and the axis y is correlated with humidity. The species on PCA plot form five groups (Fig. 3).

The first group contains species *Alopecosa cuneata, Alopecosa pulvurenta, Ozyptila atomaria, Pardosa pullata*, *Pardosa riparia, Drassyllus pusillus* associated with semi dry or meso-termophilous and xerofil open habitats (the upper right quadrat and upper left quadrat of the ordination diagram). Species associated with dry to mesophilous open habitat had relation with playground sampling site.

The second group contains species *Ceratinella brevis*, *Inermocoelotes inermis, Mansuphantes mansuetus, Tapinocyba insecta*, *Tenuiphantes cristatus, Tenuiphantes flavipes, Zodarion germanicum* associated with semi dry woodland habitats, often under the stones, in moss (and upper left quadrat of the ordination diagram). Species associated with semidry forest habitats had relation with forest sampling site.

The third group contains species *Bathyphantes gracilis, Bathyphantes nigrinus, Cybaeus angustiarum, Diplostyla concolor*, *Ero furcata, Ozyptila trux*, *Pachygnatha listeri, Pardosa amentata* associated with mesophilous unshaded habitats (the lower left quadrat of the ordination diagram near axis x). Species associated with above mentioned habitats had relation with, overgrow, field-meadow and peat sampling sites.

The fourth group contains species *Aulonia albimana, Coelotes atropos, Drassodes cupreus, Drassodes pubescens, Euophrys frontalis, Zelotes exiguus* associated with mesophilous or drier partly shaded habitats without herbal vegetation cover (the upper right quadrat of the ordination diagram near axis x). The mentioned species had a relation with gravel bars sampling site.

The fifth group contains species *Antistea elegans, Centromerus sylvaticus, Pocadicnemis juncea, Piratula hygrophila* associated with more wet types of habitats,

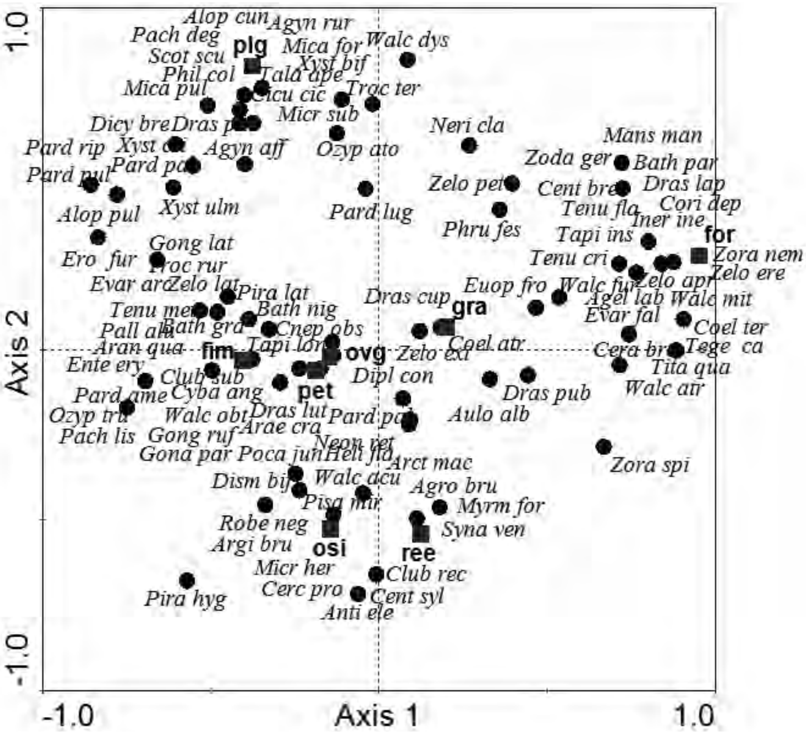


Fig. 3. Ordination diagram based on Principal Component Analysis (PCA) of ground living spiders and sampling sites, produced using CANOCO. The species are represented by blue circles with codes and study site by red squares also with codes. For codes of species see the Table 1. Codes of study sites: playground (plg); forest (for); field-meadow (fim); osier (osi); peat (pet); overgrow (ovg); gravel bars (gra); reed (ree); field-meadow (fim).

open or slightly shaded landscape (the lower left and right quadrats of the ordination diagram near axis y). Species associated with hygrophilous habitats had a relation with osier and reed sampling sites.

### Ecosozological assessment

Eight documented species have IUCN threat status in the Slovak Red list (Gajdoš and Svatoň, 2001). Gnaphosid spider *Zelotes exiguus*, collected in forest habitat, belongs to critically endangered species (CR). This spider lives under stones on sun-exposed rocky slopes. Hygrophilous linyphiid spider *Araeoncus crassiceps*, collected in peatland, has status endangered (EN (Table 1).

According to originality of habitats the majority of collected spiders belongs to species which are characteristic for original (climax) and/or semi-natural habitats. Only five documented species are strictly associated with original undisturbed habitats. Species associated with high disturbed habitats were presented by less number of species (27 species) but they were abundant in several studied habitats (e.g. ovg – 70.2%, plg – 61.7%, pet – 54.9%, fim – 47.6%, ree – 44.6%). Our results correlate with anthropogenic degradation classification provided by Igondová and Majzlan (2015) on carabid beetles at the same sampling sites. They also found strong effect of level of anthropogenic degradation on community of ground dwelling beetles.


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### References


Badík, M., 1994. Vážky (Odonata) CHN Šujské rašelinisko [Dragonflies (Odonata) of Šujské rašelinisko peatbog]. *Vlastivedný zborník Považia*, 17: 99–103.

Bitušík, P., 1998. K poznaniu pakomárov (Diptera: Chironomidae) PR Šujské rašelinisko [To the knowledge of the midges (Diptera: Chironomidae) of Šuja peatbog protected site]. *Ochrana Prírody*, 16: 131–136.

Bragg, O., Lindsay, R. (eds), 2003. *Strategy and action plan for mire and peatland conservation in Central Europe.* Wageningen: Wetlands International. vi + 94 p.

Buchar, J., 1983a. Die Klassifikation der Spinnenarten Böhmens als ein Hilfsmittel für die Bioindikation der Umwelt. *Fauna Bohemiae Septentrionalis*, 8: 119–135.

BUCHAR, J., 1983b. Bioindikační využití pavouků [The use of spiders for bioindication]. *Nika,* 4 (3): 11–14.

BUCHAR, J., 1991. The use of faunistical data for biomonitoring. *Bulletin de la Société Neuchâteloise des Sciences Naturelles*, 116: 49–57.

BUCHAR, J., RUŽIČKA, V., 2002. *Catalogue of spiders of the Czech Republic.* Praha: Peres publishers. 349 p.

BUCHHOLZ, S., 2016. Natural peat bog remnants promote distinct spider assemblages and habitat specific traits. *Ecological Indicators*, 60: 774–780.

GAJDOŠ, P., SVATOŇ, J., 2001. Červený (ekosozologický) zoznam pavúkov (Araneae) Slovenska [Red (ecosozological) List of spiders (Araneae) of Slovakia]. In BALÁŽ, D, MARHOLD, K., URBAN, P. (eds). *Červený zoznam rastlín a živočíchov Slovenska*. Ochrana prírody, 20, Suppl., p. 80–86.

IGONDOVÁ, E., MAJZLAN, O., 2015. Assemblages of ground beetles (Carabidae, Coleoptera) in peatland habitat, surrounding dry pine forest and meadows. *Folia Oecologica*, 42: 21–28.

ITV RAJEC, 2010. *Šujské rašelinisko – čierna stavba* [Šuja peatbog – unwarranted project]. YouTube, 6. 6. 2010 [cit. 2015-11-03]. https://www.youtube.com/watch?v=xnmn--xspQk

MAELFAIT, J. P., 1996. Spiders as bioindicators. In VAN STRAALEN, N. M, KRIVOLUTSKY, D. M. (eds). *Bioindicator systems for soil pollution*. Dordrecht: Kluwer Academic Publishers, p. 165–178.

MAELFAIT, J. P., BAERT, L., BONTE, D., BAKKER, D., GURDEBEKE, S., HENDRICKX, F., 2002. The use of spiders as indicators of habitat quality and antropogenic disturbance in Flanders, Belgium. In SAMU, F., SZINETÁR, Cs. (eds). *European arachnology 2002*. Budapest: Plant Protection Institute, p. 129–141.

MAELFAIT, J. P., JOCOQUE, R., BAERT, L., DESENDER, K., 1990. Heathland management and spiders. *Acta Zoologica Fennica*, 190: 261–266.

MAJZLAN, O., IGONDOVÁ, E., 2013. Epigeické chrobáky (Coleoptera) ako bioindikátor stability rezervácie PR Šujské rašelinisko [Epigeic beetles (Coleoptera) as bioindicator stability of the reserve PR Šujské rašelinisko]. *Naturae Tutela*, 18 (1): 53–68.

MAJZLAN, O., RYCHLÍK, I., KUBIČKOVÁ, P., 2004. Chrobáky (Coleoptera) Šujského rašeliniska chráneného územia Rajeckej doliny [Beetle (Coleoptera) of the Šuja peatbog protected site in Rajecká valley]. *Naturae Tutela,* 8: 7–24.

PIELOU, E.C., 1966. The measurement of diversity in different types of biological collections. *Journal of Theoretical Ecology,* 13: 131–144.

RŮŽIČKA, V., 1986. The structure of spider communities based upon the ecological strategy as the bioindicator of landscape deterioration. In PAUKERT, J., RŮŽIČKA, V., BOHÁČ, J. (eds). *Proceedings of the IVth international conference Bioindicatores Deteriorisationis Regionis, Liblice, Prague, Czechoslovakia.* České Budějovice, p. 219 – 237.

RŮŽIČKA, V., 1987. Biodiagnostic evaluation of epigeic spider communities. *Ekológia (ČSSR)*, 6: 345–357.

RŮŽIČKA, V., BOHÁČ, J., 1994. The utilization of epigeic invertebrate communities as bioindicators of terrestial environmental quality. In SALANKI, J., JEFFREY, D.W., HUGHES, G.M. (eds). *Biological monitoring of the environment*. Wallingford: CAB International in association with International Union of Biological Sciences, p.79–86.

SCOTT, A.G., OXFORD, G.S., SELDEN, P.A., 2006. Epigeic spiders as ecological indicators of conservation value for peat. *Biological Conservation*, 127: 420–428.

SHANNON, C.E., WEAVER, W., 1949. *The mathematical theory of communication.* Urbana: University of Illinois Press. 117 p.

SPELLERBERG, I.F., FEDOR, P.J., 2003. A tribute to Claude Shannon (1916–2001) and plea for more rigorous use of terms such as species richness, species diversity and the 'Shannon-Wiener' Index. *Global Ecology and Biogeography*, 12 (3): 177–179.

STANOVÁ, V., 2000. *Rašeliniská Slovenska* [Peatbogs of Slovakia]. Bratislava: Daphne – Inštitút aplikovanej ekológie. 194 p.

ŠÁCHA, D., RACKO, L., 2014. Výsledky faunistického výskumu vážok Šujského rašeliniska v roku 2013 (Insecta: Odonata) [Results of faunistic research of dragonflies of the Šujské rašelinisko peatbog in year 2013 (Insecta: Odonata)]. *Folia Faunistica Slovaca*, 19: 27–31.

TERBRAAK, C.J.F., ŠMILAUER, P., 1998. *CANOCO Reference manual and user´s guide to Canoco for Windows: software for canonical community* (version 4). Ithaca, NY, USA: Microcomputer Power. 352 p.

WORLD SPIDER CATALOG, 2015. *World Spider Catalog. Natural History Museum Bern* [cit. 2015-12-15]. http://wsc.nmbe.ch, version 16.5.



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# Wood ash effect on chemical and microbiological properties of topsoil in a Norway spruce stand one year after the treatment

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**Abstract**

Gömöryová, E., Tóthová, S., Pichler, V., Homolák, M., Kriššák, V., Gömöry, D., 2016. Wood ash effect on chemical and microbiological properties of topsoil in a Norway spruce stand one year after the treatment. *Folia Oecologica*, 43: 156–163.

Changes of soil chemical and microbial characteristics in the forest floor and in the A-horizon caused by the application of wood ash (WA) on the soil surface were studied one year after the WA application. Soil sampling was performed at three plots in a 40-year-old Norway spruce stand in Central Slovakia – at the control plot (CP) and at the plots with WA application in the spring (P1) and the autumn (P2) 2010. Soil samples were taken from the litter (L), fermentation (F), humic (H) forest floor horizons and from the A-horizon (the depth of 0−10 cm). In soil samples chemical attributes (pH, C and N concentration, extractable C and base cations concentration) as well as microbial characteristics (microbial biomass C, basal respiration, catalase activity, richness and diversity of soil microbial functional groups) were analysed. WA application was reflected in the changes of chemical properties (soil reaction, C, N and $Ca^{2}$+ concentration) only in the forest floor horizons but not in the A-horizon one year after the treatment. No significant differences between plots were found in microbial characteristics throughout the topsoil horizons. The most distinct differences in decreased C and N content compared to control plot were observed in the H-horizon. Soil acidity differed especially in the F-horizon, where the increase of pH-$H_2O$ from 4.76 to 6.85 at P1 was recorded.

**Key words**

forest floor, forest soils, soil properties, wood ash

## Introduction

In the last two decades, increasing number of studies on the effect of wood ash application on soil has appeared (Bååth et al., 1995; Demeyer et al., 2001; Liiri et al., 2002; Zimmermann and Frey, 2002; Jokinen et al., 2006; Lupwayi et al., 2009; Perruci et al., 2008; Saarsalmi et al., 2012). Wood ash (WA) is used as a liming agent in agriculture and forestry and its application on the soil has become a convenient way to recycle nutrient elements (Demeyer et al., 2001; Pitman, 2006).

The impact of WA application on soil can vary depending on the properties of WA (plant type used for burning, nature of the burn process, form of the ash), the conditions at the application site as well as other factors (Demeyer et al., 2001; Pitman, 2006). It is generally known that WA exhibits a high alkalinity and neutralizing capacity (Demeyer et al., 2001; Yusiharni and Gilkes, 2012) as a result of a large proportion of bases, especially Ca, Mg and K (Ozolinčius et al., 2005). Therefore, WA is recommended to be spread in forests to decrease soil acidification and nutrient deficiencies, and thus help to sustain long-term forest productivity in areas where acid deposition is high or whole-tree harvest has been practised (Meiwes, 1995; Jacobson, 2003). On the other side, WA may contain high concen-

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trations of heavy metals due to human activities, which can negatively affect plants and soil organisms. Especially fly ash as the lightest component can contain high concentrations of Cd, Cu, Cr, Pb and As, and therefore should not be used as a fertilizer (Pitman, 2006).

Addition of WA to soil can affect many soil properties, e.g. alkali metals content, soil salinity, soil structure, field capacity and soil aeration (Rumpf et al., 2001; Perruci et al., 2008). Especially the effect of WA on soil chemical properties is well documented (Meiwes, 1995; Saarsalmi, 2001; Ozolinčius, 2006). However, soil properties also directly and/or indirectly affect soil organisms including microorganisms and thus changes in the biomass, activity and composition of the microbial community are expected after WA application. The reports on the effects of WA on soil organisms are sometimes conflicting; WA may cause both increased and decreased microbial biomass, activity, community structure etc. (Bååth et al., 1995; Demeyer et al., 2001; Zimmermann and Frey; 2002, Bjørk et al.; 2010).

Studies about the impact of WA on soil mostly focus on the forest floor (surface organic horizon, the O-horizon) and the mineral top horizon (the A-horizon). Forest floor (FF) is an important component of forest ecosystems as it is a source of large amounts of nutrients and water for plants and soil organisms. It provides also habitat for many soil organisms and plant roots as well as a substrate for seedling establishment in forest ecosystems (Greiffenhagen et al., 2006; Šnajdr et al., 2008; Menšík et al., 2009). FF distinctly influences not only the rate of natural forest regeneration, but also the herb layer and adult trees, as their fine roots occur abundantly in this surface organic layer (Vance and Nadkarni, 1990).

The studies usually focus on the FF as a whole. However, FF is sometimes very thick especially in coniferous stands, and can be formed of several layers, which differ not only in morphology but also in their physical and chemical properties, water and temperature regimes etc. (Blume et al., 2010; Titeux and Delvaux, 2010; Gömöryová et al., 2013). This results in different ecological conditions for soil microorganisms and plant roots. In spite of this, studies dealing with the responses of soil properties, including microbiological attributes on WA addition in different FF layers are scarce. The aim of this study was thus to evaluate the influence of WA application on the changes in chemical and biological soil properties in particular layers of forest floor and the underlying mineral A-horizon in a field experiment. Such research is expected to improve our understanding of the processes undergoing in particular horizons after the treatments and the potential availability of nutrients for living organisms.

## Material and methods

Study of the FF horizon changes after the WA application was performed in a 40-year-old Norway spruce (*Picea abies* (L.) Karst.) stand located in the central part of Slovakia (48º 35,006`N, 19º 36,283`E) at the altitude of 825 m asl. The mean temperature is 5 °C and annual precipitation 800 mm. The dominant soil type is Dystric Cambisol with a loam texture. In the forest floor, litter (L), fermentation (F) and humus (H) horizons with average thickness of 1.0, 1.5 and 1.5 cm, respectively, could be recognized.

Three plots with an area of 400 $m^2$ each situated on a very gently 5° slope oriented to S were established by the researchers from the National Forest Centre in Zvolen with the primary aim to monitor the impact of wood ash on the growth of spruce stand. The design of plots resulted from this primary objective:

- CP – control plot, without WA amendment
- P1 – WA (5 t $ha^{-1}$) was applied on the soil surface in the spring 2010
- P2 – WA (5 t $ha^{-1}$) was applied on the soil surface 6 months later in the autumn 2010.

Wood ash comes from the heating station in Hriňová (5 km from the study plot) which used chemically untreated wood (both deciduous and coniferous). WA not pelleted was carefully spread handly on the FF surface at P1 and P2. WA was not mixed with FF and plot was not irrigated to ensure conditions similar to those in usual forest management practice. All three plots were located next to each other to minimize the effects of soil spatial variability. As the slope is very gentle there was no risk of spreading ash onto the control plot.

At each plot soil sampling was done at three sampling points representing the vertex of an equilateral triangle with the distance of 15 m from the centre of triangle to each vertex. At each sampling point soil samples were taken from the distinct FF horizons (L, F, H) using a 0.25 × 0.25 m frame and the A-horizon from the depth of 0–10 cm using knife and scoop in the autumn 2011. Samples from distinct plot were not mixed together but each sample was analysed separately.

In the laboratory, each soil sample was divided into two parts. One part was stored in field-moist condition at 4 °C prior to microbial analyses. The other part used for chemical analyses was air-dried.

In air-dried samples, soil acidity, total carbon (C), total nitrogen (N), extractable carbon ($C_{ext}$) and base cations ($Ca^{2+}$, $Mg^{2+}$, $K^+$) concentration were determined. Soil reaction (pH-$H_2O$) was measured potentiometrically in water suspension (10 g samples from the L, F, H horizons and/or 20 g soil from the A-horizon plus 50 ml distilled water) after 24 h. VarioMacro CNS Analyser (CNS Version: Elementar, Germany) was used to determine soil C and N concentration. The extractable C was measured in 0.5 M $K_2SO_4$ extract and quantified by the oxidation with $K_2Cr_2O_7/H_2SO_4$ and subsequent titration with $Fe(NH_4)_2(SO_4)_2$. Exchangeable $Ca^{2+}$, $Mg^{2+}$ and $K^+$ were estimated in 0.15 M $NH_4Cl$ extract using atomic absorption spectrometry (GBC Avanta AAS).

In fresh soil samples, soil water content and sample dry weight were estimated gravimetrically by oven-drying soil at 105 °C for 24 h. Microbial biomass carbon ($C_{mic}$) was assessed according to Islam and Weil (1998) using the microwave-irradiation procedure. After irradiation by microwaves, soil samples were extracted with 0.5 M $K_2SO_4$. C content in the extract was quantified the same way as $C_{ext}$. The same procedure was done with a non-irradiated sample. The microbial biomass carbon was then determined as $C_{mic} = (C_{irradiated} - C_{non\text{-}irradiated})/K_{ME}$, whereby extraction efficiency factor $K_{ME} = 0.213$.

Basal soil respiration (Resp) was measured by estimating the amount of $CO_2$ evolved during incubation of soil sample in a closed jar for 24 h (Alef, 1991). $CO_2$ absorbed in a 0.05 M NaOH was determined by the titration with 0.05 M HCl using the phenolphthalein indicator after the precipitation of carbonates by $BaCl_2$. Catalase activity (Acat) was measured 10 min after 3% $H_2O_2$ was added to soil sample. The measurement is based on the volume of discharged oxygen based on the method of Khaziev (1976). The community-level metabolic profiles of soil microbial community were estimated using BIOLOG EcoPlates (Insam, 1997). Briefly, fresh soil was resuspended in 0.9 % NaCl and supernatant was diluted $1{:}10^3$–$10^4$ depending on the microbial biomass. 150 ml of extract were incubated in microtitration plates at 37 °C during 7 days. Absorbance at 590 nm was recorded using the Sunrise Microplate Reader (Tecan, Salzburg, Austria). The richness (Richn) of the soil microbial community was assessed as the number of substrates with a non-zero response. For the estimation of functional diversity of the microbial community, Hill's diversity index (Div) was calculated (Hill, 1973).

Statistical analyses were done using the statistical package SAS/STAT (SAS, 2010). The variability of the soil characteristics between treatments and horizons was evaluated using two-way analysis variance; both horizon and treatment were considered fixed-effect factors. Pairwise differences of the means between the treatments were tested by Duncan´s test.

## Results

One year after the treatment, the effect of WA application was reflected only in some soil properties (Table 1). Significant differences were found in soil reaction, C, N and Ca concentration. No changes were observed in microbial characteristics between the treatments. Almost all measured soil characteristics except for richness and diversity of microbial functional groups differed significantly between horizons. Treatment × horizon interactions were also significant in the case of soil reaction, N concentration and functional diversity.

Table 1. Analyses of variance of soil characteristics (significance of *F*-tests)

| Factor | d.f. | pH | C | N | C/N | $C_{ext}$ | Ca | Mg | K |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | 2 | *** | ** | *** | ns | ns | * | ns | ns |
| Horizon | 3 | *** | *** | *** | *** | *** | *** | *** | *** |
| Treatment *horizon | 6 | ** | ns | * | ns | ns | ns | ns | ns |
| Error | 24 | | | | | | | | |

Table 1. Analyses of variance of soil characteristics (significance of *F*-tests) – continued

| Factor | d.f. | $C_{mic}$ | Resp | Acat | Richn | Div |
|---|---|---|---|---|---|---|
| Treatment | 2 | ns | ns | ns | ns | ns |
| Horizon | 3 | *** | *** | *** | ns | ns |
| Treatment *horizon | 6 | ns | ns | ns | ns | * |
| Error | 24 | | | | | |

Significance levels: ***P < 0.001, **P 0.001–0.01, *P 0.01–0.05; ns, non-significant.

$C_{ext}$, extractable carbon; $C_{mic}$, microbial biomass carbon; Resp, basal respiration; Acat, catalase activity; Richn, richness of soil microbial functional groups; Div, diversity of soil microbial functional groups.

WA application on the soil surface led to the decrease of acidity at the P1 and P2 in comparison to the CP (Table 2); the treated plots exhibit higher pH by approximately one unit of pH. Decreased acidity is observed in all horizons; however, the most distinct differences are found in F-horizon where pH increased from 4.76 to 6.85 at P1 (Fig. 1a). While at the CP plot pH decreases with the depth of FF profile, the treated plots do not exhibit the same pattern. One year after the WA application the highest pH is seen in the F-horizon and the smallest in the A-horizon.

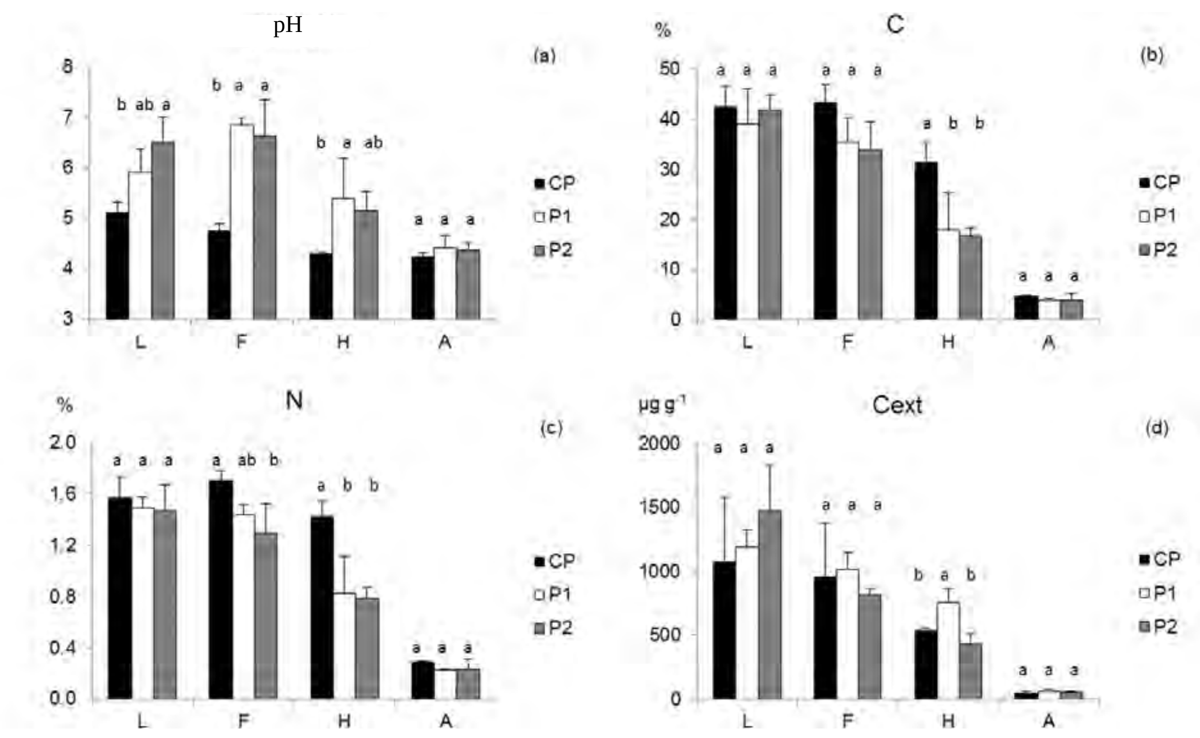
Table 2. Duncan's pairwise tests of the differences of means between treatments across horizons (treatments with the same capital letters do not differ significantly)

| Treatment | pH | C | N | C/N | $C_{ext}$ | Ca | Mg | K |
|---|---|---|---|---|---|---|---|---|
| | | % | % | | $\mu g\ g^{-1}$ | $mg\ kg^{-1}$ | $mg\ kg^{-1}$ | $mg\ kg^{-1}$ |
| CP | 4.60 B | 30.5 B | 1.24 A | 21.9 A | 650 A | 906 B | 132.7 A | 72.9 A |
| P1 | 5.64 A | 24.1 A | 0.99 B | 23.5 A | 755 A | 1,176 A | 124.9 A | 74.9 A |
| P2 | 5.67 A | 24.1 A | 0.94 C | 21.9 A | 693 A | 1,236 A | 149.9 A | 88.8 A |

Table 2. Duncan's pairwise tests of the differences of means between treatments across horizons (treatments with the same capital letters do not differ significantly) – continued

| Treatment | $C_{mic}$ | Resp | Acat | Richn | Div |
|---|---|---|---|---|---|
| | $mg\ g^{-1}$ | $\mu g\ CO_2\ g^{-1}h^{-1}$ | $ml\ O_2\ g^{-1}\ min^{-1}$ | | |
| CP | 12.66 A | 98.4 A | 5.16 A | 7.5 A | 4.4 A |
| P1 | 7.84 B | 82.0 A | 5.02 A | 6.4 A | 4.2 A |
| P2 | 10.76 AB | 86.5 A | 5.28 A | 7.1 A | 4.7 A |

$C_{ext}$, extractable carbon; $C_{mic}$, microbial biomass carbon; Resp, basal respiration; Acat, catalase activity; Richn, richness of soil microbial functional groups; Div, diversity of soil microbial functional groups.

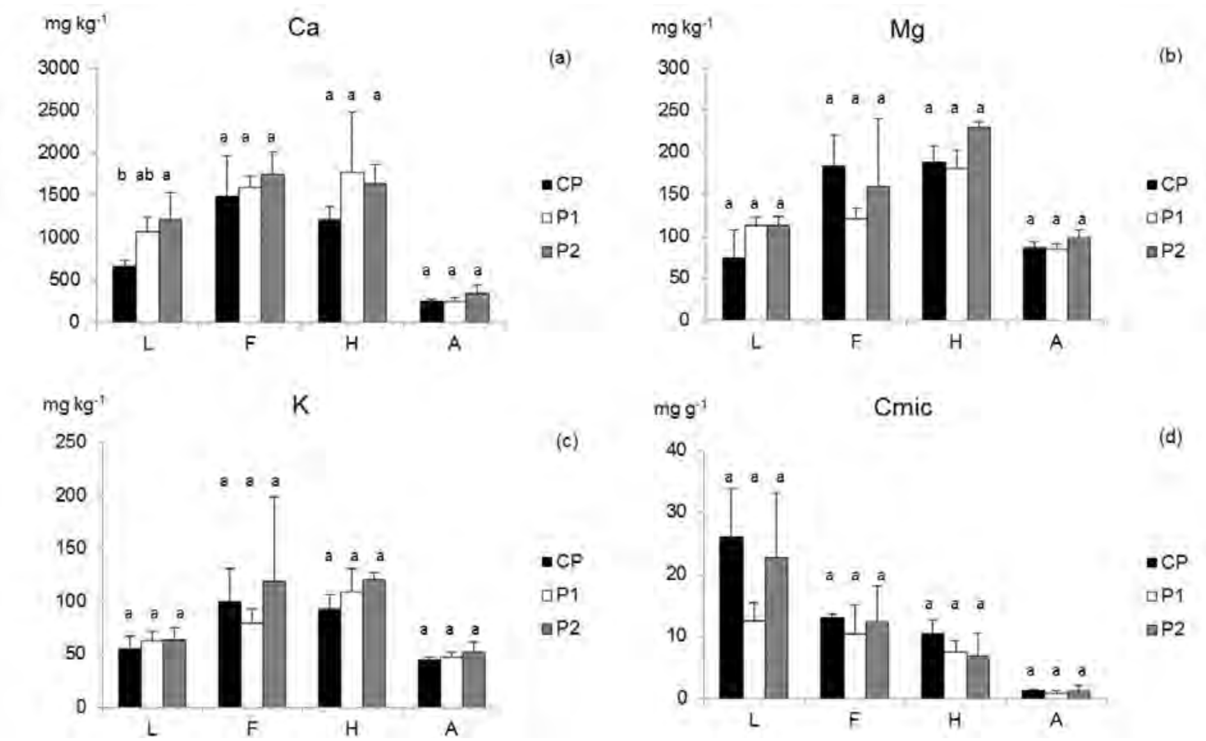



$C_{ext}$, extractable carbon.

Fig 1. Means and standard deviations of soil acidity (a), carbon (b), nitrogen (c) and extractable carbon concentration (d) sorted by treatments (CP, control plot; P1, plot with wood ash addition in spring 2010; P2, plot with wood ash addition in autumn 2010) in distinct soil horizons (L, F, H, A).

Application of WA caused a significant decrease in C and especially N concentration in the FF horizons of the P1 and P2 (Table 2). The differences in C and N concentration between CP on one side and treated plots on the other side increase down the horizons profile with maximal difference in the H-horizon where C and N concentration at P1 and P2 represents the half of that concentration at CP (Fig. 1b and c). The highest $C_{ext}$ concentration occurs in L-horizon and with a depth its amount decreases. The differences between treatments are non-significant due to high within-treatment variability (Table 2, Fig 1d).

Among the base cations only the Ca concentration showed a significant difference between the treatments with higher values at the plots with WA application; in the case of Mg and K no pattern could be identified (Table 2, Fig. 2a–c). The highest concentration of base cations was found in the F and H horizons at all plots.



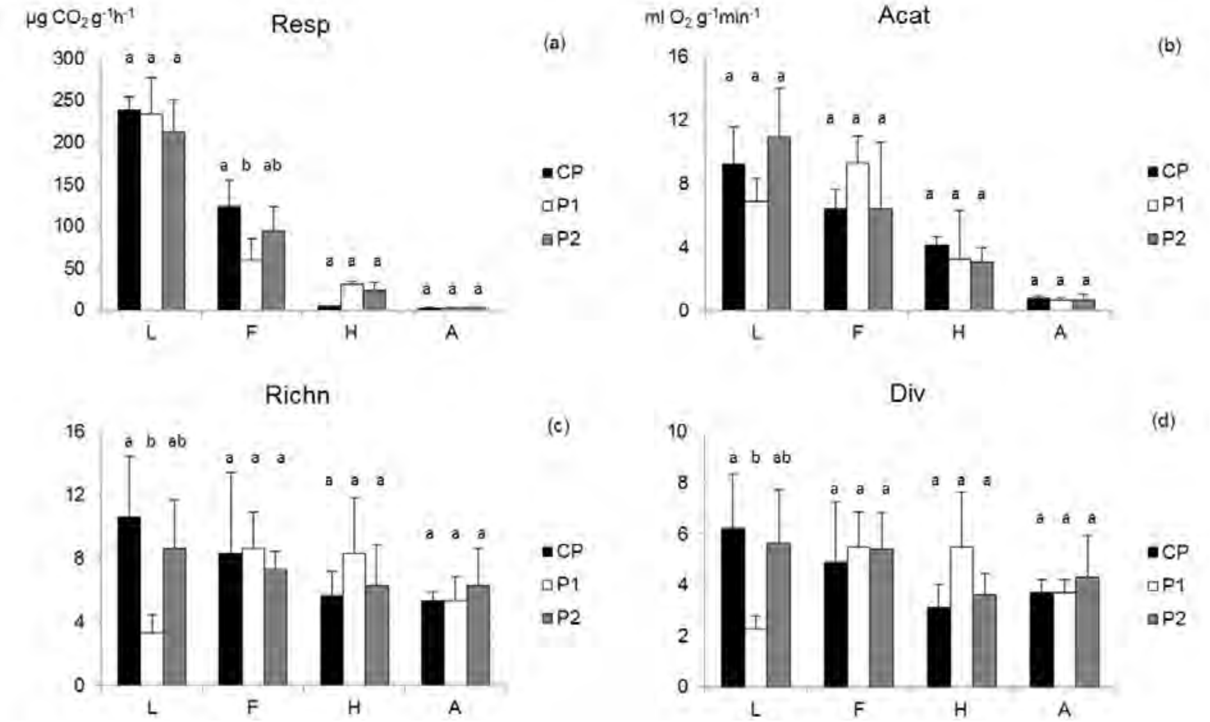

$C_{mic}$, microbial biomass carbon.

Fig 2. Means and standard deviations of calcium (a), magnesium (b) and potassium concentration (c) and microbial biomass carbon (d) sorted by treatments (CP, control plot; P1, plot with wood ash application in spring 2010; P2, plot with wood ash application in autumn 2010) in distinct soil horizons (L, F, H, A).

All microbial characteristics exhibited high within-treatment variability (Fig. 2d, 3a–d). While microbial biomass C, basal respiration and catalase activity decreased down the soil horizons profile, characteristics of microbial community structure did not differ significantly between horizons. No pattern in microbial characteristics between treatments throughout the FF and A- horizons was identified.

## Discussion

It is known that WA application leads to the decrease of soil acidity due to the input of large base cations amount contained in the WA. The extent of such changes varies distinctly depending, for instance, on the WA dose, time after the treatment or depth of the soil. Ozolinčius et al. (2006) found that pH increased from 3.45 to 6.15, which means 2.7 pH units 4 months after WA application in FF, Levula et al. (2000) observed a pH increase up to 2 pH units 5 months after WA application of 5 t ha$^{-1}$, Saarsalmi et al. (2001) found a decrease of soil acidity by 0.2–2.4 pH units in FF 1–19 years after the WA addition. In our study treated plots showed decrease in soil acidity against the CP. The most distinct difference by 2 pH units was observed in the F-horizon. As already mentioned, FF is not homogeneous and its thickness can vary distinctly. Changes in pH throughout the topsoil can thus be influenced by the properties of distinct FF horizons on one side and the water amount coming into the soil on the other side. The fact that water infiltrated and percolating through the FF profile gradually depletes in nutrients top layers and enriches the lower horizons is reflected also in pH values as illustrated in the Fig. 1a. While the differences in soil reaction between the L- and F-horizon are small one year after the treatment (CP2), half year later the L-horizon is already more acid than the underlying F-horizon (CP1). However, in the A-horizon no changes have been observed yet. Ozolinčius et al. (2006); Saarsalmi et al. (2001) showed that the WA effect on pH in the mineral soil was less pronounced in short-term and appeared much later than in forest floor. Distinct changes in mineral horizons are often observed several years after the treatment. They found that mineral soil layers (below 10 cm depth) showed very little change at




Resp, basal respiration; Acat, catalase activity; Richn, richness of soil microbial functional groups; Div, diversity of soil microbial functional groups.

Fig 3. Means and standard deviations of basal respiration (a), catalase activity (b), richness (c) and diversity (d) of soil microbial functional groups sorted by treatments (CP, control plot; P1, plot with wood ash application in spring 2010; P2, plot with wood ash application in autumn 2010) in distinct soil horizons (L, F, H, A).

7 years after ash application, but after 16 years, the increased pH values at depth indicated a slow downward transfer of activity from the topsoil over time. However, the thickness of the FF layer has a significant role as a thin O-horizon has lower capacity to hold cations applied in ash than a thick horizon.

WA is a direct source of the major elements such as P, Ca, Mg and K in soils. Among base cations only Ca concentration differed significantly between treatments at our plots, which was in agreement with Ozolinčius et al. (2006), who found that in the case of loose ash, leaching of base cations had mostly occurred during the first year after WA addition. It can be associated with the solubility of particular base cations. According to Khanna et al. (1994) the major elements can be divided into three groups based on the solubility: K (dissolves very quickly) – Ca and Mg – P (remains relatively insoluble); magnesium is more soluble than calcium (Eriksson, 1998). As K and Mg are more soluble than Ca they can be leached more rapidly from the topsoil (Ozolinčius et al., 2006).

Beside the decreased soil acidity and increased base cations content, WA application can be also reflected in the changes of decomposition rate and consequently C and N content (Demeyer et al., 2001; Maljanen et al., 2014). There are many contradictory results regarding organic matter content after the WA application. Saarsalmi et al. (2001) did not find any changes in the C and N content either in organic or mineral horizons 7 years after the treatment. On the other side, Demeyer et al. (2001) stated that WA could lead to a decrease of total C and N because of the increase in the solubility of organic C and nitrification. Contradictory results can be caused by different time of soil sampling after the treatment and also by different C and N content in soil before the WA addition. According to Rosenberg et al. (2010), WA application onto the soils rich on N can significantly influence C and N cycle while at N-poor sites the presence of WA was not reflected in the C and N content and processes associated with it. In our study, WA application onto the soil surface provoked a decrease in C and N content in distinct FF horizons. Increased differences against the control plot were found down the FF profile from L- to H-horizon; in the A-horizon the difference was again minimal. Increase of pH and the base cation content down the FF profile led to increased mineralisation of organic matter and thus C and N losses. According to Pitman (2006), WA application generally has little direct impact on N availability due to its low levels in the raw material (<0.1 per cent). However, N availability often increases indirectly as a result of ash application due to a rise in soil pH, base cation content and consequent N-mineralization.

We did not find any changes in microbial characteristics between plots. Similarly, Zimmermann and Frey (2002) found that while the effect of WA on chemical properties was long-term, in the case of microbial characteristics the effect was only short-term. On the other side, Rosenberg et al. (2010) observed increased $CO_2$ evolution still 12 years after the WA application. In a number of studies decrease as well as increase in microbial biomass, respiration, N-mineralisation, differences in the fungi:bacteria ratio were observed (Bååth at al., 1995, Demeyer at al., 2001; Aronsson and Ekelund, 2004; Jokinen et al., 2006; Ozolincius et al., 2006; Perruci et al., 2008). The observed results documented the complexity of microbial processes and their different responses to the changed environment.

## Conclusions

Wood ash application on the soil surface in a 40-year-old spruce stand led to a decrease in soil acidity and C and N concentration in particular forest floor horizons but no significant changes were found in the underlying mineral A-horizon still one year after the treatment. The extent of the changes in distinct horizons was not uniform. The most pronounced WA effect on pH was observed in the F-horizon; in the case of C and N concentration the most distinct differences were found in the H-horizon. Unlike chemical properties, no responses of microbial community to the WA addition were observed in the FF and A-horizons. Six-month temporal shift in the WA application did not lead to significant differences in soil characteristic between the treated plots. Changes in soil chemical properties throughout the forest floor profile are vertically differentiated, which needs to be considered when the processes in forest soils and habitat changes for soil organisms and plant roots occupying these particular layers are studied.

## Acknowledgement

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## References

Alef, K., 1991. *Methodenhandbuch Bodenmikrobiologie: Aktivitäten, Biomasse, Differenzierung.* Landesberg /Lech: Ecomed. 284 p.

Aronsson, K.A., Ekelund, N.G.A., 2004. Biological effects of wood ash application to forest and aquatic ecosystems. *Journal of Environmental Quality*, 33: 1959−1605.

Bååth, E., Frostegård, A., Pennanen, T., Fritze, H., 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. *Soil Biology and Biochemistry*, 27: 229−240.

Bjørk, R.G., Ernfors, M., Sikstrom, U., Nilsson, M.B., Andersson, M.X., Rutting, T., Klemedtsson, L., 2010. Contrasting effects of wood ash application on microbial community structure, biomass and processes in drained forested peatlands. *FEMS Microbial Ecology*, 73: 550−562.

Blume, H.P., Brummer, G.W., Horn, R., Kandeler, E., Kögel-Knabner, I., Kretzschmar, R., Stahr, K., Wilke, B.M., 2010. *Scheffer/Schachtschabel: Lehrbuch der Bodenkunde.* 16. Aufl. Heidelberg: Spektrum Akademischer Verlag. 569 p.

Demeyer, A., Voundi Nkana, J.C., Verloo, M.G., 2001. Characteristics of wood ash and influence on soil properties and nutrient uptake: an overview. *Bioresource Technology*, 77: 287−295.

Eriksson, J., 1998. Dissolution of hardened wood ashes in forest soils: studies in a column experiment. *Scandinavian Journal of Forest Research*, 2:23−32.

Gömöryová, E., Střelcová, K., Škvarenina, J., Gömöry, D., 2013. Responses of soil microorganisms and water content in forest floor horizons to environmental factors. *European Journal of Soil Biology,* 55: 71−76.

Greiffenhagen, A., Wessolek, G., Facklam, M., Renger, M., Stoffregen, H., 2006. Hydraulic functions and water repellency of forest floor horizons on sandy soils. *Geoderma*, 132: 182–195.

Hill, M.O., 1973. Diversity and evenness: A unifying notation and its consequences. *Ecology,* 54: 427−432.

Insam, H., 1997. A new set of substrates proposed for community characterization in environmental samples. In Insam, H., Rangger, A. (eds). *Microbial communities. Functional versus structural approaches*. Heidleberg: Springer, p. 260−261.

Jacobson, S., 2003. Addition of stabilized wood ashes to Swedish coniferous stands on mineral soils – effects on stem growth and needle nutrient concentrations. *Silva Fennica*, 37: 437–450.

Jokinen, H.K., Kiikkila, O., Fritze, H., 2006. Exploring the mechanisms behind elevated microbial activity after wood ash application. *Soil Biology and Biochemistry*, 38: 2285−2291.

Khanna, P.K., Raison, R.J., Falkiner, R.A., 1994. Chemical properties of ash derived from Eucalyptus litter and its effects on forest soils. *Forest Ecology and Management*, 66: 107−125.

Khaziev, F.Kh., 1976. *Fermentativnaja aktivnost' pochv* [Fermentative activity of the soils]. Moskva: Metodicheskoje posob'e. 180 p.

Levula, T., Saarsalmi, A., Rantavaara, A., 2000. Effects of ash fertilization and prescribed burning on macronutrient, heavy metal, sulphur and $^{137}Cs$ concentrations in lingonberries (Vaccinium vitis-idea). *Forest Ecology and Management*, 126: 269−277.

LIIRI, M., HAIMI, J., SETÄLÄ, H., 2002. Community composition of soil microarthropods of acid forest soils as affected by wood ash application. *Pedobiologia*, 46: 108−124.

LUPWAYI, N.Z., ARSHAD, M.A., AZOOZ, R.H., SOON, Y.K., 2009. Soil microbial response to wood ash or lime applied to annual crops and perennial grass in an acid soil of northwestern Alberta. *Canadian Journal of Soil Science*, 89: 169−177.

MALJANEN, M., LIIMATAINEN, M., HYTÖNEN, J., MARTIKAINEN, P.J., 2006. The effect of granulated wood-ash fertilization on soil properties and greenhouse gas (GHG) emissions in boreal peatland forests. *Boreal Environment Research*, 19: 295−309.

MEIWES, K.J, 1995. Application of lime and wood ash to decrease acidification of forest soils. W*ater, Air and Soil Polution*, 85: 143−152.

MENŠÍK, L., KULHAVÝ, J., KANTOR, P., REMEŠ, M., 2009. Humus conditions of stands with different proportion of Douglas fir in the Hůrky Training District and Křtiny training Forest enterprise. *Journal of Forest Science*, 55: 345−356.

OZOLINČIUS, R., VARNAGIRYTÉ, I., ARMOLAITIS, K., KARLTUN, E., 2005. Initial effects of wood ash fertilization on soil, needle and litterfall chemistry in a Scots pine (Pinus sylvestris L.) stand. *Baltic Forestry*, 11: 59–67.

PERRUCI, P., MONACI, E., ONOFRI, A., VISCHETTI, C., CASUCCI, C., 2008. Changes in physico-chemical and biochemical parameters of soil following addition of wood ash: a field experiment. *European Journal of Agronomy*, 28: 155−161.

PITMAN, R.M., 2006. Wood ash use in forestry – a review of the environmental impacts. *Forestry*, 79: 563−588.

ROSENBERG, O., PERSSON, T., HÖGBOM, L., JACOBSON S., 2010. Effects of wood-ash application on potential carbon and nitrogen mineralisation at two forest sites with different tree species, climate and N-status. *Forest Ecology and Management*, 260: 511−518.

RUMPF, S., LUDWIG, B., MINDRUP, M., 2001. Effect of wood ash on soil chemistry of a pine stand in Northern Germany. *Journal of Plant Nutrition and Soil Science*, 164: 569−575.

SAARSALMI, A., SMOLANDER, A., KUKKOLA, M., MOILANEN, M., SARAMÄKI, J., 2012. 30-year effects of wood ash and nitrogen fertilization on soil chemical properties, soil microbial processes and stand growth in a Scots pine stand. *Forest Ecology and Management*, 278: 63−70.

SAS, 2009. *SAS/STAT® User's Guide* [cit. 2016-01-15]. http://support.sas.com/documentation/onlinedoc/stat/index.html.

ŠNAJDR, J., VALÁŠKOVÁ, V., MARHAUTOVÁ, V., HERINKOVÁ, J., CAJTHAML, T., BALDRIÁN, P., 2008. Spatial variability of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. *Soil Biology and Biochemistry*, 40: 2068−2075.

TITEUX, H., DELVAUX, B., 2010. Properties of successive horizons in a thick forest floor (mor) reflect a sequence of soil acidification. *Geoderma*, 148: 291−298.

VANCE, E.D., NADKARNI, N.M., 1992. Root biomass distribution in a moist tropical montane forest. *Plant and Soil*, 142: 31–39.

YUSIHARNI, E., GILKES, R., 2012. Minerals in the ash of Australian native plants. *Geoderma*, 189: 369−380.

ZIMMERMANN, S., FREY, B., 2002. Soil respiration and microbial properties in a acid forest soil: effect of wood ash. *Soil Biology and Biochemistry*, 34: 1727−1737.

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# *Panellus ringens* and *P. violaceofulvus* (Agaricales, Mycenaceae) from Slovakia: morphological and ecological aspects

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**Abstract**

Jančovičová, A., Blanár, D., Glejdura, S., Kunca, V., 2016. *Panellus ringens* and *P. violaceofulvus* (Agaricales, Mycenaceae) from Slovakia: morphological and ecological aspects. *Folia Oecologica,* 43: 164–175.

Species *Panellus ringens* and *P. violaceofulvus* are similar in some respects and different in others. They look alike macroscopically, especially in having pleurotoid habit and violet-brown and/or red-brown colours of basidiomata, but differ microscopically, distinctly in size of spores. For each species, description and illustration of macroscopic and microscopic characters are provided based on the Slovak collections. Some misidentifications are mentioned and crucial morphological characters stressed. Compared and commented are also the characters used for differentiation of both species in the selected literature. The added distributional and ecological data concern the area of Slovakia and Europe. *Panellus ringens* is proposed to be included in a new version of the Red list of fungi of Slovakia.

**Key words**

Basidiomycota, Europe, fungi, identification, microscopy

## Introduction

*Panellus* P. Karst. is a genus within Mycenaceae (Agaricales, fungi) characterized by a pleurotoid habit of basidiomata, i.e. with lateral or absent stipe. Comparing with *Sarcomyxa* P. Karst. and *Tectella* Earle (the other genera within Mycenaceae with pleurotoid habit), pileus is thin-fleshed, up to 2 mm thick, veil absent, and basidiomata are coloured in shades of white, yellow-brown, violet-brown and/or red-brown (Vesterholt, 2012).

In Europe, four species of the genus *Panellus* occur: *P. mitis* (Pers.) Singer with whitish-coloured basidiomata, *P. stipticus* (Bull.) P. Karst. with yellow-brown basidiomata and *P. ringens* (Fr.) Romagn. and *P. violaceofulvus* (Batsch) Singer with violet-brown and/or red-brown basidiomata (Vesterholt, 2012). In Slovakia, *P. mitis* and *P. stipticus* seem to be common and well-known species, e.g. there are about 40 records of *P. mitis* and 280 for *P. stipticus* in Škubla (2003).

The knowledge on *P. ringens* and *P. violaceofulvus* is, however, scant. We knew only three published collections of *P. ringens* from the years 1972 and 2006 (Kautmanová, 2006) and 17 of *P. violaceofulvus* from the years 1914–2006 (Ripková et al., 2007) before our study.

What is the reason for such a low number of collections? Are these species rare or overlooked? Do they have special requirements for habitats and/or substrate? Are they clearly delimitated? Can they be simply recognized using prevalent literature for identification of fungal taxa?

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To answer these questions – in order to better understand the morphological features and ecological requirements of *P. ringens* and *P. violaceofulvus*, we aimed our work to: 1) gather recent information about the species, i.e. to search for more published records and to process new (mostly our own) collections, 2) describe and illustrate macro- and microscopic characters of *P. ringens* and *P. violaceofulvus* based on the Slovak collections, 3) stress crucial morphological characters for their differentiation, 4) compare the distinguishing characters with those used in the literature, 5) summarise the occurrence and ecological requirements of the species in Slovakia, 6) consider reasons for including the species into a new version of Red list of fungi of Slovakia, 7) add data on species occurrence, ecology and threat in Europe.

## Material and methods

The studied material includes specimens of *Panellus ringens*, *P. violaceofulvus* and *Phyllotopsis nidulas* collected in Slovakia. The specimens are kept in the institutional herbaria SAV, SLO and private herbaria PVKU (herbarium of V. Kunca) and PSG (herbarium of S. Glejdura). The herbarium acronyms follow Thiers (2016). The description of macroscopic characters is based on fresh material. Three selected dried specimens were used to describe microscopic characters of *Panellus ringens* (SLO 787, SLO 788, and SLO 790) and of *P. violaceofulvus* (SLO 789, SLO 1449, and SAV 10487). Microscopic mounts were prepared in Congo red after a short pre-treatment in 3% aqueous solution of KOH and observed under an Olympus BX41 and CX41 light microscopes with an oil-immersion lens at a magnification of 1,000 ×. Drawings of all microscopic structure, with exception of spores, were made with a camera lucida using an Olympus U-DA drawing attachment at a projection scale of 2,000 ×. Spores were drawn from scanned and enlarged pictures by an Artray Artcam 300 MI camera. Statistic calculations of all microscopic characters are based on 20–30 measurements per specimens and given as minimum, maximum (in parentheses), average ± standard deviation and average (av.) values. Abbreviations: Q = ratio of length and width of spores, L = number of lamellae reaching the point of attachment, l = number of lamellulae between each pair of lamellae. Descriptive terminology is adopted from Vellinga (1988). Nomenclature of fungal taxa follows Knudsen and Vesterholt (2012).

## Results and discussion

***Panellus ringens*** **(Fr.) Romagn., Bull. Trimest. Soc. Mycol. Fr. 61: 38 (1945)**

**Description** (Figs 1, 2)

Fig. 1. *Panellus ringens*: basidiomata (Stolické vrchy Mts, 21 September 2012, PVKU 747). Photo V. Kunca.

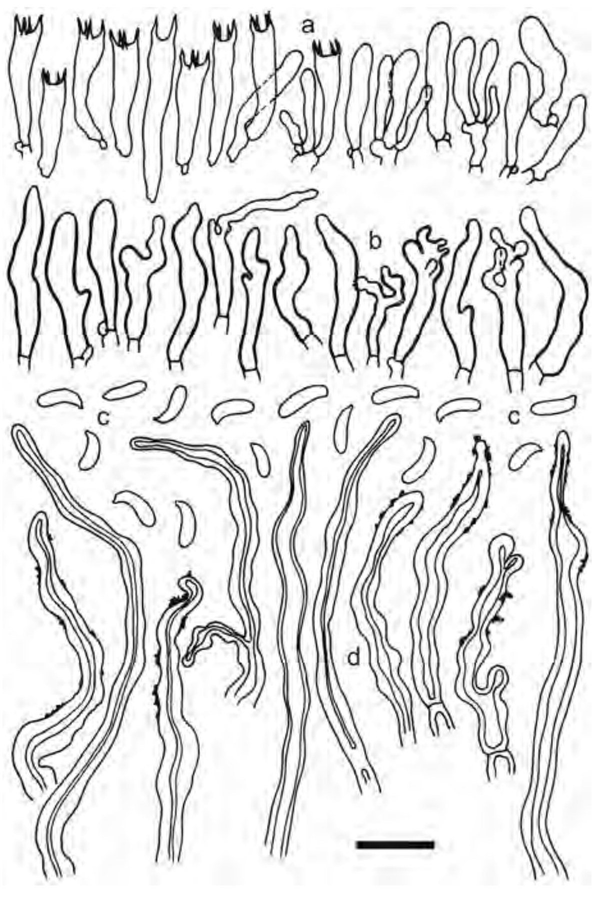


Fig. 2. *Panellus ringens*: a – basidia and basidioles, b – cheilocystidia, c – spores, d – terminal cells in the pileipellis (Stolické vrchy Mts, 25 March 2006, SLO 787). Scale bar = 10 µm for spores, 15 µm for other structure. Del. S. Jančovičová.

Basidiomata in groups, growing out from the substrate separately or in clusters of 2–8 (10) basidiomata. Stipe absent, basidiomata attached to the substrate dorsally or laterally (by stem-like base). Pileus (3) 5−10 (20) mm, irregularly circular, flabelliform, rounded flabelliform, spathuliform or reniform (from above), hemispherical convex to plano-convex (from aside), hygrophanous; margin involute, with age inflexed to straight (aspect), crenulated, sometimes lobed (shape), translucently striate (distinctly when wet); surface whitish granulose-fibrillose to tomentose (fibrils and hairs equally all over the surface or somewhat denser towards the point of attachment and margin; in some cases almost invisible when wet, but distinct when dry), background red brown to brown (usually darker towards the point of attachment), occasionally with violet shades when young. Trama up to 1 mm thick, off-white, taste indistinct or somewhat bitter, smell indistinct. Lamellae L = 11–17, l = 3, 5 or 7, with wide 0.5−1 mm, radiating from central to eccentric spot or from lateral stem-like base, ventricose, adnexed, light brown, brown, red brown, sometimes with pink hue, edge even, concolorous or darker.

Spores cylindrical, narrowly cylindrical, allantoid, (5) 5.9 – 7.3 (8) × (1.5) 1.7 − 2 µm, av. 6.6 × 1.8 µm, Q = (2.8) 3.2 – 4 (4.7), av. Q = 3.6, smooth, colourless and amyloid. Basidia 4-spored, rarely 2-spored, clavate, narrowly clavate, (15) 17–23.4 (33) × 4–5 (6.5) µm, av. 20.2 × 4.5 µm, colourless to yellowish, thin-walled. Basidioles clavate, narrowly clavate, narrowly cylindrical, (12) 14.9–22.7 (33) × (2.5) 3.1–4.6 (5.5) µm, av. 18.8 × 3.8 µm, colourless to yellowish, thin-walled. Cheilocystidia clavate, narrowly clavate, narrowly cylindrical, narrowly fusiform, narrowly utriform, flexuous, some with lateral outgrowth or with branching uppermost part, (16) 21.1–32.7 (42) × (2) 3.9–6.3 (7.5) µm, av. 26.9 × 5.1 µm, colourless to yellowish, thick-walled or thin-walled, in one mount embedded in yellowish mucus. Subhymenium 10–18 µm deep, of dense, intricate, irregularly oriented, 1.5–3.5 µm wide hyphae, well delimited from trama. Trama in lamellae composed of parallel to sub-parallel, thick-walled hyphae, 3.5–7 (10) µm wide, with wall thickness 1–3 µm, occasionally branched and anastomosed, colourless, in clamp-connections with circular hole up to 5 µm wide. Pileipellis an intricate trichoderm, the erect hyphae (arising from decumbent hyphae) thick-walled, 5–11 µm wide, with wall thickness 0.5–3 µm, flexuous to twisted, some nodulouse, tapering towards the end, often intricate, but not fascicled, branched, mostly incrusted. Trama in pileus similar to lamellar trama. Gelatinous matrix inconspicuous. In some mounts, yellowish crystals about 2–15 µm in diam. were observed in all tissues. Clamp connections present.

***Panellus violaceofulvus*** (Batsch) Singer, Beih. Botan. Centralbl., Abt. B 56: 142 (1936)

**Description** (Figs 3, 4)

Fig. 3. *Panellus violaceofulvus*: basidiomata (Kremnické vrchy Mts, 16 February 2013, PVKU 884). Photo V. Kunca.

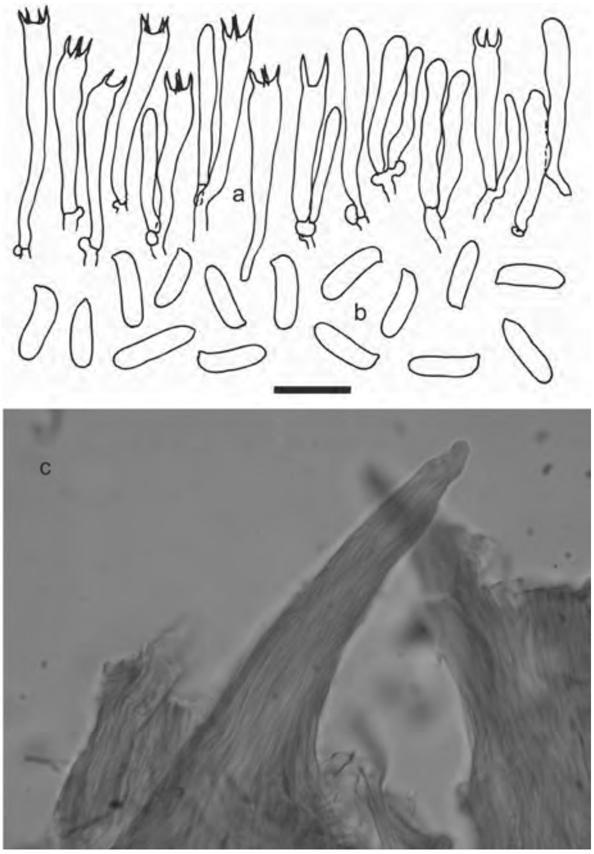


Fig. 4. *Panellus violaceofulvus*: a – basidia and basidioles, b – spores, c – terminal cells in the pileipellis (conical structure) (Javorníky Mts, 1 April 2009, SAV 10487). Scale bar = 10 µm for spores, 15 µm for basidia and basidioles, 20 µm for conical structure. Del. and photo S. Jančovičová.

Basidiomata in groups, growing out from the substrate in clusters of 2–12 basidiomata or separately. Stipe absent, basidiomata attached to the substrate laterally (by stem-like base) or dorsally. Pileus 5−30 mm, irregularly circular, flabelliform, rounded flabelliform or reniform (from above), hemispherical convex to plano-convex (from aside), hygrophanous; margin involute, with age inflexed, straight to reflexed (aspect), crenulated, sometimes lobed (shape), translucently striate (distinctly when wet); surface whitish granulose-fibrillose, tomentose to strigose (fibrils and hairs denser towards the point of attachment, distinct when dry), background red brown, violet brown to black violet. Trama up to 1.5 mm thick, off-white, taste bitter, smell indistinct. Lamellae L = 11–20, l = 3, 5 or 7, with wide 0.5−1.5 mm, radiating from lateral stem-like base or from central to eccentric spot, ventricose, adnexed, light violet, light brown, violet brown, red brown, often with pink hue, edge even, concolorous, lighter or darker.

Spores cylindrical, (6.5) 7.8–10.2 (11.5) × (2.5) 2.8–3.2 (3.5) µm, av. 9 × 3 µm, Q = (2) 2.6–3.2 (3.3), av. Q = 2.9, smooth, colourless and amyloid. Basidia 4-spored, rarely 2-spored, narrowly clavate, (25) 29.4–37.2 (43) × (4.5) 5.2–6.5 (7.5) µm, av. 33.4 × 5.9 µm, colourless, thin-walled. Basidioles narrowly clavate, narrowly cylindrical, (19) 23.5–34.2 (45) × (2.8) 3.3–5.4 (7) µm, av. 28.9 × 4.3 µm, colourless, thin-walled. Cheilocystidia not observed. Subhymenium 16–23 µm deep, of dense, intricate, irregularly oriented, 2–3.5 µm wide hyphae, well delimited from trama. Trama in lamellae composed of parallel to sub-parallel, thick-walled hyphae, 3–8 µm wide, with wall thickness 0.5–1.5 (2) µm, occasionally branched and anastomosed, colourless, in clamp-connections circular hole up to 1.5 µm wide. Pileipellis a transition between cutis and (ixo) trichoderm; a cutis layer consists of ± repent hyphae (some of them branching and anastomosing), thick-walled, 3–6 µm wide, with wall thickness 0.5–1 µm, straight to flexuous, with distance between septa more than 100 µm; the erect hyphae stand either ± individually or are fused together and form some conical (pyramidal) structures (these hyphae are unbranched, at the apex tapered and thin-walled); length and quantity of hyphae forming such cones differ (the highest observed conus measured 210 µm). In some mounts, conical structures embedded in the gelatinous matrix. Trama in pileus similar to lamellar trama. Clamp connections present.

**Specimens studied**

***Panellus ringens*:** Muránska planina Plateau, SE of the village Telgárt, locality "Za Skalicou", 48°50'16.7N", 20°12'7.36"E, 1,015 m asl, mire willow scrub dominated by *Salix cinerea*, on bark of fallen decaying branch of *Salix cinerea*, c. 0.5 cm in diam., 18 November 2003, leg. D. Blanár (SLO 790). – Ibidem, on bark of fallen decaying branch of *Salix pentandra*, c. 1 cm in diam., 18 November 2003, leg. D. Blanár (SLO 791).

– Stolické vrchy Mts, c. 1.5 km ENE of the city Tisovec, near the Šťavica spring, alluvium of the Skalička Stream, 48°41'11.28"N, 19°57'47.23"E, c. 460 m asl, stand with *Salix*, *Alnus* and *Fraxinus*, on bark of fallen decaying branch of *Salix* sp., 1–2 cm in diam., 25 March 2006, leg. S. Jančovičová (SLO 787); published by Ripková et al. (2007) as *Panellus violaceofulvus*. – NNE of the village Muránska Zdychava, saddle "Šumiacka priehyba", 48°47'38.37"N, 20°10'24.14"E, 1,170 m asl, species rich *Nardus* grassland (*Nardo-Agrostion tenuis*), on bark of branch growing from the standing trunk of *Salix caprea*, c. 2 cm in diam., 17 January 2009, leg. D. Blanár (SLO 788). – About 2.6 km N of the village Muránska Zdychava, alluvium of the Zdychavka (Zdychava) Stream, 48°45'40.36"N, 20°8'37.24"E, 658 m asl, submontane riparian mixed forest with *Picea abies* and *Alnus incana*, less *Salix fragilis*, *S. purpurea*, *Corylus avallana* a. o., on bark of branch growing from the standing trunk of *Salix caprea*, c. 1 cm in diam., 21 September 2012, leg. S. Jančovičová (SLO 1427, PVKU 747). – About 800 m SW of the village Dobrý Potok, 48°32'58.19"N, 19°40'41.94"E, 395 m asl, mixed stream bank forest with *Salix* sp., *Alnus glutinosa* and *Corylus avellana*, on bark of decaying branches of *Salix* sp., fallen on ground or tangled among other trunks, c. 1.5 cm in diam., 4 January 2014, leg. M. Smiková (PVKU 1140). – About 2.5 km NW of the city Revúca, 48°41'59.99"N, 20°5'51.67"E, 330 m asl, remnant of submontane mixed riparian forest with dominance of *Alnus glutinosa*, on bark of standing decaying trunk of *Padus avium*, c. 8 cm in diam., 25 October 2015, leg. V. Kunca (PVKU 1564).

– Revúcka vrchovina Mts, city Revúca, conflux of the Zdychavka (Zdychava) Stream and the Muránka (Muráň) River, alluvium, 48°40'47.44"N, 20°6'30.52"E, 310 m asl, willow stand with *Salix fragilis* (*Salicion albae*), on bark of standing thin trunk of *Salix cinerea*, 2–3 cm in diam., 20 November 2010, leg. D. Blanár (SLO 984); published by Mihál and Blanár (2014) as *Panellus* cf. *ringens*.

– Liptovská kotlina Basin, 1.3 km NE of the village Žiar, 49°8'8.01"N, 19°41'13.15"E, 845 m asl, mixed stream bank forest, on bark of standing decaying thin trunk of *Salix* sp., 11 November 2013, leg. M. Paulíny (PVKU 1117); referred by Peiger et al. (2015). – NE of the village Pavčina Lehota, c. 50 m W of the Nature Reserve Jelšie, 49°2'24.07"N, 19°33'51.06"E, 670 m asl, mixed stream bank forest with dominance of *Salix* sp. and *Alnus glutinosa*, on bark of broken decaying thin branch of *Salix* sp. with one end touching the ground, 5 January 2014, leg. P. Tomka (PSG 5341); referred by Peiger et al. (2015).

– Popradská kotlina Basin, c. 1 km SW of the village Gánovce, 49°1'22.09"N, 20°18'57.81"E, 660 m asl, intensively managed open remnant of submontane riparian forest with dominance of *Salix* sp., on bark of broken decaying branch of *Salix* sp. (in vertical position), c. 1.5 cm in diam., 22 February 2016, leg. M. Ondo (PVKU 1606).

– Horehronské podolie Basin, village Tále, 2 km SSW of the hotel Stupka, 48°51'55.62"N, 19°35'28.47"E, 676

m asl, old and sparse, not intensively managed forest of *Picea abies* and *Abies alba* with admixed *Corylus avellana*, *Populus tremula* and *Salix* sp., on bark of decaying branches of uprooted *Salix* sp., branches c. 1 m above the ground, c. 6 cm in diam., 23 January 2014, leg. R. Rutkowski (PSG 5377).
– Zvolenská kotlina Basin, c. 1.5 km S of the village Zolná, 48°35'11.05"N, 19°13'31.55"E, 345 m asl., remnant of submontane mixed riparian forest with dominance of *Salix* sp., on bark of decaying branch of *Salix* sp. tangled among tree-branches, c. 2 cm in diam., 4 December 2015, leg. V. Kunca (PVKU 1585).

***Panellus violaceofulvus*:** Veporské vrchy Mts, c. 7 km SSE of the village Čierny Balog, National Nature Reserve Dobročský prales, 48°40'48.26"N, 19°40'19.14"E, 870 m asl, on bark of fallen decaying branch of *Picea abies*, c. 1 cm in diam., 17 November 2003, leg. V. Kučera (SLO 789). – Central part of the National Nature Reserve Dobročský prales, 48°40'54.31"N, 19°40'48.12"E, c. 935 m asl, old-growth forest with *Fagus sylvatica*, *Abies alba* and *Picea abies*, on bark of fallen decaying branches of *Abies alba*, 3–4 cm in diam., 28 May 2010, leg. S. Glejdura, V. Kunca (PVKU 244, PSG 3684). – Village Čierny Balog, near the train station Svätý Ján, 48°46'50.36"N, 19°35'34.31" E, 530 m asl, managed forest with *Abies alba* and *Picea abies*, on bark of fallen decaying branch of *Abies alba*, c. 3 cm in diam., 17 April 2013, leg. M. Peiger (PVKU 915).
– Stolické vrchy Mts, village Muránska Zdychava, settlement Števkov diel, 48°45'34.8"N, 20°9'27.8"E, 824 m asl, managed forest with *Fagus sylvatica*, *Picea abies* and *Abies alba*, on bark of fallen thin branch of *A. alba*, 9 November 2013, leg. S. Glejdura (PSG 5195). – About 2.2 km NW of the village Šoltýska, ski and recreation resort Kokava-Háj, 48°35'36.29"N, 19°43'23.31"E, 780 m asl, managed forest with *Picea abies*, *Abies alba* and *Fagus sylvatica*, on bark of fallen decaying branch of *Abies alba*, c. 5 cm in diam., 9 January 2014, leg. P. Smik (PVKU 1144).
– Starohorské vrchy Mts, c. 900 m NNE of the church in the village Staré Hory, 48°50'36.33"N, 19°7'15.05"E, 640 m asl, managed special purpose forest with *Fagus sylvatica*, *Abies alba* and *Picea abies*, on unbarked wood of fallen decaying branch of *Abies alba*, c. 3 cm in diam., 28 November 2006, leg. S. Glejdura (PSG 3011). – About 1.2 km NE of the church in the village Staré Hory, 48°50'47.21"N, 19°7'45.94"E, c. 670 m asl, on bark of fallen decaying branches of *Abies alba* and *Picea abies*, 3–5 cm in diam., 11 December 2009, leg. S. Glejdura, V. Kunca (PVKU 184, PSG 1253); published (with wrong data) by Hagara (2014).
– Village Uľanka, near the train station Uľanka, 48°47'44.32"N, 19°6'12.92"E, 485 m asl, protective forest with *Picea abies* and *Abies alba*, on bark of fallen decaying branches of *Abies alba*, 2.5–3 cm in diam., 14 May 2013, leg. V. Kunca (PVKU 960). – About 0.2 km E of the church in the village Staré Hory, 48°50'2.46"N, 19°7'0.37" E, 520 m asl, managed forest with *Abies alba* and *Picea abies*, on bark of fallen decaying branches of *Abies alba*, c. 3 cm in diam., 23 October 2015, leg. V. Kunca (PVKU 1560).
– Západné Tatry Mts, right ridge of the Žiarska dolina valley, 1,123 m asl, managed forest with *Picea abies* and *Pinus sylvestris*, on bark of stump of *Picea abies*, c. 25 cm in diam., 4 October 2012, leg. M. Peiger, V. Kunca (PVKU 770); referred by Peiger et al. (2015).
– Vysoké Tatry Mts, village Podbanské, Kamenistá dolina valley, c. 1 km NW of the hotel Kriváň, 49°08'57.25"N, 19°53'14.79"E, c. 1,050 m asl, *Picea abies* forest with admixed *Abies alba* a. o., on bark of fallen decaying trunk of cf. *Abies alba*, 24 October 2012, leg. S. Jančovičová (SLO 1449).
– Nízke Tatry Mts, c. 400 m W of the village Malužiná, 48°58'48.63"N, 19°45'38.11"E, 850 m asl, protective forest with *Picea abies* and *Abies alba*, on wood (unbarked part) of fallen decaying trunk of *Abies alba*, 70–80 cm in diam., 4 September 2013, leg. M. Paulíny (PVKU 1009); referred by Peiger et al. (2015).
– About 80 m E of the village Jarabá, 48°53'20.86"N, 19°41'24.98"E, 895 m asl, managed forest with *Picea abies*, *Abies alba* and *Fagus sylvatica*, on bark of fallen decaying branch of *Abies alba*, c. 1 cm in diam., 9 March 2016, leg. V. Kunca (PVKU 1613).
– Kremnické vrchy Mts, c. 2 km NW of the spa in the village Kováčová, 48°37'16.81"N, 19°4'34.06" E, c. 360 m asl, managed special purpose forest with *Abies alba*, *Picea abies* and *Fagus sylvatica*, on bark of tree-top of fallen decaying young trunk of *Abies alba*, c. 3 cm in diam., 16 April 2010, leg. S. Glejdura, V. Kunca (PVKU 219). – Ibidem, on bark of fallen decaying branch of *Abies alba*, c. 4 cm in diam., 26 November 2010, leg. V. Kunca (PVKU 435). – Ibidem, on bark of fallen decaying branch and on bark of tree-top of fallen decaying young trunk of *Abies alba*, 3–5 cm in diam., 21 December 2011, leg. V. Kunca (PVKU 592).
– About 1.8 km NW of the spa in the village Kováčová, 48°37'0.88"N, 19°4'42.25"E, c. 350 m asl, managed special purpose forest with *Abies alba*, *Picea abies* and *Fagus sylvatica*, on bark of fallen decaying branch of *Abies alba*, c. 4 cm in diam., 15 July 2012, leg. V. Kunca (PVKU 701). – About 1.4 km NW of the church in the village Budča, 48°35'5.12"N, 19°3'5.09"E, c. 410 m asl, managed special purpose forest with *Fagus sylvatica* and *Abies alba*, on bark of cut decaying branches of *Abies alba*, 2–4 cm in diam., 10 November 2012, leg. V. Kunca (PVKU 855). – About 2 km NW of the church in the village Sielnica, 48°38'47.68"N, 19°5'4.51"E, 460 m asl, managed forest with *Abies alba* and *Picea abies*, on bark of cut decaying branches of *Abies alba*, 3–4 cm in diam., 16 February 2013, leg. V. Kunca (PVKU 884). – Village Kováčová, valley of Kováčovský potok Stream, 1.5 km NE of Stará Kováčová Hill, 48°37'18.9"N, 19°4'33.4"E, 379 m asl, managed special purpose forest with *Abies alba*, *Picea abies* and *Fagus sylvatica*, on bark of fallen trunk of *Abies alba*, 17 cm in diam., 7 April 2013, leg.

S. Glejdura (PSG 5194). – Village Ihráč, right slope of valley of the Vápenný potok Stream, 48°39'42.4''N, 18°58'37''E, 716 m asl, old-growth forest Dolný Chlm, on bark of fallen decaying branch of *Abies alba*, c. 4 cm in diam., 14 November 2013, leg. S. Glejdura (PSG 5170). – About 2 km NW of the spa in the village Kováčová, 48°37'32.33''N, 19°4'37.92''E, c. 395 m asl, managed special purpose forest with *Fagus sylvatica*, *Quercus petraea* and *Abies alba*, on bark of fallen decaying branch of *Abies alba*, c. 3 cm in diam., 22 February 2015, leg. V. Kunca (PVKU 1371). – About 7 km NW of the village Sielnica, 48°40'3.61''N, 19°2'56.88''E, 590 m asl, managed forest with *Abies alba*, on bark of fallen decaying branch of *Abies alba*, 2–3 cm in diam., 1 March 2015, leg. V. Kunca (PVKU 1372). –Village Badín, beyond the border of the NW part of the National Nature Reserve Badínsky prales, 48°40'3.61''N, 19°2'56.88'' E, c. 770 m asl, natural forest with *Fagus sylvatica* and *Abies alba*, on bark of fallen decaying branch of *Abies alba*, c. 4 cm in diam., 11 March 2015, leg. R. Rutkowski (PVKU 1379).

– Poľana Mts, city Hriňová, settlement Snohy, E part of the National Nature Reserve Zadná Poľana, 48°38'42.72''N, 19°30'21.18''E, 1,086 m asl, old-growth forest with *Fagus sylvatica*, *Abies alba* and *Acer pseudoplatanus*, on bark of fallen decaying branches of *Abies alba*, 2–3 cm in diam., 18 September 2013, leg. V. Kunca, S. Glejdura (PVKU 1019, PSG 5184).

– Javorie Mts, city Zvolen, c. 1.3 km S of the Môťová water reservoir, 48°32'20.36''N, 19°10'32.21''E, 465 m asl, managed special purpose forest with *Quercus petraea*, *Abies alba* and *Fagus sylvatica*, on bark of tree-top of fallen decaying young trunk of *Abies alba*, c. 3 cm in diam., 8 March 2013, leg. V. Kunca (PVKU 891). – About 1 km NEE of the village Ostrá Lúka, 48°33'5.73''N, 19°4'30.74''E, 400 m asl, protective forest with dominance *Abies alba*, on bark of fallen decaying branch of *Abies alba*, c. 3 cm in diam., 17 March 2013, leg. V. Kunca (PVKU 893).

– Zvolenská kotlina Basin, c. 1 km SEE of the village Dúbravica, 48°40'21.95''N, 19°17'15.88''E, 600 m asl, managed forest with *Fagus sylvatica*, *Picea abies* and *Abies alba*, on bark of tree-top of fallen decaying young trunk of *Abies alba*, on small unbarked area between bark and wood, c. 4 cm in diam., 22 November 2013, leg. V. Kunca (PVKU 1123). – About 2.5 km NW of the village Poniky, 48°43'25.72''N, 19°15'20.55''E, 610 m asl, managed forest with *Fagus sylvatica*, *Abies alba* and *Picea abies*, on bark of fallen decaying branch of *Abies alba*, c. 3 cm in diam., 5 May 2015, leg. V. Kunca (PVKU 1411).

– Javorníky Mts, c. 7.5 km NW of the church in the village Papradno, settlement Badačov grúnik (also known as settlement Macháčovce), 49°16'37.91''N, 18°20'12.44''E, 748 m asl, forested area dominated by *Fagus sylvatica* and *Acer* spp., with admixed *Abies alba*, *Larix decidua*, *Pseudotsuga menziesii* a. o., on bark of fallen decaying trunk of *Pseudotsuga menziesii*, 1 April 2009, leg. V. Kučera (SAV 10487). – Village Dešná, c. 500 m NNW of the village part Beňadín, 49°14' 6.79''N, 18°9'39.47''E, 580 m asl, managed forest with *Picea abies* and *Abies alba*, on bark of fallen decaying branch of *Abies alba*, c. 3 cm in diam., 3 January 2014, leg. V. Pšenka (PVKU 1139). – About 1 km SSW of the village Vysoká nad Kysucou, 49°22'0.46''N, 18°32'2.79''E, 655 m asl, managed forest with *Picea abies* and *Abies alba*, on bark of fallen decaying branch of *Abies alba*, 8–10 cm in diam., 21 February 2014, leg. M. Zajac (PVKU 1168).

– Podbeskydská brázda Furrow, village Oravská Polhora, 500 m E of the settlement Slaná Voda, 49°31'19.2''N, 19°28'54.7''E, 744 m asl, juvenile managed forest with *Picea abies* and *Abies alba*, on bark of fallen decaying branch of *A. alba*, 13 January 2014, leg. R. Rutkowski (PSG 5342).

– Bukovské vrchy Mts, village Osadné, National Nature Reserve Udava, 49°10'44.34''N, 22°12'59.71''E, 640 m asl, old-growth forest with *Fagus sylvatica* and *Abies alba*, on bark of fallen decaying thin branches of *Abies alba*, 13 April 2013, leg. J. Pavlík (PVKU 907).

– Laborecká vrchovina Mts, village Nižný Komárnik, National Nature Reserve Komárnická jedlina, 49°22'53.63''N, 21°44' 47.68''E, 510 m asl, old-growth forest with *Fagus sylvatica*, *Abies alba* and *Ulmus glabra*, on bark of fallen decaying branch of *Abies alba*, c. 2 cm in diam., 9 March 2016, leg. V. Kunca (PVKU 1610).

***Phyllotopsis nidulans***: Nízke Tatry Mts, village Malužiná, at the bank of the Boca River near the mouth of Skribňovo valley, 730 m asl, on dead stump of *Salix alba*, 16 April 2006, leg. V. Kautman (BRA CR 8435); published by Kautmanová (2006) and Hagara (2014) as *Panellus ringens*. – Ibidem, on bark of fallen decaying branch of *Salix alba*, c. 10 cm in diam., 27 April 2011, leg. V. Kučera (SLO 828).

**Published records from Slovakia**

Two first collections of *Panellus ringens* from Slovakia are those from 1972 kept in the herbarium PRM (one from Levočské vrchy Mts, and the second one from Ľubovnianska vrchovina Mts). They were published by Škubla (2003) and Kautmanová (2006). The third record of *P. ringens*, namely the collection from 2006, Nízke Tatry Mts, was published by Kautmanová (2006). This collection [regarded as *P. ringens* also by Hagara (2014)], however, represents *Phyllotopsis nidulans* (= BRA CR 8435 in the Specimens studied). Since then, many other collections of *P. ringens* have been recorded (see Specimens studied), but only a few published: Ripková et al. (2007) published one collection from 2006, Stolické vrchy Mts (= SLO 787, incorrectly identified as *Panellus violaceofulvus*); Mihál and Blanár (2014) published one collection from 2010, Revúcka vrchovina Mts (= SLO 984 as *P.* cf. *ringens*); and Peiger et al. (2015) referred to four collections from 2012–2015, Liptovská kotlina Basin (two of them = PVKU 1117 and PSG 5341). Altogether, we have found out eight published collections of *P. ringens* from Slovakia.

Ripková et al. (2007) published 17 collections of *Panellus violaceofulvus* from Slovakia, concerning also data by Kuthan (1989), Kuthan et al. (1999), Škubla(2003) and Adamčík et al. (2007). The latest of these collections from 2006, Stolické vrchy Mts has been re-identified as *P. ringens* in this study (= SLO 787). The rest of these *P. violaceofulvus* collections are from the years 1914–2003, kept in the herbaria BRA, PRM, GENT and private herbarium of J. Heilmann-Clausen; one collection is from Oravská vrchovina Mts, Malá Fatra Mts, Štiavnické vrchy Mts, and Liptovská kotlina Basin, two from Nízke Tatry Mts and Levočské vrchy Mts, three from Laborecká vrchovina Mts and five from Bukovské vrchy Mts. Number of *P. violaceofulvus* records has almost tripled since then, but only a few of them were published: Mihál et al. (2011) published one collection from 2008, Stolické vrchy Mts (this collection from *Salix caprea* could represent *P. ringens*; however, the specimen does not exist and we have not counted it); Hagara (2014) published one collection from 2006, Starohorské vrchy Mts (= PVKU 184); and Peiger et al. (2015) referred to one collection from 2012, Západné Tatry Mts (= PVKU 770) and one from 2013, Nízke Tatry Mts (= PVKU 1009). Altogether, we have found out 21 collections of *P. violaceofulvus* published from Slovakia up to this time.

**Species differentiation based on the morphological characters observed on the Slovak collections**

Observing Slovak collections of *Panellus ringens* and *P. violaceofulvus*, we have found out differences in their macro- and micromorphological characters.

Macroscopically, both species are very similar. In our material, basidiomata of *P. ringens* are somewhat smaller (max. size 20 mm in diam.) than of *P. violaceofulvus* (max. size 30 mm in diam.). In colour of pileus, red-brown dominate in *P. ringens* and violet-brown in *P. violaceofulvus*, but shades of both colours could be seen in *P. ringens* as well *P. violaceofulvus*. Especially when dry, surface of both species is distinctly hairy, but the hairs are usually somewhat thicker and longer in *P. violaceofulvus* (especially at the point of attachment). Despite of some differences, we do not treat macroscopic features as determining (Figs 1, 3).

Microscopically, both species distinctly differ in their spores characters. While *P. ringens* has spores cylindrical, narrowly cylindrical and allantoid, av. 6.6 × 1.8 µm, av. Q = 3.6, *P. violaceofulvus* cylindrical, av. 9 × 3 µm, av. Q = 2.9. In hymenium of *P. ringens*, we have observed cheilocystidia of various shapes, thick-walled or thin-walled, with av. 26.9 × 5.1 µm; such cystidia, according to our observations absent in *P. violaceofulvus*. Basidia also seem to be of taxonomic importance: basidia of *P. ringens* with av. 20.2 × 4.5 µm are distinctly smaller comparing with those of *P. violaceofulvus* with av. 33.4 × 5.9 µm. Pileipellis of both species is also different: in *P. ringens*, the erect hyphae are thick-walled, 5–11 µm wide, with wall thickness 0.5–3 µm, flexuous to twisted, some nodulouse, often intricate, but not fascicled, mostly incrusted; while in *P. violaceofulvus*, the erect hyphae are thick-walled, 3–6 µm wide, with wall thickness 0.5–1 µm, flexuous, standing either ± separately, or fused together to form some conical structures. The differences are also in trama: tramal hyphae of pileus and lamellae in *P. ringens* are 3.5–7 (10) µm wide, with wall thickness 1–3 µm, in clamp-connections with circular hole up to 5 µm wide; in *P. violaceofulvus*, they are 3–8 µm wide, with wall thickness 0.5–1.5 (2) µm, in clamp-connections with circular hole up to 1.5 µm wide (Figs 2, 4).

**Species differentiation based on the morphological characters used in the literature**

In literature, *Panellus ringens* and *P. violaceofulvus* are distinguished using various characters. We have compared our observations and measurements with those in the selected literature. It is the literature in which both studied species are included and which is/was traditionally used to identify fungal taxa: Moser (1983), Pöder (1985), Watling and Gregory (1989), Breitenbach and Kränzlin (1991), Käärik (1992) and Vesterholt (2012) (Table 1).

Out of macroscopic characters, colour and size of pileus are mostly compared. No author described violet colour in *P. ringens* and Pöder (1985) even stressed that this colour never occurred in this species. As to pileus size, the authors described *P. violaceofulvus* to be smaller or of similar size as *P. ringens*. However, it contradicts our observations.

Most authors used size of spores as the first character in the key. We also agree that spores characters are of high taxonomic importance. Spores of *P. ringens* and *P. violaceofulvus* do not overlap in their width and only minimally in their length. Our measured values of spore length are somewhat higher, but we think it is consistent with the delimitation and variability of both species.

Cheilocystidia are controversial. Some authors described them in *P. ringens*, some other, contrary, in *P. violaceofulvus*. We are not able to explain this conflict, as we have observed cheilocystidia only in material of *P. ringens*.

To distinguish *P. ringens* from *P. violaceofulvus*, Moser (1983) and Käärik (1992) used subhymenium characters. Without any specification, these authors stated that subhymenium was well developed in *P. ringens* in comparison to poorly developed subhymenium in *P. violaceofulvus.* Pöder (1985) described subhymenium in *P. ringens* as ± well differentiated, in *P. violaceofulvus* ± slender, gelatinous. According to our observations, subhymenium is well delimited from

Table 1. The comparison of the characters used to differentiate *Panellus ringens* and *P. violaceofulvus* in the selected literature and our own observations

| | *Panellus ringens* | *Panellus violaceofulvus* |
|---|---|---|
| Pileus size (mm) and colour | | |
| Our observations | 3–20, red brown to brown (occasionally with violet shades when young) | 5–30, red brown, violet brown to black violet |
| MOSER (1983) | 5–25, brownish red, flesh brownish | 5–15, violet brown, violet |
| PÖDER (1985) | 5–15, red flesh red, red brown (without any violet shades) | 5–15, violet purple, black violet, yellow ochre shades towards base |
| WATLING and GREGORY (1989) | 7.5–25, light purple, purple drab, vinaceous, vinaceous grey, vinaceous buff, clay pink | –, similar colours as *P. ringens* |
| BREITENBACH and KRÄNZLIN (1991) | –, flesh brownish | 8–25, black violet, brown violet |
| KÄÄRIK (1992) | 5–25, flesh | 5–15, vinaceous purple, violet |
| VESTERHOLT (2012) | 5–30, purplish brown, vinaceous | 5–15, violet to purple, purplish brown |
| Spores (μm) | | |
| Our observations | 5–8 × 1.5–2 | 6.5–11.5 × 2.5–3.5 |
| MOSER (1983) | 4–6 × 1.5–2 | 6–10 × 2–4 |
| PÖDER (1985) | 4–5 × 1.5–2 | 6–10 × 2.5–3 |
| WATLING and GREGORY (1989) | 4–7 × 1.2–2 | 6.5–10 × 2.2–3.5 |
| BREITENBACH and KRÄNZLIN (1991) | 4.5–6 × 1.5–2 | 6.5–9.5 × 2.6–4.2 |
| KÄÄRIK (1992) | 4–6 × 1.5–2 | 6–10 × 2–4 |
| VESTERHOLT (2012) | 5–7 × 1–2 | 6.5–9.5 × 2.5–3.5 |
| Cheilocystidia (μm) | | |
| Our observations | 16–42 × 2–7.5, clavate, narrowly (n.) clavate, n. cylindrical, n. fusiform, n. utriform, flexuous, some nodulouse or branched | not observed |
| PÖDER (1985) | absent | ± basidium like, rarely with short diverticules |
| WATLING and GREGORY(1989) | 25–40 × 4.2–5.5, clavate, cylindrical, fusiform | – |
| BREITENBACH and KRÄNZLIN (1991) | – | 22–35 × 3–4, fusiform, flexuous, sometimes with short outgrowths |
| KÄÄRIK (1992) | rare, hyphoid | – |
| VESTERHOLT (2012) | absent or inconspicuous | 22–35 × 3–5 cylindrical to subclavate, sometimes irregular with lateral outgrowths |
| Subhymenium | | |
| Our observations | 10–18 μm deep, of dense, intricate, irregularly oriented, 1.5–3.5 μm wide hyphae | 16–23 μm deep, of dense, intricate, irregularly oriented, 2–3.5 μm wide hyphae |
| PÖDER (1985) | ± well differentiated | ± slender, gelatinous |
| MOSER (1983) | clearly differentiated from cap flesh (dense) | hyphae in subhymenium and in trama swollen-gelatinous, in subhymenium somewhat thinner |
| KÄÄRIK (1992) | well developed | poorly developed |

Size of structures in our observation is given as minimum and maximum. –, character not mentioned. If the authors are not listed, they did not describe the character.

trama in both species and differs minimally: it is 10–18 μm deep and formed of 1.5–3.5 μm wide hyphae in *P. ringes* and 16–23 μm deep and composed of 2–3.5 μm wide hyphae in *P. violaceofulvus*.

With exception of Pöder (1985), the authors described the pileipellis briefly or not at all. According to Pöder (1985), pileipellis of *P. ringens* consists of thick-walled, 4–6 (7) µm wide hyphae, originating from a layer of radially laying, pigmented, gelatinous, and 3–4 µm wide hyphae. In *P. violaceofulvus*, pileipellis is a trichoderm, formed of thick-walled, often irregularly flexuous, branched, 3–4 µm wide hyphae, arising from radially deposed, gelatinous hyphae. Following descriptions by Pöder (1985), differences between *P. ringens* and *P. violaceofulvus* are a little bit ambiguous. We consider the pileipellis characters to be determining.

Tramal characters seem to be also important. Out of all compared works, it was only Pöder (1985) who described trama more detailed. According to him (Pöder, 1985), trama in pileus of *P. ringens* consists of thick-walled, 3–6 µm wide hyphae, often with intercalar or apical inflations up to 30 µm wide (called sclerocystidia in the legend to the figure); trama in lamellae consists of 3–4 µm wide hyphae. In *P. violaceofulvus*, Pöder (1985) described hyphae of trama in pileus as thick-walled, 3 µm wide, with intercalar or apical inflations up to 28 µm wide (sclerocystidia); hyphae of trama in lamellae as thick-walled, 3–5 m wide. We have not observed such sclerocystidia in our material, but the differences in wall thickness of hyphae and width of hole in clamp-connections are evident.

**Ecology, occurrence and threat in Slovakia**

In this study, we have gathered information about 17 collections of *Panellus ringens* (eight published and nine new, i.e. introduced in this paper) and 53 of *P. violaceofulvus* (21 published and 33 new) collected in Slovakia. Based on these collections, the species have different ecological requirements: *P. ringens* prefers hardwoods, *P. violaceofulvus* conifers.

*Panellus ringens* was recorded on willow trees and bushes, such as *Salix caprea*, *S. cinerea*, *S. pentandra* and *S.* sp.; only once on *Padus avium*. Basidiomata were produced on bark of branches and trunks. Branches of 0.5–6 cm in diam. were a) laying on the ground, b) broken and touching the ground only with some part or c) without any contact with the ground, i.e. growing from the standing trunks, from the uprooted trunks or broken and tangled among tree-branches or other trunks. The trunks of 2–8 cm in diam. were standing, still living or dead. The collections are from September to March. We think that the basidiomata production in winter to early spring could be a reason why some collections were sterile (without matured spores).

The most collecting sites of *P. ringens* had wetland character – they were the stands along streams and rivers where *Salix* species were dominant or admixed, i.e. the stands with *S. fragilis* and/or other willows; as altitude increase, they were the stands with *Alnus glutinosa* and *Fraxinus excelsior*; then the stands with *Alnus incana* and *Picea abies*; the scrub with dominant *Salix cinerea*; and the species rich *Nardus* grassland. One collection was from the *Picea abies* forest with admixed *Abies alba*. The majority of stands belong to the agricultural land resources and it means that trunks having a tree girth up to 40 cm (such like trees are present almost in all collecting sites) can be cut at any time (without any administrative permission) (Act of NC SR No. 543/2002 on Nature and Landscape Protection). The altitude of the collecting sites ranges from 310 to 1,170 m asl.

*Panellus ringens* is not included in the Red list of fungi of Slovakia (Lizoň, 2001). Although the number of records of this species has been increasing, we think its habitats need preservation. As said above, *P. ringens* grows in the stands which are under the risk to be cut. In general, wetlands belong among the most endangered habitats all over the world. Many wetlands were destroyed after stream regulations and building water-storage reservoirs, and the other have been decreasing due to development of industrial zones, recreation centres and highways (Hájek and Ditě, 2009). Because of these risk factors, we propose *P. ringens* to be red-listed in future as endangered species.

*Panellus violaceofulvus* was mostly recorded on *Abies alba*, rarely on *Picea abies* and once on *Pseudotsuga menziesii*. Basidiomata grew on branches, less on trunks and sporadically (two collections) on stumps (both on *Picea abies* stumps). Branches of 1–5 (10) cm in diam. were laying on the ground – they fell naturally or (in two cases) were piled into heaps after cutting. Fallen trunks were of 3–80 cm in diam. and the thinnest ones (3–5 cm in diam.) were the broken tree-tops. Most substrates (branches, trunks, stumps) were covered by bark; only once, basidiomata grew on wood (unbarked part) of trunk and once on wood of branch. The collections are from September to May (predominantly from colder months). Collections from summer months July and August are exceptional. *Panellus violaceofulvus* seems to be a forest species. It was found in basin *Quercus petraea* forests with *Abies alba*, in submontane *Fagus sylvatica* and *Quercus petraea* forests and *Fagus sylvatica* forests with *Abies alba*, in homogenous *Abies alba* forests, in typical Carpathian *Fagus sylvatica*, *Abies alba* and *Picea abies* forests, and in mountainous *Picea abies* forests with *Abies alba* and *Picea abies* forests with *Pinus*. One collection is from the forested area ("the area of environment management") dominated by *Fagus sylvatica* and *Acer* spp., with admixed *Abies alba*, *Larix decidua*, *Pseudotsuga menziesii* and others.

According to Holec et al. (2015b), *P. violaceofulvus* prefers old-growth forests. We have found out that intensity of forest management probably does not play important role in the occurrence of this fungus. Generally, there are three categories of forests in Slovakia: a) commercial forests which are intensively managed, b) protection forests which are (resp. were) not managed or managed with specific intention to fulfil preventative function of abiotic structures in landscape, and c) special-purpose forests which are managed with different intensity (often by selective cutting) or, in the case of national nature reserves, they are left to spontaneous development without any intervention (Anonymus, 2015). Our records of *P. violaceofulvus* were more or less proportionally distributed all over these forest categories. However, supporting Holec et al. (2015b), about third of the stands can be classified as old-growth forests (cf. forest naturalness classification by Holec et al., 2015a). The altitude of the collecting sites ranges from 350 to 1,123 m asl.

*Panellus violaceofulvus* is not included in the Red list of fungi of Slovakia (Lizoň, 2001) and we have no arguments to propose it to be red-listed even in future. In management view, *P. violaceofulvus* occurs in all forest categories. Our targeted search for *P. violaceofulvus* in forests with at least 10% representation of *Abies alba* succeeded in almost every stand. Although *Abies alba* occurs in the forests of Slovakia only with the representation of 4%, it is not a rare tree species (Anonymus, 2015). In ten last years, the number of collections of *P. violaceofulvus* has almost tripled.

**Ecology, occurrence and threat in Europe**

Comparing our ecological findings on *Panellus ringens* and *P. violaceofulvus* with those of published in the selected literature (Table 2), they more or less correspond to each other. Some differences are in the host spectrum: Pöder (1985) presented as hosts of *P. ringens* also *Betula* and *Alnus incana*; Vesterholt (2012) included among hosts of *P. ringens* also conifers and for *P. violaceofulvus Salix* spp.

Table 2. Comparison of the ecological characteristics of *Panellus ringens* and *P. violaceofulvus* in the selected literature and our own observations

| Ecological characteristics | *Panellus ringens* | *Panellus violaceofulvus* |
| --- | --- | --- |
| Our observations | on bark of fallen or standing branches and trunks of *Salix caprea*, *S. cinerea*, *S. pentandra*, *S.* sp., (once) *Padus avium*; September–March | on bark (rarely wood) of fallen branches, trunks, (rarely stumps) of *Abies alba*, *Picea abies*, (once) *Pseudotsuga menziesii*; September–May (July, August) |
| Moser (1983) | on deciduous wood; in winter | especially coniferous wood |
| Pöder (1985) | on bark of deciduous trees, especially *Betula* spp. *Alnus incana*, *Salix* spp. | on bark of conifers, especially *Abies alba* and *Picea abies* |
| Watling and Gregory (1989) | on twigs, sticks and branches of frondose trees | – |
| Breitenbach and Kränzlin (1991) | on hardwoods, especially *Salix*, winter half of the year | on dead branches or small trunks of *Abies alba*; winter to spring |
| Käärik (1992) | on bark of living or dead deciduous trees | – |
| Vesterholt (2012) | on deciduous wood, often on standing trees or still attached branches, rarely on conifers; autumn to late autumn | on *Salix*, in Central Europe also on conifers; autumn |

As shown in Table 3, *Panellus ringens* and *P. violaceofulvus* are red-listed and/or occur in several European countries.

Table 3. Occurrence and threat of *Panellus ringens* and *P. violaceofulvus* in Europe

| | A | CH | CZ | D | F | FIN | GB | I | LT | N | PL | S | UA |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Panellus ringens* | O | RL | O | RL | O | O | O | O | RL | O | RL | O | ? |
| *Panellus violaceofulvus* | RL | O | RL | RL | O | ? | ? | O | RL | RL | RL | ? | O |

RL – red-listed species, O – species occurs in the country, ? – we are not sure if the species occurs in the country.

***Panellus ringens.*** A – Austria: O (Austrian Macological Society, pers. comm.), CH – Switzerland: RL (Senn-Irlet et al., 2007), CZ – the Czech Republic: O (Svrček, 1954), D – Germany: RL (Benkert et al., 1996), F – France (Roux, 2006), FIN – Finland (Vesterholt, 2012), GB – Great Britain: O (Watling and

Gregory, 1989), I – Italy: O (Pöder, 1985), LT – Latvia: RL (Andruđaitis, 1996), N – Norway (Vesterholt, 2012), PL – Poland: RL (Wojewoda and Ławrynowicz, 2006), S – Sweden: O (Vesterholt, 2012).

***Panellus violaceofulvus.*** A – Austria: RL (Krisai-Greilhuber, 1999), CH – Switzerland: O (Breitenbach and Kränzlin, 1991), CZ – the Czech Republic: RL (Holec and Beran, 2006), D – Germany: RL (Benkert et al., 1996), F – France: O (Roux, 2006), I – Italy: O (Pöder, 1985), LT – Latvia: (Andruđaitis, 1996), N – Norway: RL (Brandrud, 2015), PL – Poland: RL (Wojewoda and Ławrynowicz, 2006), UA – Ukraine: O (Holec, 2008).

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**References**

Adamčík, S., Christensen, M., Heilmann-Clausen, J., Walleyn, R., 2007. Fungal diversity in the Poloniny National Park with emphasis on indicator species of conservation value of beech forests in Europe. *Czech Mycology*, 59: 67–81.

Andruđaitis, G. (ed.), 1996. *Latvijas Sarkanâ Grâmata. Retâs un izzűdođâs augu un dzîvnieku sugas. 1. sçjums* [Red data book of Latvia. Rare and endangered species of plants and animals, Vol. 1]. Rîga: LU Bioloģijas institūts.

Anonymus, 2015. *Správa o lesnom hospodárstve v Slovenskej republike za rok 2014 (Zelená správa)* [Report on the status of forestry in the Slovak Republic of 2014 (Green Report)]. Bratislava: Ministerstvo pôdohospodárstva a rozvoja vidieka Slovenskej republiky. 86 p.

Benkert, D., Dörfelt, H., Hardtke, H.J., Hirsch, G., Kreisel, H., Kriegelsteiner, G.J., Lüderitz, M., Runge, A., Schmidt, H., Schmitt, J.A., Winterhoff, W., Wöldecke, K., Zehfuss, H.D., 1996. Rote Liste der Großpilze Deutschlands. *Schriftenreihe für Vegetationskunde*, 28: 377–426.

Brandrud, T.E. (ed.), 2015. Sopper [Fungi]. In Henriksen, S., Hilmo, O. (eds). *Norsk rødliste for arter 2015*. Norge: Artsdatabanken, p. 132–156. [cit. 2016-03-17]. http://www.artsdatabanken.no/File/2308/Norsk%20r%C3%B8dliste%20for%20arter%202015.

Breitenbach, J., Kränzlin, F., 1991. *Fungi of Switzerland, Vol. 3. Boletes and Agarics, Part 1.* Luzern: Verlag Mykologia. 361 p.

Hagara, L., 2014. *Ottova encyklopédia húb* [Otto's encyclopedia of fungi]. Praha: Ottovo nakladatelství, s. r. o. 1152 p.

Hájek, M., Dítě, D., 2009. Vodné a mokraďové biotopy Karpát [Water and wetland biotopes of the Carpathians]. In Královičová, A., Herianová, S. (eds). *Svet Karpát. Príručka k environmentálnej výchove*. Bratislava: Daphne – Inštitút aplikovanej ekológie, p. 103–128.

Holec, J., 2008. Interesting macrofungi from the Eastern Carpathians, Ukraine and their value as bioindicators of primeval and near-natural forest. *Mycologia Balcanica*, 5: 55–67.

Holec, J., Beran, M. (eds), 2006. *Červený seznam hub (makromycetů) České republiky* [Red list of fungi (macromycetes) of the Czech Republic]. Příroda, 24. Praha: Agentúra ochrany přirody a krajiny. 282 p.

Holec, J., Kříž, M., Beran M., Kolařík M., 2015a. Chromosera cyanophylla (Basidiomycota, Agaricales) – a rare fungus of Central European old-growth forests and its habitat preferences in Europe. *Nova Hedwigia*, 100: 189–204.

Holec, J., Kříž, M., Pouzar, Z., Šandová, M., 2015b. Boubínsky prales virgin forest, a Central European refugium of boreal-montane and old-growth forest fungi. *Czech Mycology*, 67: 157–226.

Kautmanová, I., 2006. Panellus ringens rediscovered after 30 years. *Catathelasma*, 8: 33.

Käärik, A., 1992. Panellus Karst. In Hansen, L., Knudsen, H. (eds). *Nordic macromycetes, Vol. 2.* Copenhagen: Nordsvamp, p. 175–176.

Knudsen, H., Vesterholt, J. (eds), 2012. *Funga Nordica*. Copenhagen: Nordsvamp. 1083 p.

Krisai-Greilhuber, I., 1999. Rote Liste gefährdeter Großpilze Österreichs. In Niklfeld, H. (ed.). *Rote Listen gefährdeter Pflanzen Österreichs*. Graz: Bundesministerium für Umwelt, Jugend und Familie, p. 229–266.

Kuthan, J., 1989. Soupis makromycetů sbíraných na průzkumných exkurzích (s myk. NDR) vednech 3. IX. – 9. IX. 1988 a na exkurzích „Setkání českých a slovenských mykologů pod Tatrami“ dne 10. IX. – 18. IX. na lokalitách v oblasti Vysokých a Nízkých Tater a v Slovenském rudohoří [The list of macromycetes collected during the reconnaissance excursions (with mycol. NDR) on the 3.IX.–9.IX. 1988 and during the excursions "Setkání českých a slovenských mykologů pod Tatrami" on the 10.IX.–18.IX. at the localities in the Vysoké Tatry Mts, Nízke Tatry Mts and Slovenské rudohorie Mts]. In Kuthan, J. (ed.). *Houby rašelinišť a bažinatých lesů v Československu. Zborník referátů*. Praha: ČSVSM, p. 63–89.

Kuthan, J., Adamčík, S., Antonín, V., Terray, J., 1999. *Huby Národného parku Poloniny* [Fungi of the National Park Poloniny]. Liptovský Mikuláš: Správa národných parkov SR, Snina: Správa Národného parku Poloniny. 197 p.

Lizoň, P., 2001. Červený zoznam húb Slovenska, 3. verzia (december 2001) [Red list of fungi of Slovakia, the 3rd draft (December 2001)]. *Ochrana Prírody*, 20 (Suppl.): 6–13.

Mihál, I., Blanár, D., 2014. Fungi and slime molds of alder and willow alluvial forests of the upper part of the Muránka river (central Slovakia). *Folia Oecologica*, 41: 153–172.

Mihál, I., Glejdura, S., Blanár, D., 2011. Makromycéty (Zygomycota, Ascomycota, Basidiomycota) v masíve Kohúta (Stolické vrchy) [Macromycetes (Zygomycota, Ascomycota, Basidiomycota) in the massif of the Kohút Mountain (the Stolické vrchy Mts). *Reussia*, 6: 1–44.

Moser, M., 1983. *Keys to agarics and boleti (Polyporales, Boletales, Agaricales, Russulales).* 4th ed. London: Roger Phillips. *535 p.*

Peiger, M., Tomka, P., Paulíny, M., 2015. *Huby Liptova* [Fungi of Liptov]. Ružomberok: Liptovské múzeum v Ružomberku. 168 p.

Pöder, R., 1985. Panellus ringens (Fr.) Romagn. e P. violaceofulvus (Batsch: Fr.) Sing. due rare tricholomataceae. *Bolletino del Gruppo Micologico G. Bresadola,* 28 (3–4): 101–106.

Ripková, S., Adamčík, S., Kučera, V., 2007. New, rare and less known macromycetes in Slovakia II. *Czech Mycology*, 59: 185–199.

Roux, P., 2006. *Mille et un champignons* [A thousand and one mushrooms]. Sainte-Sigolène: Édition Roux. 1224 p.

Senn-Irlet, B., Bieri, G., Egli, S., 2007. *Rote Liste der gefährdeten Grosspilze der Schweiz.* Bern: Umwelt-Vollzug. 92 p.

Svrček, M., 1954. Druhý příspěvek k poznání mykoflory Českého středohoří [The second contribution to the knowledge of funga of the České stredohoří Mts]. *Česká Mykologie*, 8: 129–134.

Škubla, P., 2003. *Mycoflora Slovaca*. Šaľa: Self-editing. 1103 p.

Thiers, B., 2016, continuously updated. *Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium* [cit. 2016-02-02]. http://sweetgum.nybg.org/ih

Vellinga, E.C., 1988. Glossary. In Bas, C., Kuyper, T.W., Noordeloos, M.E., Vellinga, E.C. (eds). *Flora Agaricina Neerlandica, Vol. 1*. Rotterdam, Brookfield: A.A. Balkema, p. 54–64.

Vesterholt, J., 2012. Panellus P. Karst. In Knudsen, H., Vesterholt, J. (eds). *Funga Nordica*. Copenhagen: Nordsvamp, p. 442–443.

Watling, R., Gregory, N.M., 1989. Crepidotaceae, Pleurotaceae and other pleurotoid agarics. In Henderson, D.M., Orton, P.D., Watling, R. (eds). *British fungus flora. Agarics and Boleti, Vol. 6*. Edinburg: Royal Botanic Garden, p. 1–157.

Wojewoda, W., Ławrynowicz, M., 2006. Red list of macrofungi in Poland. In Mirek, Z., Zarzycki, K., Wojewoda, W., Szeląg, Z. (eds). *Red list of plants and fungi in Poland*. Kraków: W. Szafer Institute of Botany, Polish Academy of Sciences, p. 53–70.

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# Soil reaction and tick abundance *Ixodes ricinus*

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**Abstract**

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Tick *Ixodes ricinus* is a carrier of several diseases. At present, its activity in Slovakia was discovered in the winter months. It also extends to higher altitudes. Therefore it is necessary to know the ecology and optimal conditions suitable for its existence. Although considerable attention is devoted to the health research of tick, little is known about its claim on the soil environment, because as a representative of a temporary edaphone initial ontogenetic stages (eggs and larvae) survives in the soil. For this reason, the research focused on the soil pH as an important environmental factor that can influence the development and abundance of tick. The research was carried out on the plots with tick high abundance in the Veľká Fatra Mts at an altitude of - 760 meters asl. We found that mull humus form with a thin film of last year's litter fall (the Oon necrotic subhorizon) with a pH narrow range of 5.9–6.0 (rounded) was convenient for developmental stages of this tick. Below this horizon the soil type of cambisol with pH values in the upper range of 5.45–5.79 was produced. The soil reaction was therefore a slight acid. In this environment, a relatively high density of tick was in May, with the secondary mild autumn peak.

**Key words**

Cambisol, ecology, humus form, *Ixodes ricinus*, soil properties, Veľká Fatra Mts

## Introduction

*Ixodes ricinus* – tick ordinary is epidemiologically most important tick of the families Ixodidae, one of 20 tick species occurring in Slovakia. Its danger lies in the ability of its body to transmit pathogens that cause Lyme disease, anaplasmosis, tick-borne encephalitis, and babesiosis.

Geographic and vertical expansion of this tick is dependent on a number of biotic and abiotic factors. The *Ixodes ricinus* tick is mesophilic, spring activity begins when the temperature rises to 4 to 5 °C (Hubálek et. al., 2003). The temperature required for the metamorphosis of the larva to the eggs is between 8 to 11 °C (Daniel, 1993). Activities *of Ixodes ricinus* correlate positively with a moisture content exceeding 80%, with the exception of parasitic hosting (Eisen and Lane, 2002).

Under the vertical occurrence, certain changes are detected, which originate from climate socio-economic factors. While Mačička (1955) indicates the occurrence of this tick at an altitude of 600–900 m asl in Slovakia, Bullová et al. (2008) monitored the incidence of *Ixodes ricinus* in Choč Mountain at a height of 1,230 m asl. Hrkľová (2010) describes the occurrence of all stages of the development of *Ixodes ricinus* on the area of Malinô Brdo, Veľká Fatra Mts at 1,044 m asl. Majláthová et al. (2015) reported the incidence of the tick above 1,400 m asl in the Veľká Fatra Mts and a relatively high density of *Ixodes ricinus* on sites up to an altitude of 1,000 m (60–120 ticks/an hour collection).

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The presence and density of tick´s hosts, and vegetation structure are important biotic factors for the incidence of ticks. The essential factor is primarily the structure of vegetation mainly within the habitat. Gray et al. (1998) describe the importance of the litter fall (fallen leaves) that protects ticks against cold and dry, and then creates optimal climatic conditions with high humidity. Some nymphs are lain down to the stage of rest generally in the upper layer of soil. Eisen and Lane (2002) have studied the ontogenesis of ticks in the laboratory and found spawning in a number of 1,000 eggs at *I. ricinus*.

Within the morphology frame of an ordinary tick a significant sexual dimorphism was observed in adults, the female body surface is covered with a scutum, which reaches from 1/3 to 1/2 the length of the body and allows the increased size of the body. Chelicerae responsible for penetration through the skin of the host are placed on the head at the terminal position. Paired cheliceras and pedipalps serve as sensory organs and centrally located *hypostom*, with backward pointing little teeth fixing the tick body in the skin of the host. On the first pair of limbs there is Haller organ which detects $CO_2$, heat and a host motion (Černý and Rosický, 1971).

*Ixodes ricinus* belongs to outer ticks. All developmental stages (larvae, nymphs and adults) are active blood-sucking forms. The female lays eggs in the topsoil or in fallen leaves where the ongoing development and metamorphosis of the individual stages run (Filippova, 1977). Tick belongs to temporary zooedaphone as geophiles i.e. edaphophils (Stašiov, 2006). Therefore ticks form part of zooedaphone, whom currently an increased attention is generally paid to (Stašiov, 2009; Stašiov and Svitok, 2014).This research of *Ixodes ricinus* was carried out in the soil type of Cambisol. Literary sources have not so far stated details relating to the soil pH and the occurrence and abundance of *I*xod*es ricinus.*

The main aim of the study is to determine what soil reaction (pH) occurs at locations with high abundance and density of incidence of the ordinary tick *I. ricinus*, thus on the locations with its optimal occurrence. Characterization of humus form and some properties of environment on localities with high tick abundance is the next goal.

## Materials and methods

The research was carried out on the plot of Škutovky, northwest of the village of Liptovská osada, in the Veľká Fatra Mts located at an altitude of 760 m asl. The research area has eastern exposure with an inclination of 5°. Dimensions of investigated meadows were 80 × 15 m and the edge of the forest parallel with the meadow was wide about six meters.

On weathered granite massif there were mostly sandy-loamy soils broaden. On the rocks crystalline core and also on our research areas Cambisol was developed containing three horizons. The land is inhabited by a number of organisms, while in general, the greater the number and diversity, the greater the stability of the soil as an ecosystem (Stašiov, 2006).

The factors that affect the life cycle of ticks include rock, soil, climate, geographical factors, plants and animals. Studied sites belong to temperate and cold climate areas. The territory has a mountainous precipitation regime with a total annual rainfall of 1,000 to 1,400 mm (Miklós, 2002), which ranks among the wettest areas of Slovakia. The average annual temperature was 7 °C. An average temperature in July was 16 °C and in January –5 °C.

Geological substrate, landforms, soil and climatic conditions of Veľká Fatra Mts, together with other factors have contributed to the development of diverse habitats. Different plant and animal species (Halaša and Šľachta, 1968) have developed on them. Mainly particular forest communities maintain its natural character which demonstrates the development of forest communities of the Carpathian type with the occurrence of numerous rare and endangered species. The forests were mixed *Acer pseudoplatanus*, *Acer platanoides*, *Fraxinus excelsior*, *Sorbus aucuparia*, *Tilia cordata* and *Tilia platyphyllos*. Beech forests occupied the largest forest land. Plant communities of rocky habitats with rare taxa were of a relict nature. Mountain orientation and limestone bedrock caused that more thermophilic species were spread in the Veľká Fatra Mts compared to other high mountains.

### Fieldwork

The research was focused on ecotone, i.e. a temporary band mixed, hardwood-conifer forest with rich undergrowth of scrub (a forest edge habitat) and a parallel meadow ecosystem (a meadow habitat). From April to November 2015, host seeking nymphal and adult ticks were sampled once a month depending on local weather conditions by the methods of flag dragging through vegetation using a flannel flag (1 × 0.7 m) in the morning considering the temporal and spatial appetence behaviour of ticks, i.e. during the highest questing activities (timed dragging). The number of ticks collected using the flag during one hour collection represented the apparent density of ticks. Timed dragging was conducted by teams of 4 or 5 persons, with each person dragging for a respective time, making together 1 hour. According to Peťko et al. (1996) an hour collection represents an area of 600 $m^2$ (in the pastures and woods with shrubs) to 800 $m^2$ (the meadows and parks).

Soil sampling was focused on surface soil whose characteristics most influence the abundance and a life cycle of ticks. For the research, one transect was chosen on the meadow and forest edge ecotones. On each transect three trench soil pits were dug up. Soil samples were taken from each horizon of the soil pit – surface humus horizon (the Oo horizon), followed by a layer of 5–10 cm (the A horizon) and a depth of 20–25 cm (the Bv horizon), making together 18 soil samples. Soil samples were dried at a laboratory temperature and processed according to the pH appropriate method.

#### Laboratory work

The soil pH was measured in a soil suspension. The common ratio of soil to water was 1:2.5 (10 g of soil to 25 ml of distilled water). At surface horizon organic matter, this ratio was 2:25 (2 g of soil sample was added to a 100 ml beaker and mixed with 25 ml of distilled water). After stirring, the soil sample suspension was left standing for 24 hours motionlessly. After this time, the soil pH was measured using multifunction equipment 340i WTW on 2 decimal places.

The data were analysed using the Statistical Package of SPSS 19.0. A data normal distribution was treated by the Kolmogorov-Smirnov test. When processing the pH data, basic statistical characteristics were calculated – an arithmetic mean, a standard deviation, coefficient of variation, variation margin, and a median. Since the tick data did not follow a normal distribution, variable was analysed using a nonparametric test. The difference in the tick abundance between the forest edge and meadow was tested for significance using the non-parametric Friedman test. A p-value of 0.05 was considered as statistically significant.

## Results

### The results of fieldwork

#### Meadow

The meadow had 100% coverage of grassland vegetation (grasses, herbs, mosses – *Entodon schreberi*). Humus form was turf mull. The Oon litter fall sub-horizon was practically absent, residues of dead plant organs occurred rarely. On this habitat, 3 trench soil pits were dug up with the following characteristics:

- Meadow 1 – the humus form was Mull, a thickness of Au was 11 cm.
- Meadow 2 – the humus form was Mull, a thickness of Au was 15 cm.
- Meadow 3 – the humus form was Mull, a thickness of Au was 17 cm.

Unlike the soil in ecotone (the forest edge), the Au horizon was weakly humic, gray-brown, sharply demarcated from the substrate horizon – this meadow was in the past probably plowed to a depth of 25 cm, a content of skeleton was about 20%.

The meadow average relative abundance of *Ixodes ricinus* was 2.1 ± 3.7 individuals per one dragging hour. Composition of ticks collected was 71.4% and 28.5% for nymphs and adults, respectively.

#### The edge of the forest

An ecotone coat of stand was overgrown with shrubs and hazel (*Corylus avellana)*, Goat willow (*Salix caprea)*, Sloe (*Prunus spinosa*) and Midland hawthorn (*Crataegus laevigata)*. In the undergrowth, there were forest and meadow grasses and herbs – St. John's Wort (*Hypericum perforatum)*, European sanicle – (*Sanicula europaea*) etc. The herbaceous layer covers 100% of the surface of the site.

On this stand compared to other habitats, the relatively high average abundance of 11.7 ± 9.6 was found, in the range of 9–104 ticks per one dragging hour. Composition of ticks collected was 85.4% and 14.5% for nymphs and adults, respectively. Results showed that this study area had convenient conditions for the tick life cycle. The research of seasonal dynamics has pointed to two peaks of the tick frequency over the studied period (Fig. 1). It is certainly linked with the occurrence of hosts in this habitat.

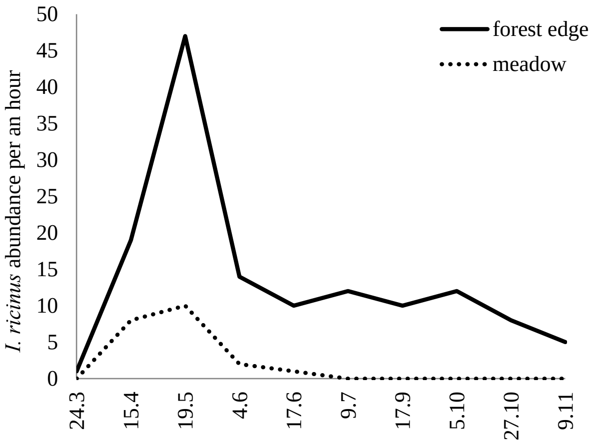


Fig. 1. Seasonal dynamics of *Ixodes ricinus* (timed dragging).

The difference in tick abundance between the forest edge and meadow habitats was statistically evaluated. Due to the fact that the result data did not follow a normal distribution (the Kolmogorov-Smirnov test), this difference was tested using the non-parametric Friedman test.

On forest edge habitat, 3 trench soil pits were dug up with the following characteristics:

- Edge of forest 1 – the Oon horizon was predominantly formed nearly with decomposed litter of grasses and shrubs. The humus form was a mull with a thickness of 1 cm. The Au horizon was 12 cm thick.
- Edge of forest 2 – the humus form was also mull with a total thickness up to 1 cm. In undergrowth synusia, in addition to herbs and grasses, there was also moss (*Entodon schreberi*) presented which might indicate a certain acidification. The Au horizon was 11 cm thick.
- Edge of forest 3 – the humus form was a mull with a thickness up to 1 cm. The Au horizon was 11 cm thick. The composition of the surface vegetation was analogous to the plots 1 and 2.

In the meadow ecosystem, there was Cambisol with surface diagnostic horizons Oo (sub-horizon Oon), Umbrian surface diagnostic horizons Au and subsurface diagnostic horizons Bv. The humus form was turf mull.

In ecotone of the forest edge, there was also Cambisol with surface diagnostic horizons Oo (sub-horizon Oon), Umbrian surface diagnostic horizons Au and subsurface diagnostic horizons Bv. The humus form was mull.

**The results of laboratory work**

The basic statistical characteristics of soil pH (Table 1) comprised mean data, standard deviations and coefficients of variation of different soil profiles present. Each value represents the arithmetic mean calculated from sub-horizon Oon and two mineral horizons (A, B). Thus the arithmetic mean was given by properties of organic Oo and organo-minerals A and mineral B horizons. Despite the different structure and composition of biota, results' variability was low. The coefficient of variation was less than 8%, the standard deviation "s", did not exceed a value given by the product of 0.43× pH and the span of pH arithmetic means for the Cambisol fluctuated in an interval of 5.4–5.8 (rounded).

Table 1. Statistical values of pH of trench probe, including Oo surface horizons

| Locality | Area | $\overline{x}$ | s | $v_k$ |
|---|---|---|---|---|
| Škutovky | Meadow 1 | 5.777 | 0.0902 | 1.56% |
| Škutovky | Meadow 2 | 5.780 | 0.2425 | 4.19% |
| Škutovky | Meadow 3 | 5.790 | 0.3959 | 6.83% |
| Škutovky | Forest edge 1 | 5.700 | 0.3905 | 6.85% |
| Škutovky | Forest edge 2 | 5.453 | 0.3066 | 5.62% |
| Škutovky | Forest edge 3 | 5.643 | 0.4206 | 7.45% |

$\bar{x}$, arithmetic mean; s, standard deviation; $v_k$, coefficient of variation.

The arithmetic mean, median, standard deviations, coefficients of variation and outliers for the Oon surface humus sub-horizon on individual research areas were compared (Table 2). This horizon was found on all areas and localities, therefore its values were compared with each other. In addition, it was a horizon whose properties were very important to the developmental cycle of the tick. It was formed of the litter which was already subjected to some changes and therefore this horizon differed terminologically and was designated as a horizon or a layer of litter. It was directly influenced by a composition of the litter (necromass) of biocenose (plants, herbs, grasses etc.). Analysis results rounded up to one decimal place were of very low variability (5.9 to 6.0 pH) with a coefficient of variation below 5%.

Table 2. Statistical pH values of Ool horizons of meadow habitats and forest edges

| Area | $\overline{x}$ | $\tilde{x}$ | s | $v_k$ (%) | Min-max |
|---|---|---|---|---|---|
| Meadow | 5.78 | 5.77 | 0.24 | 4.99 | 5.64–6.20 |
| Forest edge | 6.00 | 6.05 | 0.19 | 3.10 | 5.79–6.15 |
| Together | 5.93 | 5.91 | 0.21 | 4.04 | 5.64–6.20 |

$\bar{x}$, arithmetic mean; s, standard deviation, $v_k$, coefficient of variation.

The pH statistical characteristics of the trench soil pits were compared (Table 1), no statistically significant higher pH values were found on the forest edge (pH 6.00). Neither statistically significant lower pH values were found on the meadow, for more acid environment with a pH of 5.78 (Table 2).

Significance test of the arithmetic mean difference of the pH by using the analysis of variance is given in Table 3. The analysis confirmed that the difference of arithmetic mean of the pH on the research areas was not statistically significant and therefore, properties of areas are very similar. No significant differences were achieved with the 95% probability.

Table 3. Hypothesis testing the differences of arithmetic means of pH values at individual stations

| | Meadow 1 | Meadow 2 | Meadow 3 | Forest edge 1 | Forest edge 2 | Forest edge 3 |
|---|---|---|---|---|---|---|
| Meadow 1 | | 0.99503 | 0.98013 | 0.88612 | 0.54662 | 0.80334 |
| Meadow 2 | 0.99503 | | 0.98509 | 0.8812 | 0.54251 | 0.79854 |
| Meadow 3 | 0.98013 | 0.98509 | | 0.86649 | 0.53028 | 0.78416 |
| Forest edge 1 | 0.88612 | 0.8812 | 0.86649 | | 0.6453 | 0.91569 |
| Forest edge 2 | 0.54662 | 0.54251 | 0.53028 | 0.6453 | | 0.72279 |
| Forest edge 3 | 0.80334 | 0.79854 | 0.78416 | 0.91569 | 0.72279 | |

## Discussion

There are cumulative factors which influence the relation of the ticks and the host as possible vector of range spectra pathogens. Globally these factors influence the incidence of tick-transmitted diseases (Lindgren, 1998; Sarah and Jonathan, 2010). The non-parasitic stage of ticks requires the special condition of environment. The abiotic factors as temperature, precipitation influence plant communities, the host animals as well as the occurrence of people in landscapes. The metamorphosis of the ticks takes place in the surface layers of soil or forest litter (Daniel et al., 2015). Certainly the micro-environment influences the development of the stage of ticks and consequently their abundance. Despite of many works about influence of environmental condition on the ticks abundance there is missing a systematic study of the soil environment.

Tick metamorphosis begins in the Oon organic surface sub-horizon. From soil and genetic point of view, it is called the necrotic sub-horizon of slightly altered to strongly decomposed residues of forest and a meadow litter fall which represents stages of its decomposition and transformation in the direction from top to bottom (Societas pedologiga slovaca, 2014). The thickness of the Oo litter fall horizon is more than 3 mm, more than 20% of combustible organic matter and at least one of sub-horizons (Oon necrotic – Oof fermentation (detritus) – Ooh humic sub-horizon) is presented. Only Oon litter sub-horizon was found on our research areas, it is basically a layer of the last year's litter fall.

The humus form of mull that was found on our research plot was produced in mild to warm climate on soils adequately supplied with nutrients and sufficiently moist. Cambisol on our study areas was formed from non-carbonate parent rocks. Proper soil-forming substrate was rich in skeleton and therefore a sufficient mass of soil material was quite susceptible to weathering, thus constantly releasing nutrients. As for the grain size, the soil on the research plots was loamy according to a field trial.

Mountains orientation of a north-south direction, bedrock, soil and climatic conditions provided good conditions for thermophilic plant and animal species in the Veľká Fatra Mts. Favourable effect of temperature and soil moisture also explained why the ticks throve so well in this environment. Moreover, our research plots border with shrub undergrowth of hazel, hawthorn and blackthorn and just such an environment was according to current knowledge appropriate for the survival of ticks, their hosts and their developmental stages.

The pH is one of the most important properties of the soil as it affects not only the solubility of the substances in the soil and their utility for biota, but also the sorption of cations and the structure of the soil, and thus soil physical properties and biochemical reactions. Soil pH substantially affects living conditions for soil organisms, including ticks. Therefore one of the goals of our research was to determine what a soil reaction (pH) occurs at locations with a high abundance of ticks. It appeared that the pH of the Oon necrotic sub-horizon suited well to ticks in a confidence interval of pH 5.6–6.2 with a 95% probability that was in the slightly acidic range. Mentioned range may be considered convenient for the incidence of ticks.

In addition, we found that the pH of the Oon necrotic sub-horizon was in a very narrow range of arithmetic means (5.87 to 6.00). The coefficient of variation fluctuated around 4% and the standard deviation was equalled to 0.21× pH at a variation range 0.56× pH (Table 2). Extreme readings were in the range pH from 5.64 to 6.20. Below the Oo litter fall horizon, Cambisol was produced with a pH from 5.45 to 5.79 in a mineral soil in a depth of up to 25 cm, confirming again that a maximum of tick-abundance was in a slightly acidic pH. Since our investigations were the first study on the analysis of the tick distribution associated with a soil reaction, our genuine pH results couldn't be compared with those of literature sources.

The occurrence of the tick *Ixodes ricinus* was characterised by seasonally curve (Fig. 1) with two peaks in May and in October (the incidence of ticks was lower compared to that in May). This bimodal seasonal activity dynamics of host-seeking *I. ricinus* was also found in the study of Schwarz et al. (2009) who studied the tick distribution based on plant communities, with peaks in June and October. This shift of tick abundance peak in June may be given by local climatic conditions. *I. ricinus* starts its host-seeking activity at 2.5 °C and 45% relative humidity (Hubálek et al., 2003; Lindgren et al., 2000), that was why the tick lowest occurrence was recorded on 24 March. The abundance peaks were next associated with soil relative moisture, which was reflected in the statistically significant large difference in the average tick occurrence between the forest edge habitat (11.7 ± 9.6 ticks per hour) and the meadow habitat (2.1 ± 3.7 ticks per hour). The seasonal questing activity of *I. ricinus* may be associated with major variations in biotic (host species, host abundance and behavior, and vegetation structure) as well as abiotic factors (climate) (Gern et al., 2008). From this point of view, ticks were sampled by flagging at temperature up to 24 °C (Perret et al., 2000). The high air temperature above 24 °C leads to decrease in numbers of questing ticks due to the low desiccation resistance of ticks (Martin et al., 1990). Ticks descend more often from the vegetation into the soil to rehydrate. If ticks frequently have to leave their questing places due to rehydration, they will rapidly exhaust their energy reserves before they find a host and die (Gern et al., 2008). The relative humidity directly above the soil is given by the soil water content. As the ticks have to rehydrate in the soil, the number of collected ticks correlate to the soil water content (Guerra et al., 2002).

From July till the end of sampling period, no *I. ricinus* ticks were found on the meadow habitat which can be advocated by a dry period, while in the forest edge habitat with the higher relative soil moisture the numbers of collected ticks were above 10, except for November. This result was in coincidence with the finding of Maetzel et al. (2005) stating that no ticks were found in open habitats like agricultural fields. Similar seasonal dynamics were also found in other species zooedaphone (Macko, 2008; Rendoš et al., 2012).

Favoured habitats of this tick are coniferous and mixed deciduous woodlands (Lindstrom and Jaenson, 2003). The tree composition of a forest is determined by a moisture gradient involving soil aeration, soil nutrient supply, and microclimatic features (Curtis, 1959). The factors interacting at the microclimatic level within the topsoil and leaf litter sub-horizon appeared to have an important influence on tick survival. Soil texture, in addition to the topography, determines the extent of drainage, and the level of moisture of the ground layer (Guerra et al., 2002). Excessive moisture in the soil may be deleterious to tick survival since they overwinter in the topsoil and leaf litter sub-horizon. Leaf litter is a requisite component for the survival of immature stages of ticks (Schulze et al., 1995; Gray et al., 1998).

Ticks are found everywhere where environmental factors are suitable to them. With climate warming, ticks are even getting to higher positions above 1,000 m asl. In the course of research, a rare occurrence of ticks was confirmed even in the NNR Skalná Alpa at an altitude of 1,450 m asl.

It was discovered that edges of forests satisfied ticks in a better way, thus forest ecotone is more convenient for them than meadow ecosystems (Maetzel et al., 2005). Ticks live mainly on the edges of deciduous and coniferous forest in the shrub and herbaceous undergrowth. Especially nymphs use an Oon sub-horizon (a layer of litter) for its development. (Schulze et al., 1995).

## Conclusion

By assessment of selected abiotic factors, soil pH, composition of forest ecotones and meadows in the mountain area of Veľká Fatra Mts, we have come to a finding that they provide favourable conditions for the occurrence of ticks and a real danger for the development of infections of humans and animals (grazing) – Lyme disease, tick-borne encephalitis, anaplasmosis and other tick-borne infectious and parasitic diseases of humans. Forest ecotone satisfies all the requirements needed to maintain the life cycle of the tick. Its conditions are very suitable for the survival of hosts needed for the livelihood of ticks. As these were the first and original results of ecopedological conditions for the occurrence of the tick *Ixodes ricinus*, further research is needed to clarify our findings.

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## References

Bullová, E., Lukáň, M., Víchová, B., Majláthová, V., Peťko, B., 2008. Kliešť obyčajný (Ixodes ricinus) vo vyšších nadmorských výškach – nové riziko turistických oblastí na Slovensku? [Common tick (Ixodes ricinus) at higher altitudes – the risk of new tourist areas in Slovakia?]. In *VIII. české a slovenské parazitologické dny. 19. – 23. května 2008, Sezimovo Ústí. Sborník abstraktů*. České Budějovice: Česká parazitologická společnost, p. 13.

Curtis, J.T., 1959. *The vegetation of Wisconsin*. Madison: University Wisconsin Press. 637 p.

Černý, V., Rosický, B., 1971. Podřád Klíšťata-Ixodides [Tick subgroup of Ixodes]. In Daniel,M., Černý, V. (eds). *Klíč zvířeny ČSSR IV*. Praha: Academia. 604 p.

Daniel, M., 1993. Influence of the microclimate on the vertical distribution of the tick Ixodes ricinus in central Europe. *Axarologica*, 34: 105–113.

Daniel, M., Malý, M., Danielová, V., Kříž, B., Nuttal, P., 2015. Abiotic predictors and annual seasonal dynamics of Ixodes ricinus, the major disease vector of Central Europe. *Parasites and Vectors*. 8, 478:1–19.

Eisen, L., Lane, R.S., 2002. Vectors of Borrelia burgdorferi sensu lato. In Gray, J., Kahl,O., Lane, R.S., Stanek, G. (eds). *Lyme borreliosis: biology and control*. Wallingford: CABI, p. 91–116.

Filippova, N.A., 1977. Iksodovyje klešči podsem. Ixodinae. Fauna SSSR [Ixodid ticks of the subfamily Ixodinae. Fauna of the U.S.S.R.]. In *Paukoobraznyje, IV (4)*. Leningrad: Nauka, 396 p.

Gern, L., Cadenas, M., Burri, C., 2008. Influence of some climatic factors on Ixodes ricinus ticks studied along altitudinal gradients in two geographic regions in Switzerland. *International Journal of Medical Microbiology*, 298(S1): 55-59.

Gray, J.S., Kahl, O., Robertson, J.N., Daniel, M., Estrada-Pena, A., Gettinby, G.,Jaenson, T.G.T., Jensen, P., Jongejan, F., Korenberg, E., Kurtenbach, K., Zeman, P., 1998. Lyme borreliosis habitat assessment. *Zentralblatt für Bacteriologie. International Journal of Medical Microbiology*, 287: 253–265.

Guerra, M., Walker, El, Jones, C., Paskewitz, S, Cortinas, R., Stancil, A, Beck,L.,Bobo, M., Kitron, U., 2002. Predicting the risk of Lyme disease: habitat suitability for Ixodes scapularis in the North Central United States. *Emerging Infectious Disseases*, 8: 289–297.

Halaša, J., Šľachta, F., 1968. *Veľká Fatra* [Veľká Fatra Mts]. Bratislava: Obzor. 168 p.

Hrkľová, G., 2010. *Vybrané ekologické a epidemiologické aspekty kliešťov v horských oblastiach Slovenska* [Selected ecological and epidemiological aspects of ticks in mountain areas in Slovakia]. Košice: Prírodovedecká fakulta UK v Bratislave, Parazitologický ústav SAV. 121 p.

Hubálek, Z., Halouzka, J., Juřicová, Z., 2003. Host--seeking activity of ixodid ticks in relation to weather variables. *Journal of Vector Ecology,* 28, 2: 159-165.

Lindgren, E., 1998. Climate and tick-borne encephalitis. *Conservation Ecology* (online), 2 (1): 5.

Lindgren, E., Tellekling, L., Polfeldt, T., 2000. Impact of climatic change on the northern latitude limit and populations density of the disease-transmitting European tick Ixodes ricinus. *Environmental Health Perspectives,* 108: 119–123.

Lindstrom, A., Jaenson, T.G.T., 2003. Distribution of the common tick, Ixodes ricinus (Acari: Ixodidae), in different vegetation types in Southern Sweden. *Journal of Medicinal Entomology,* 40: 375–378.

Macko, J., 2008. Pôdna fauna 8 LVS Západných Tatier [Soil fauna of 8 forest Vegetation zones in the Western Tatras]. *Phytopedon (Bratislava),* 7 (1–2): 149–154.

Mačička, O., 1955. O výškovom rozvrstvení kliešťa obyčajného (Ixodes ricinus L. 1758) vo Vysokých Tatrách [About vertical stratification of tick common (Ixodes ricinus L. 1758) in the High Tatras]. *Zoologické a Entomologické listy,* 4: 384–388.

Maetzel, D., Maier, W.A., Kampen, H., 2005. Borrelia burgdorferi infection prevalences inquesting Ixodes ricinus ticks (Acari: Ixodidae) in urban and suburban Bonn, western Germany. *Parasitology Research,* 95: 5–12.

Majláthová, V., Majláth, I., Kočíková, B., Vargová, B., Remetová, L., Molčány, T.,Raši, T., Peťko, B., 2015. Kliešte a kliešťami prenášané patogény vo vysokohorských oblastiach [Ticks and tick-borne pathogens in mountain areas]. In *Parazity v mestách pod vplyvom globálnych zmien. Zborník abstraktov. Košice 28. – 29. 5.* Košice: Slovenská akadémia vied, Parazitologický ústav. 35 p.

Martin, P., Bigaignon, G., Gillion, P., Thirion, A., Fain, A., 1990. Fréquence de Borrelia burgdorferi (Maladie de Lyme) et répartition de son vecteur Ixodes ricinus (Acari: Ixodidae) dans le district mosan en Belgique. *Bulletin de la Société Française de Parasitologie*. 8 : 331–338.

Miklós, L., 2002. *Atlas krajiny Slovenskej republiky* [Landscape atlas of the Slovak Republic]. Bratislava: Ministerstvo životného prostredia SR. 342 p.

Perret, J.L., Guigoz, E., Rais, O., Gern, L., 2000. Influence of saturation deficit and temperature on Ixodes ricinus tick questing activity in a Lyme borreliosis-endemic area (Switzerland). *Parasitology Research,* 86: 554–557.

Peťko, B., Štefančíková, A., Tresová, G., Peterková, J., Škardová, I., Prokopčáková,H., Čisláková, L., 1996. Kliešť obyčajný (Ixodes ricinus) ako zdroj infekcie ľudí a psov pôvodcom lymeskej boreliózy [Common tick (Ixodes ricinus) as the source of infection of humans and dogs, originator of Lyme disease]. *Slovenský Veterinársky Časopis,* 21: 313–319.

Rendoš, M., Mock, A., Jászay, T., 2012. Spatial and temporal dynamics of invertebratesd welling karstic mesovoid shallow substratum of Sivec National Nature Reserve (Slovakia), with emphasis on Coleoptera. *Biologia, Bratislava,* 67: 1143–1151.

Sarah, H.O., Jonathan, A.P., 2010. *Global environment change and tick-borne disease*. *Draft background paper written to stimulate discussion for the Institute of Medicine Committee on Lyme Disease and Other Tick-Borne Diseases: The State of the Science workshop entitled: "Critical needs and gaps in understanding prevention, amelioration, and resolution of lyme and other tick-borne diseases: The short-term and long-term outcomes". Washington, D. C., October 11-12, 2010*. National Academy of Sciences (US) [cit. 2015-12-20].

Schwarz, A., Maier, W.A., Kistemann, T., Kampen, H., 2009. Analysis of the distribution of the tick Ixodes ricinus L. (Acari: Ixodidae) in a nature reserve of western Germany using geographic information. *International Journal of Hygiene and Environmental Health,* 212: 87–96.

Societas pedologica Slovaca, 2014. *Morfogenetický klasifikačný systém pôd Slovenska:bazálna referenčná taxonómia* [Morphogenetic soil classification system of Slovakia: basal reference taxonomy]. 2nd ed. Bratislava: Výskumný ústav pôdoznalectva a ochrany pôdy, 2014. 96 p.

Stašiov, S., Kováč, Ľ., Krumpál, M., 2006. *Ekológia pôdnych organizmov: metódy výskumu mezo- až megazooedafónu* [Ecology of soil organisms: methods of meso- and mega-zooedaphon research]. Zvolen: Technická univerzita vo Zvolene. 70 p.

Stašiov, S., 2009. Millipede (Diplopoda) communities in mixed oak-hornbeam forest stands:effect of selected site factors. *Polish Journal of Ecology,* 57: 785–792.

Stašiov, S., Svitok, M., 2014. The influence of stand density on the structure of centipede (Chilopoda) and millipede (Diplopoda) communities in the submountain beech forest. *Folia Oecologica*, 41: 195–201.

Schulze, T.L., Jordan, R.A., Hung. R.W., 1995. Suppression of subadult Ixodes scapularis (Acari: Ixodidae) following removal of leaf litter. *Journal of Medicinal Entomology,* 32:730–733.

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# Bioindication value of tar spot on maple trees in industrial areas: the case of Ostrava region, the Czech Republic

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**Abstract**
Náplavová, K., Gáper, J., 2016. Bioindication value of tar spot on maple trees in industrial areas: the case of Ostrava region, the Czech Republic. *Folia Oecologica*, 43: 183–192.

*Rhytisma acerinum* is considered to be a bioindicator of air quality and therefore the occurrence of tar spot corresponding with the level of site pollution can be used as a tool for estimation of environmental pollution. The aim of this study was to assess the bioindication value of individual maple taxa. The research was established on fieldwork in the City of Ostrava (Czech Republic) and on the investigation of 1,247 trees. Four main habitat types were selected according to assumed (high or low) levels of air pollution and type of vegetation and land use. Different occurrence of symptoms of fungal pathogen in different categories of vegetation was found. Our analysis provides evidence that trees with lower diameter at breast height (DBH) suffered from higher infestation of tar spot. Airborne dust ($PM_{10}$) was identified as the air pollutant with the significant negative effect on stroma occurrence. Our results also reveal that infestation of maple leaves was significantly affected by cultivar. Therefore the most susceptible taxa to tar spot (*Acer pseudoplatanus*, *A. pseudoplatanus* ‘Atropurpureum’, *A. platanoides* ‘Cleveland’, *A. platanoides* ‘Globosum’) can be the best candidates for monitoring air pollution.

**Key words**
*Acer*, air pollution monitoring, *Rhytisma acerinum*, urban areas

## Introduction

Tar spot occurring on the surface of maple leaves is caused by parasitic fungus – *Rhytisma acerinum* (Pers.) Fr. It is one of the most easily distinguishable fungal leaf diseases and it is distributed throughout temperate northern hemisphere. It belongs to Ascomycota and forms black flat macroscopic stromata on the leaf blades of maple trees (Weber and Webster, 2002). Detailed biology of this fungal pathogen has been previously described (Jones, 1925; Hudler et al., 1987; Weber and Webster, 2002). It overwinters on fallen leaves and then infects adaxial leaf surface in spring during the moist conditions. Leaf spots are first yellowish green but by mid to late summer a tar-like appearance occurs. Stromata are present mainly on leaves of *Acer pseudoplatanus* L. (Sycamore maple) and *Acer platanoides* L. (Norway maple) and less often in industrial and urbanized areas (Sutton, 1980; Farr et al., 1989; Weber and Webster, 2002; Mihál, 2004; Kosiba, 2007). Several studies were dedicated to examining effects of environmental pollution on tar spot distribution (Bevan and Greenhalgh, 1976; Vick and Bevan, 1976; Greenhalgh and Bevan, 1978; Gosling

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et al., 2016). LEITH and FOWLER (1987) and GOSLING et al. (2016) also investigated other factors influencing its occurrence such as presence of overwintered leaf material or the exposure to wind. None of those studies was considering host specificity of tar spot among maple cultivars. So the hypothesis of the present study was that individual maple taxa have different bioindication value. It was based on monitoring of tar spot symptoms and its occurrence in the area of Northern Moravia, Czech Republic (especially in the surroundings of Ostrava City), evaluating its distribution through different categories of vegetation and examining if ontogeny of host trees plays any role in stroma quantity.

Currently one of the most pressing problems of the environment of Ostrava City, as an industrial agglomeration, is the bad air quality. The big industrial factories, engineering and metallurgical companies, automobile traffic and small emission sources have significant influence on air pollution (ZDRAVOTNÍ ÚSTAV, 2008).

## Material and methods

The research was carried out during 2 years (2013 and 2014) in the area of Ostrava City districts (Fig. 1). Study areas have been selected according to the nature character and land use.


Fig. 1. A scheme of the Ostrava City districts area (www.ostrava.cz, 49°50’08”N, 18°17’33”E, area 214 km$^2$).

## List of study areas

*“Moravská Ostrava a Přívoz” city district*
parks – Dr. Milada Horáková Park, Komenského sady Park, Bezručův sad Park, Husův sad Park; housing estates – cross roads of Českobratrská, Hornopolní, Varenská Streets, housing estate at Vítkovická Street close to Kaufland supermarket, area around Ostrčilova Sreet, area in front of Librex house near Černá louka exhibition ground, Jilemnice Square, area next to Aquapark Vodní svět situated close to Sokolská třída Street; parking lots – Soukenická Street, Varenská Street around Futurum shopping mall, area in front of Kaufland supermarket close to Vítkovická Street, area next to elementary school Ostrčilova; low traffic roads – Na jízdárně Street, Veleslavínova Street, Hus Square, Blahoslavova, Žerotínova, Gregorova, Budečská, Sadová Streets; high traffic roads – Českobratrská Street close to Janáček conservatory, Novinářská Street in front of Futurum shopping mall, 28. října Street between tram stops Dům energetiky and Krajský úřad, Nádražní Street, area around Černá louka exhibition ground;

*“Slezská Ostrava” city district*
part of Trojické Valley near to Ema heap, private gardens – Na Najmanské, Hýbnerova, Občanská, Keltičkova Streets; parking lot – close to Hladnovská Street in front of Penny hypermarket and Tylův sad Park;

*“Mariánské hory a Hulváky” city district*
part of Hulvácký Forest along U koupaliště Street; Smetanův sad Park; private garden at Boleslavova Street; housing estate near Rtm. Gucmana, Gen. Hrušky and Pflegrova Streets, between Vršovců and Václavská Streets; parking lot near Grmelova Street in front of Kaufland and Scontomarkets; along high traffic road – Přemyslovců Street;

*“Ostrava - Jih” city district*
part of Bělský Forest; park within Gen. Svoboda Park; private gardens – Chalupníkova, Klegova Streets; housing estates close to areas around Provaznická and Klegova Streets; parking lot between Horní and Jaromíra Matuška Streets; low traffic roads – Česká, Slezská, Klegova, Chalupníkova, Beskydská Streets; medium traffic road – u Haldy Street;

*“Svinov” city district*
greenery of parkland type between Stanislavského and Jandáskova Streets near Husův sbor Church, area around Dr. Braun Square, Sad míru Park; private garden at Polanecká Street; housing estate around Bílovecká Street; low traffic load along Kuršova Street; medium traffic load along Bílovecká Street;

*“Poruba” city district*
parks near Nálepka Square and Havlíček Square; greenery of parkland type – Španielova Street, area close to elementary school at Ukrajinská and Alžírská Streets; housing estate near to Mongolská Street; parking lots at Alšova, Kopeckého, Bulharská, Francouzská Streets; low traffic roads – Bohuslava Martinů, Čs. exilu, Budovatelská, Urxova, V závětří, Španielova Streets; medium traffic loads – Polská, Francouzská Streets, Nábřeží Svazu protifašistických bojovníků promenade; along high traffic roads – 17. listopadu, Porubská, Opavská Streets;

*"Nová Ves" city district*
area along 28. října road aprox. 400 m from tram stop Nová Ves vodárna direction to Svinov;

*"Polanka nad Odrou" city district*
part of Protected Landscape Area Poodří; greenery of parkland type next to Atlas house close to the bus stop Hraničky and Václav Nehýbl Park, couple of private gardens.

The only helpful source of information about occurrence of maple taxa in Ostrava City was a map portal (www.stromypodkontrolou.cz) which includes several parameters (species, diameter at breast height – DBH, tree height, health status and recommended treatment technology) about trees in the area of different city districts. Fieldwork included quantification of tar spot infection. This part of the research was conducted during the period of 14 days in 2013 (30 September–13 October) and 12 days in 2014 (3–14 October). Measurement of the DBH was carried out during the period of mid-October to early December. In addition, the estimation of total leaf number of each tree has been done. All gained data were supplemented by information about air pollution taken from official web pages of the Czech Hydrometeorological Institute (Chmi, 2014).

We divided number of infected leaves by total estimated number of leaves on each tree to allow comparison between different-sized trees. The relationship between the relative proportion of tar spot as response variable and the particular tree species, habitat type, DBH, concentration of $PM_{10}$ (air pollutant) and year was examined using a generalized linear model with binomial error distribution and logit link function. Standard errors were corrected using quasi-likelihood function. Step-wise selection based on the lowest AIC was used to choose the best transformation function of the explanatory variable. We used an F-test to determine the significance of each variable because it is a robust test for overdispersed data. Data were analysed using R software (R Development Core Team, 2013).

## Results

Out of 18 different maple taxa in total number of 1,247 tree individuals, 510 trees were characterized by presence of symptoms of tar spot, 737 were with no sign of infection in 2013. During 2014 there were 591 maples infected and 656 non-infected trees. The occurrence of symptoms was recorded on 9 taxa as well as 9 other taxa were asymptotic (Table 1). *Acer pseudoplatanus*, then *A. pseudoplatanus* 'Atropurpureum' and *A. platanoides* 'Cleveland' were taxa with the highest infestation (Fig. 2, Table 2). Significantly higher infection by tar spot was observed in 2014 (Fig. 3, Table 2).

Table 1. Overview of taxa frequency (with and without tar spot symptoms) recorded in Ostrava City during 2013 and 2014

| | | Occurrence | | | |
|---|---|---|---|---|---|
| | | 2013 | | 2014 | |
| Taxon | Total | yes | no | yes | no |
| *Acer campestre* L. | 71 | 0 | 71 | 0 | 71 |
| *Acer campestre* 'Elsrijk' | 90 | 0 | 90 | 0 | 90 |
| *Acer negundo* L. | 114 | 0 | 114 | 0 | 114 |
| *Acer negundo* 'Variegatum' | 5 | 0 | 5 | 0 | 5 |
| *Acer palmatum* Thunb. | 45 | 0 | 45 | 0 | 45 |
| *Acer palmatum* 'Dissectum' | 3 | 0 | 3 | 0 | 3 |
| *Acer platanoides* 'Cleveland' | 74 | 30 | 44 | 55 | 19 |
| *Acer platanoides* 'Columnare' | 3 | 0 | 3 | 3 | 0 |
| *Acer platanoides* 'Crimson King' | 36 | 9 | 27 | 17 | 20 |
| *Acer platanoides* 'Deborah' | 43 | 21 | 22 | 34 | 9 |
| *Acer platanoides* 'Drummondii' | 19 | 0 | 19 | 0 | 19 |
| *Acer platanoides* 'Globosum' | 424 | 347 | 77 | 366 | 57 |
| *Acer platanoides* 'Schwedleri' | 25 | 3 | 22 | 7 | 18 |
| *Acer pseudoplatanus* L. | 78 | 65 | 13 | 70 | 8 |
| *Acer pseudoplatanus* 'Atropurpureum' | 40 | 31 | 9 | 35 | 5 |
| *Acer pseudoplatanus* 'Leopoldii' | 4 | 4 | 0 | 4 | 0 |
| *Acer saccharinum* L. | 90 | 0 | 90 | 0 | 90 |
| *Acer tataricum* subsp. *ginnala* (Maxim.) Wesm. | 83 | 0 | 83 | 0 | 83 |
| Total | 1,164 | 510 | 654 | 591 | 573 |

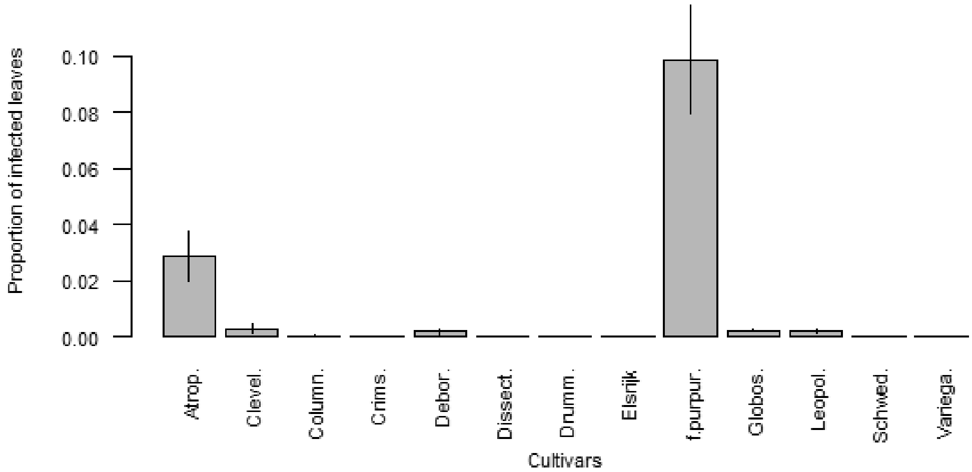


Fig. 2. Relative proportion of infected leaves by tar spot – *Rhytisma acerinum* according to individual taxa of *Acer* spp.; Atrop. – *A. pseudoplatanus* 'Atropurpureum', Clevel. – *A. platanoides* 'Cleveland', Column. – *A. platanoides* 'Columnare', Crims. – *A. platanoides* 'Crimson King', Debor. – *A. platanoides* 'Deborah', Dissect. – *A. palmatum* 'Dissectum', Drumm. – *A. platanoides* 'Drummondii', Elsrijk – *A. campestre* 'Elsrijk', f. purpur. – *A. pseudoplatanus,* Globos. – *A. platanoides* 'Globosum', Leopol. – *A. pseudoplatanus* 'Leopoldii', Schwed. – *A. platanoides* 'Schwedleri', Variega. – *A. negundo* 'Variegatum'.

Table 2. Result of testing of significancy of explanatory variables

| | Df | Deviance | AIC | F-value | Pr (>F) | |
|---|---|---|---|---|---|---|
| <none> | | 164365 | 168223 | | | |
| cult | 12 | 324480 | 328313 | 133.538 | < | 0.001*** |
| year | 1 | 337082 | 340937 | 1,728.582 | < | 0.001*** |
| microloc | 10 | 196408 | 196408 | 32.069 | < | 0.001*** |
| loc | 6 | 190082 | 193927 | 42.895 | < | 0.001*** |
| bs(d) | 3 | 187860 | 191712 | 78.381 | < | 0.001*** |
| alike | 3 | 175955 | 179806 | 38.664 | < | 0.001*** |
| $PM_{10}$ | 1 | 166738 | 170593 | 23.747 | < | 0.001*** |

cult – maple taxa, year – year of tar spot symptoms observation, microloc – microlocality, loc – locality (different Ostrava City districts), bs(d) – trunk diameter, alike – generalized categorization of habitats, $PM_{10}$ – airborne dust $PM_{10}$.

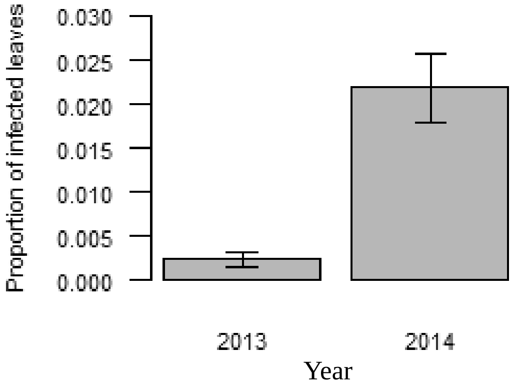


Fig. 3. Comparison of relative proportion of infected leaves by tar spot – *Rhytisma acerinum* during 2013 and 2014.

The highest infection rate was observed in Protected Lanscape Area (Fig. 4). For better comparison of natural and urbanized sites all the studied habitat microlocalities were divided into 4 categories (Fig. 5) according to similarities and character of the landscape: "forest" (contains forest-like habitat type – suburban forest, Protected Landscape Area), "ornamental" (includes parks, greenery of parkland type, private gardens), "traffic" (summarizes areas along weakly, moderately and highly frequented roads) and "urban" (contains microlocalities of housing estates and parking lots). Microlocality containing 4 individuals of *Acer pseudoplatanus* (with red-violet underside) growing along the agricultural area was not incorporated into any category. Generalized linear model revealed that habitat type significantly affected tar spot infestation among different maple taxa (Table 2).

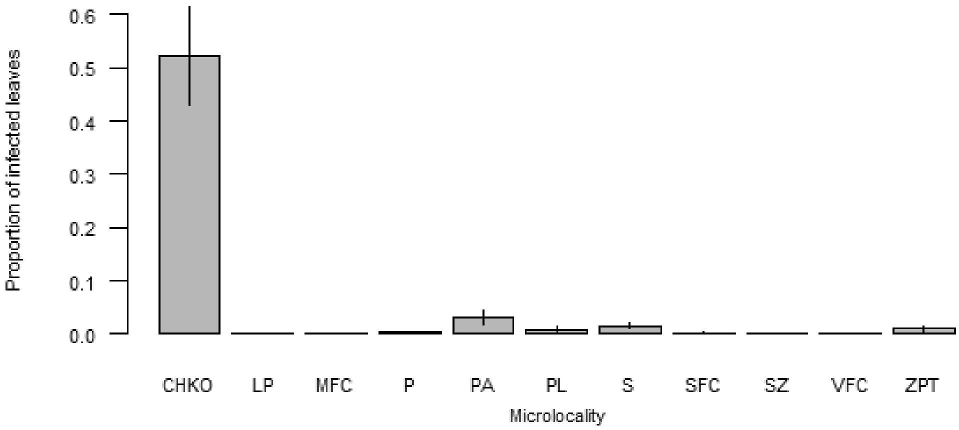


Fig. 4. Relative proportion of infected leaves by tar spot – *Rhytisma acerinum* according to the habitat type (microlocality); CHKO – Protected Landscape Area, LP – recreational forest, MFC – along medium traffic road, P – parking lot, PA – park, PL – suburban forest, S – housing estate, SFC – along low traffic road, SZ – private property (garden), VFC – along high traffic road, ZPT – greenery of parkland type.

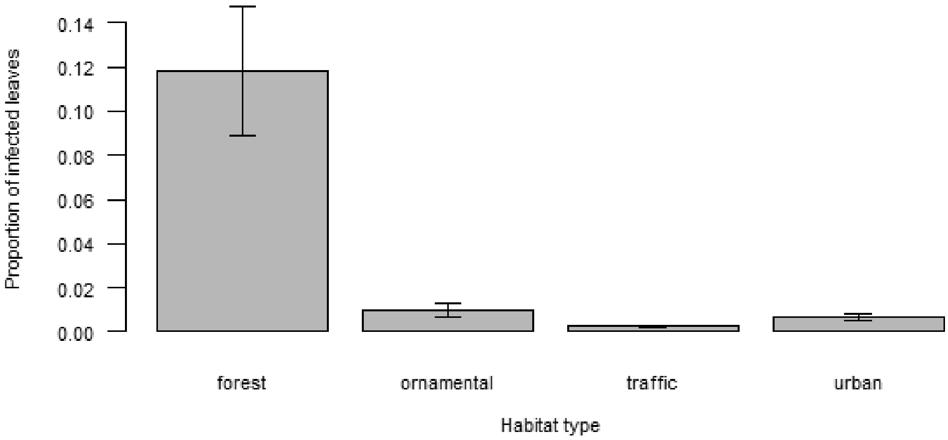


Fig. 5. Relative proportion of infected leaves by tar spot – *Rhytisma acerinum* according to generalized categorization of habitats.

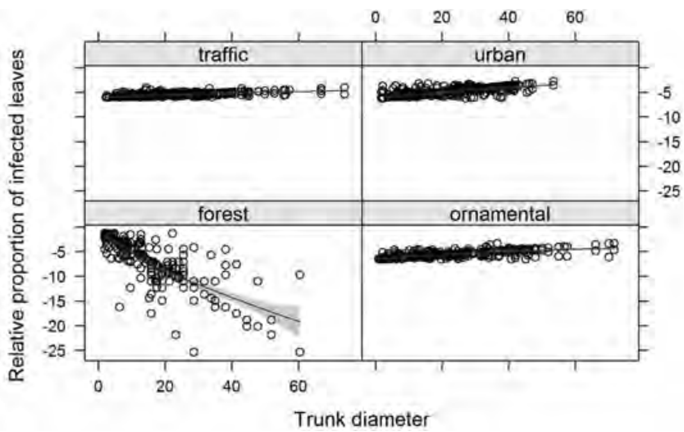


Fig. 6. Relative proportion of infected leaves by tar spot – *Rhytisma acerinum* according to the trunk diameter (DBH) and generalized categorization of habitats

Next we evaluated dependence of symptoms of tar spot on ontogeny of host trees by measuring tree girth. The result showed (Fig. 6, Table 2) that bigger trunk diameter correlates with lower quantity of stromata (non-linear dependence), especially in areas closer to the nature.

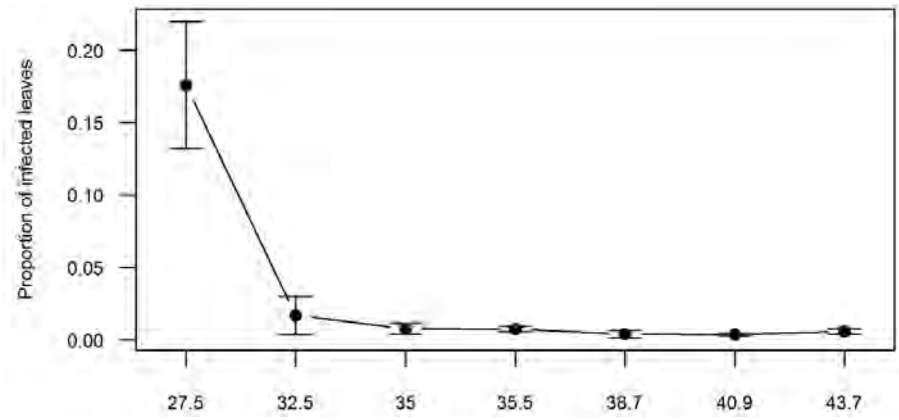


Fig. 7. Relative proportion of infected leaves by tar spot – *Rhytisma acerinum* according to the average annual concentration of $PM_{10}$ (µg $m^{-3}$).

In addition, we studied the annual average concentration of airborne dust ($PM_{10}$) in the City of Ostrava and its surroundings. We found that lower concentrations of $PM_{10}$ correlate with higher degree of infestation (Fig. 7, Table 2).

**Discussion**

**Habitat type**

Our survey found different distribution of tar spot dependent on microlocalities. Rate of infestation was the highest in the Protected Landscape Area (CHKO). This result is consistent with fact that infection occurs mainly in natural areas (Weber and Webster, 2002). This microlocality is also close to the Odra River, where moisture and shade play a positive role in infection (Hudler et al., 1987; Leith and Fowler, 1987; Kosiba, 2007).

Generally, less infected leaves were found in areas of urban type. This may be influenced by density of tree growth. Lower incidence of stromata is expected in solitary individuals or alleys when individuals are exposed to the high insolation, wind and generally low air humidity compared to individuals located in dense forest communities in wet and shaded areas (Kosiba, 2007). It could be caused by the maintenance of urban greenery when removal of fallen leaves is regularly practiced in fall because lack of fallen leaves reduces potential of infection by tar spot (Healy, 2007; Gillman, 2011). The infection was higher in Protected Landscape Area than in areas located in the city center, where parks, gardens and other greenery are periodically maintained.

We observed significantly higher infection in 2014. This is probably due to different amount of precipitation. There was higher rainfall two months before actual observation of stroma quantity in 2014 (Table 3).

Table 3. The average monthly precipitation in 2013 and 2014 in Ostrava City (mm)

| Year/Month | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2013 | 65.70 | 37.75 | 51.50 | 24.20 | 113.75 | 133.50 | 15.15 | 44.80 | 90.10 | 21.50 | 27.80 | 14.05 |
| 2014 | 25.60 | 20.80 | 19.75 | 35.70 | 106.95 | 59.05 | 73.30 | 131.10 | 91.15 | 48.60 | 34.65 | 22.85 |

Warm and wet winters cause decay of sclerotia and failure of germination in spring (Leith and Fowler, 1987). On the other hand humidity and rainy weather are favorable conditions (Heald, 1926; Manion, 1981). But although rain and humidity are the key factors to the outbreak, they must be present in small amounts and in regular intervals (Kosiba, 2007). Other factors influencing infection of maple leaves by tar spot such as moisture on the leaf surface, wind speed, temperature are described by Bevan and Greenhalgh (1976).

Also it is known that drying of the tree extends from the top to the bottom, so leaves located closer to the surface should have a higher probability of germination of ascospores than leaves located on the upper parts of the tree (Leith and Fowler, 1987). This fact was not taken into account but on the basis of observation during the fieldwork we can assume that this is so.

The external stimulus is needed for ascospore release from the wet mature stromata, such as shaking of the leaf or falling drops of water onto the stromatal surface (Leith and Fowler, 1987). This fact may explain fewer infections in areas protected from wind and rain or, on the contrary, the greater rate in sites with higher human activity, in housing estates or in parks.

**Maple species**

In our study there were tar spot attacks during both years at the species level only on *Acer pseudoplatanus* with reddish underside. *Rhytisma acerinum* parasitizes *Acer platanoides* L. (Norway maple), *A. pseudoplatanus* L. (sycamore), *A. saccharum* Marshall (sugar maple), *A. macrophyllum* Pursh (big-leaf maple), *A. rubrum* L. (red maple) and on *A. saccharinum* L. (silver maple), (Edmonds et al., 2000). However, recent studies reveal that a new species of leaf-spotting fungus occurring in America – *Rhytisma americanum* Hudler & Banik was found on red maple and silver maple (Hudler et al., 1998; Hsiang and Tian, 2007), (in accordance with result – 90 individuals of silver maple with no spots). Box elder (*Acer negundo* L.) was reported to host *R. americanum* (Healy, 2007). On the other hand, there are reports that on box elder *Rhytisma punctatum* (Pers.) Fr. parasitizes (Edmonds et al., 2000).

In our investigation symptoms were not observed on *Acer campestre* L. (field maple), however, it can also be a host of tar spot (Hsiang and Tian, 2007). According to Fungal Databases (Farr and Rossman, 2014) symptoms were registered in Denmark, Germany, Greece, Iran, Ukraine and United Kingdom. The Fungal Records Database of Britain and Ireland – hereafter FRDBI (Kirk and Cooper, 2014) also contains records about occurrence of symptoms. Out of total 71 individuals none of them was infected. This could be explained by a series of environmental interactions – altitude, temperature, precipitation, etc. On *Acer tataricum* subsp. *ginnala* (Maxim.) Wesm. (Amur maple) there were no stromata but Gilman and Watson (1993a) and Gillman (2011) reported that black spots and other leaf blisters on this maple species occurred. Fungal Databases (Farr and Rossman, 2014) confirmed its presence in Canada and China. On the other hand, FRDBI does not contain any information about organisms associated with this maple species.

**Maple cultivars**

Proportion of infected leaves by tar spot according to the individual taxa of *Acer* spp. was the highest on *Acer pseudoplatanus* (formerly known as *Acer pseudoplatanus* f. *purpureum*). *Acer pseudoplatanus* f. *purpureum* has been a form of sycamore which differs in leaf color of the reverse side (red-violet color) and it is located mainly in microlocalities of forest type. High infection rate was also found on *Acer pseudoplatanus* 'Atropurpureum' cultivar, probably due to interaction with the environment. These two maple taxa can be considered as the best indicators of air pollution (along with Norway and sycamore maples). The infection rate of the disease is influenced by microclimate and stress from the external environment as well as by tree genetic factors (Douglas, 2009).

Almost all cultivars of Norway maple are infected regularly by tar spot and by a variety of other leaf spots (Gilman and Watson, 1993b-k). In this research stromata were found on all the Norway maple cultivars except for *Acer platanoides* 'Drummondii'. Only 5 specimens of cultivar *Acer negundo* 'Variegatum' were investigated. We can assume that if there is no infection on the species *A. negundo,* then probably symptoms will not occur on its cultivar *A. negundo* 'Variegatum' either. However, the literature indicates (Gilman and Watson, 1993l), that the black spots on box elder were documented. Also Fungal Databases (Farr and Rossman, 2014) contain records of tar spot on box elder, but only from Canada. On the other hand, FRDBI (Kirk and Cooper, 2014) does not include any data about box elder infection. Regarding *A. negundo* 'Variegatum' similar cultivar – *A. negundo* 'Elegans' was observed with tar spot (Gilman and Watson, 1993m).

*Acer palmatum* 'Dissectum' was present in a number of only 3 specimens. Again, we can assume that if tar spot symptoms appear on none of *A. palmatum* individuals (Japanese maple), symptoms will not occur on its cultivar either. This is also cultivar primarily bred for high ornamental value, so it would be undesirable to obtain lower aesthetic quality due to black spots on leaves – nevertheless the occurrence it is not excluded – according to data in Fungal Databases (Farr and Rossman, 2014) symptoms were observed in Japan and Korea and according to FRDBI (Kirk and Cooper, 2014) 9 records of tar spot symptoms on Japanese maples exist. On the other hand, with proper care and maintenance, as it is a custom of most of gardeners, this condition is often avoided (Douglas, 2009).

**Diameter at breast height (DBH)**

Tar spot disease itself is not health threatening but sometimes the occurrence of numerous stromata makes the tree unsightly (Flynn, 1996, 1998; Gillman, 2011) and in some cases it can lead to the premature leaf drop (Healy, 2007). If premature defoliation happens several years in a row, it can lead to the stress and subsequently cause susceptibility to other problems. Infection has little or no effect on the growth of sycamore maples (Leith and Fowler, 1987). According to the most recent available study, the outbreak of the disease causes decline in Norway maple saplings and tree growth

(Lapointe and Brisson, 2011). In all studies previously mentioned the influence of infection on host growth was tested, but the host age influence on the occurrence of symptoms of fungal pathogen was not studied. The result of this work shows the correlation between trunk diameter and quantity of stromata. This was reflected in habitats close to the nature, where spontaneous spread of seeds happens. In urban areas, young individuals are deliberately planting and thanks to the urban greenery care there is not uncontrolled spread of seeds. This outcome could be influenced by the occurrence of older specimens in more polluted areas.

### Air pollution

This research was conducted within an urbanized area and its neighborhood. The area of the city is characterized by its microclimate, the air is more heated up and drier because of the buildings and paved surfaces, it contains dust and microorganisms and also air pollutants due to the traffic (Tello at al., 2005; Juhásová et al., 2008; Gáperová, 2009; Wong and Chen, 2010). All these elements could affect occurrence of stromata. To compare results with urban and non urban areas the Protected Landscape Area was also included in the research.

Polluted air inhibits occurrence of tar spot (Vick and Bevan, 1976). Distribution of symptoms of *Rhytisma acerinum* is associated with amount of sulphur dioxide in the air, which is produced by burning fossil fuels (Vick and Bevan, 1976) as well as with amount of nitrogen dioxide in the air (Gosling et al., 2016). Tar spot is less frequent in urban and industrial areas (Weber and Webster, 2002). Tolerance limit of this fungus is approximately 90 $\mu g\ m^{-3}$ $SO_2$ (Bevan and Greenhalgh, 1976) and about 20 $\mu g\ m^{-3}$ $NO_2$ respectively (Gosling et al., 2016). Leith and Fowler (1987) found that the average annual concentration of 50 $\mu g\ m^{-3}$ did not have any impact on occurrence of symptoms. Currently, the limit value for sulphur dioxide (to protect ecosystems and vegetation) is 20 $\mu g\ m^{-3}$ (Chmi, 2014). In 2012 the air quality limits for $SO_2$ for 1 hour concentration and for 24-hour concentration were not exceeded at any measuring station (Chmi, 2014). Due to the lack of information about concentrations of $SO_2$ in various localities in Ostrava City and its surroundings, this factor could not be statistically evaluated. The highest average annual concentration recorded in 2012 was 9.3 $\mu g\ m^{-3}$ – in the city district Ostrava-Jih (Chmi, 2014). Therefore, we can assume that the occurrence of fungal disease is influenced by other factors, not only by the amount of $SO_2$.

Another problem related to the air quality in Ostrava City is high dust level (Gacka, 2014). The result of analysis regarding the effect of $PM_{10}$ implies that its presence reduces occurrence of black spots on maple leaves. Solid emissions (such as $PM_{10}$) have a negative effect on plants (Rai and Kulshreshtha, 2006). It may cause clogging of stomata, reduction of photosynthesis, respiration and transpiration. So we can conclude that there may be blockage of stomata preventing penetration of ascospores into epidermal cells of the leaf, thereby halting the initial infection. Inhibition of transpiration and thus a reduction of moisture on the leaf blade may also cause lower chances of infection.

Another factor influencing amount of tar spot in such a characteristic urban environment could be a soil contamination. High content of nitrogen and sulfur ($SO_x$ and $NO_x$) and heavy metals affect its occurrence. In areas with a low content of these elements in the soil, the high infestation of black spots was recorded (Kosiba, 2007).

## Conclusions

By studying over 1,200 individuals of *Acer* spp. it was found that there are significant differences in occurrence of symptoms of tar spot in its number dependent on both, site and host species. Out of 18 different taxa the symptoms were recorded on a half of them. The most susceptible taxa to tar spot (*Acer pseudoplatanus*, *A. pseudoplatanus* ‘Atropurpureum’, *A. platanoides* ‘Cleveland’, *A. platanoides* ‘Globosum’) can be the best candidates for monitoring air pollution (especially in urban areas). The highest frequency of stromata was observed in the Protected Landscape Area and generally in non-urban areas (typically less polluted). Thus we can state that *Rhytisma acerinum* prefers areas with a lower degree of air pollution. Moreover, our analysis provides evidence that trees with lower diameter of trunk suffered from higher infestation of tar spot. Further research focused on alternative environmental factors in urban area (such as soil contamination by chemicals, etc.) affecting occurrence of tar spot should be conducted. It would be appropriate to make further observations and analysis of the impact of hosts ontogeny on the occurrence of symptoms.


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## References


Bevan, R.J., Greenhalgh, G.N., 1976. Rhytisma acerinum as a biological indicator of pollution. *Environmental Pollution*, 10: 271–285.

Chmi, 2014. *Znečištění ovzduší a atmosférická depozice v datech, Tabelární ročenky* [*Air pollution and atmospheric deposition in numbers, Tabular yearbook*]. Czech Hydrometeorological Institute [cit. 2014-03-28]. http://portal.chmi.cz/files/portal/docs/uoco/isko/tab_roc/tab_roc_CZ.html

DOUGLAS, S.M., 2009. *Common diseases of maple*. Fact sheet (The Connecticut Agricultural Experiment Station, Department of Plant Pathology and Ecology). 7 p.

EDMONDS, R.L., AGEE, J.K., GARA, R.I., 2000. *Forest health and protection*. Minnesota: McGraw Hill. 630 p.

FARR, D.F., ROSSMAN, A.Y., 2014. *Fungal databases*. Systematic Mycology and Microbiology Laboratory, ARS, USDA [cit. 2016-03-24]. http://nt.ars-grin.gov/fungaldatabases.

FARR, D.F., BILLS, G.F., CHAMURIS, G.P., ROSSMAN, A.Y., 1989. *Fungi on plants and plant products in the United States*. St. Paul: APS Press. 1252 p.

FLYNN, P., 1996. Tar spot on maple. *Horticulture & Home Pest News*, IC-475 (24).

FLYNN, P., 1998. Tar spot – maple. *Horticulture & Home Pest News*, IC-479 (22).

GACKA, M., 2014. *Ovzduší* [Air]. Statutory City of Ostrava – Official portal [cit. 2016-03-22]. http://www.ostrava.cz/cs/o-meste/zivotni-prostredi/ovzdusi.

GÁPEROVÁ, S., 2009. *Hniloby pagaštana konského Aesculus hippocastanum L. na Slovensku* [Rots of horse chestnut Aesculus hippocastanum L. in Slovakia]. Banská Bystrica: FPV UMB. 98 p.

GILLMAN, D., 2011. *Giant tar spot*. Fact sheet (UMassAmHerst, Landscape, Nursery & Urban Forestry Program) [cit. 2016-03-22]. http://extension.umass.edu/landscape/fact-sheets/giant-tar-spot.

GILMAN, E.F., WATSON, D.G., 1993a. *Acer ginnala: Amur Maple*. Fact sheet ST-14 (Environmental Horticulture Department, Florida Cooperative Extension Service – Institute of Food and Agricultural Sciences, University of Florida).

GILMAN, E.F., WATSON, D.G., 1993b. *Acer platanoides: Norway Maple*. Fact sheet ST-28 (Environmental Horticulture Department, Florida Cooperative Extension Service – Institute of Food and Agricultural Sciences, University of Florida).

GILMAN, E.F., WATSON, D.G., 1993c. *Acer platanoides 'Cleveland': 'Cleveland'Norway Maple*. Fact sheet ST-30 (Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida).

GILMAN, E.F., WATSON, D.G., 1993d. *Acer platanoides 'Columnare': 'Columnare' Norway Maple*. Fact sheet ST-31 (Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida).

GILMAN, E.F., WATSON, D.G., 1993e. *Acer platanoides 'Emerald Queen': 'Emerald Queen' Norway Maple*. Fact sheet ST-33 (Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida).

GILMAN, E.F., WATSON, D.G., 1993f. *Acer platanoides 'Erectum': 'Erectum' Norway Maple*. Fact sheet ST-34 (Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida).

GILMAN, E.F., WATSON, D.G., 1993g. *Acer platanoides 'Globosum': 'Globosum' Norway Maple*. Fact sheet ST-35 (Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida).

GILMAN, E.F., WATSON, D.G., 1993h. *Acer platanoides 'Olmsted': 'Olmsted' Norway Maple*. Fact sheet ST-36 (Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida).

GILMAN, E.F., WATSON, D.G., 1993i. *Acer platanoides 'Schwedleri': 'Schwedleri' Norway Maple*. Fact sheet ST-37 (Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida).

GILMAN, E.F., WATSON, D.G., 1993j. *Acer platanoides 'Superform': 'Superform' Norway Maple*. Fact sheet ST-39 (Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida).

GILMAN, E.F., WATSON, D.G., 1993k. *Acer platanoides 'Almira': 'Almira' Norway Maple*. Fact sheet ST-29. Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

GILMAN, E.F., WATSON, D.G., 1993l. *Acer negundo: Boxelder*. Fact sheet ST-20 (Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida).

GILMAN, E.F., WATSON, D.G., 1993m. *Acer negundo 'Elegans': 'Elegans' Boxelder*. Fact sheet ST-21 (Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida).

GOSLING, L., ASHMORE, M., SPARKS, T., BELL, N., 2016. Citizen science identifies the effects of nitrogen dioxide and other environmental drivers on tar spot of sycamore. *Environmental Pollution*, 214: 549–555.

GREENHALGH, G.N., BEVAN, R.J., 1978. Response of Rhytisma acerinum to air pollution. *Transactions of the British Mycological Society*, 71: 491–523.

HEALD, F.D., 1926. *Manual of plant diseases: introduction to plant pathology*. York: Agricultural Sciences, The Maple Press Company. 964 p.

HEALY, S., 2007. Minor leaf spot diseases of maple: tar spot and leaf blister. *Horticulture & Home Pest News*, IC-497(20).

HSIANG, T., TIAN, X.L., 2007. Sporulation and identity of tar spot of maple in Canada. *Acta Silvatica & Lignaria Hungarica*, Spec. Edition: 71–74.

HUDLER, G.W., BANIK, M.T., MILLER, S.G., 1987. Unusual epidemic of tar spot on Norway maple in upstate New York. *Plant Dissease*, 71: 65–68.

HUDLER, G.W., JENSEN-TRACY, S., BANIK, M.T., 1998. Rhytisma americanum sp. nov.: a previously undescribed species of Rhytisma on maples (Acer spp.). *Mycotaxon*, 68: 405–416.

JONES, S.G., 1925. Life history and cytology of Rhytisma acerinum (Pers.) Fries. *Annals of Botany*, 39: 41–75.

JUHÁSOVÁ, G., ADAMČÍKOVÁ, K., KOBZA, M., 2008. Ohrozenosť druhov rodu Quercus a Castanea hubou Cryphonectria parasitica [The threat of the Quercus and Castanea species by the Cryphonectria parasitica fungus]. *Lesnícky časopis - Forestry Journal*, 54, Suppl.1: 21–28.

KIRK, P., COOPER, J., 2014. *The fungal records database of Britain and Ireland*. British Mycological Society [cit. 2014-24-08]. http://www.fieldmycology.net/FRDBI/assoc.asp

KOSIBA, P., 2007. Impact of air pollution on the occurrence of Rhytisma acerinum „Tar Spot" on maple leaves. *Acta Societatis Botanicorum Poloniae*, 76: 333–343.

LAPOINTE, M., BRISSON, J., 2011. Tar spot disease on Norway maple in North America: Quantifying the impacts of a reunion between an invasive tree species and its adventive natural enemy in an urban forests. *Ecoscience*, 18: 63–69.

LEITH, I.D., FOWLER, D., 1987. Urban distribution of Rhytisma acerinum (Pers.) Fries (tar spot) on sycamore. *New Phytologist*, 108: 175–181.

MANION, P.D., 1981. *Tree disease concepts*. New Jersey: Prentice Hall. 399 p.

MIHÁL, I., 2004. Príspevok k poznaniu mykoflóry Veľkého Rozsutca a Vrátnej v Národnom parku Malá Fatra [A contribution to the knowledge of mycoflora of the Veľký Rozsutec Mts. and Vrátna valley within the Malá Fatra National Park]. *Lesnícky časopis - Forestry Journal*, 50: 85–90.

R DEVELOPMENT CORE TEAM, 2013. R. *A language and environment for statistical computing (Version 3.0.2)*. Vienna: R Foundation for Statistical Computing. http://www.R-project.org.

RAI, A., KULSHRESHTHA, K., 2006. Effect of particulates generated from automobile emission on some common plants. *Journal of Food, Agriculture & Environment*, 4: 253–259.

SUTTON, B.C., 1980. *The Coelomycetes: fungi imperfecti with pycnidia, acervuli and stromata*. Kew: Commonwealth Mycological Institute. 696 p.

TELLO, M.L., TOMALAK, M., SIWECKI, R., GÁPER, J., MOTTA, E., MATEO-SAGASTA, E., 2005. Biotic urban growing conditions – threats, pests and diseases. In KONIJNENDIJK, C.C., NILSSON, K., RANDRUP, B. T., SCHIPPERIJN J. (eds). *Urban forests and trees*. Berlin-Heidelberg: Springer-Verlag, p. 325–365.

VICK, CH.M., BEVAN, R., 1976. Lichens and tar spot fungus (Rhytisma acerinum) as indicators of sulphur dioxide pollution on Merseyside. *Environmental Pollution*, 11: 203–216.

WEBER, W.S.R., WEBSTER, J., 2002. Teaching techniques for mycology: 18. Rhytisma acerinum, cause of tar-spot disease of sycamore leaves. *Mycologist*, 16: 120–123.

WONG, N-H., CHEN, Y., 2010. The role of urban greenery in high-density cities. In EDWARD, NG (ed). *Designing high-density cities for social and environmental sustainability*. London: The Cromwell Press Group, p. 227–262.

ZDRAVOTNÍ ÚSTAV, 2008. *Analýza kvality ovzduší na území města Ostravy a legislativa v ochraně ovzduší* [*Analysis of air quality in Ostrava and air protection legislation*]. Ostrava: Zdravotní ústav se sídlem v Ostravě. 97 p.

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# Share of scattered woody vegetation in landscape ecological stability and agriculture sustainability

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**Abstract**

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Scattered woody vegetation in the agricultural landscape includes more or less fragments of original biotopes and cultivated cultural vegetation as are groves, windbreaks, and infiltration forest belts on slopes, tree alleys, as well as orchards and vineyards. The contribution describes their effectiveness in agriculture sustainability through defined categories of functions and services. At the studied territory, the development of landscape structure was assessed in three time periods of 1869, 1949 and 2010s with regard to the share of scattered vegetation and woody species composition. In 2010, 23 vegetation formations were surveyed, from these 20 are in linear formations and the others in areal formations. Regarding species composition, 47 woody plant species were identified, from these 28 native, 12 alien and 7 fruit trees. The share of scattered vegetation has been proposed to be increased from 5% to 6%. The studied scattered vegetation should be considered as an important contribution to biodiversity conservation, climate, water and soil quality amelioration, pest and disease control and finally crop yield increasing.

**Key words**

agriculture landscape, scattered woody vegetation, sustainable support

## Introduction

Agricultural landscape of Slovakia has passed over many changes during the last 50 years related to land ownership, technologies of agriculture and landscape structure. The wide-ranging land consolidation of small private plots to large cultural blocks ranging from an area of 10 to 200 ha is visible in structural landmarks. Industrial chemicals as fertilizers, pest and diseases control as well as large agricultural machines and tools were involved during the intensification process. The arable land share reached up to 70%, less on uplands and more in lowlands and a new surface water system was constructed and managed. Many wetlands and marches as specific biotopes were drained (Supuka et al., 2013).

Such substantial changes have reflected on the improvement of crop yield production, but were accompanied with many negative factors impacting underground water, soil quality and climate characteristics. Similar situation related to agriculture intensification, land consolidation and successive landscape structural changes have passed in developed western countries as is described by Forman and Godron (1986).

A significant decreasing of scattered woody vegetation after land consolidation has caused potential

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and real damages to agricultural land by surface erosion, when 47.7% of the overall agriculture land is endangered by water erosion and 6.2% by wind erosion (Grešová and Streďanský, 2011; Miština, 2009). Soil erosion and large scale land structural changes have decreased of ecological stability and agriculture sustainability. With an effort to reduce this degradation features of predominantly lowland countryside in the sense of wind erosion and decreased ecological stability, 6,082 ha of linear woody vegetation were planted as windbreaks in the period between 1950s and 1964s, later between 1980 and 1989, 800 km of linear-form woody vegetation were planted in Slovakia (Zachar and Tešliar, 1989). In the present, the scattered woody vegetation achieves different proportions depending on land use forms, relief conditions and historical development of the landscape.

In the hilly region of Kysuce, more than 10% of share in landcover was inventoried (Špulerová et al., 2011). In lowland of Trnava region on 11 cadastal territories, a share of 1–4% was identified (Hreško and Guldanová, 2012). The optimal areal share of scattered vegetation in the agriculture landscape of Slovakia was stated of 3–7% for lowlands and 8–12% for hillands according to different relief and land-use conditions (Supuka et al., 2008). In many European documents e.g. High Nature Value Farmland (HNVF) as a part of the European Environmental Agency (EEA), there is recommended close to 7% share of scattered woody vegetation (Andersen et al., 2004; Špulerová et al., 2011).

Concerning woody plant species composition in linear-form vegetation at south-western Slovakia, 56 deciduous woody plant species were identified and from these 19 native and 21 alien species (Varga et al., 1999). At the Žitný ostrov near the Danube River, 28 woody plant species were identified in 19 windbreaks, from these 11 species were alien (Supuka et al., 2013).

The scattered woody vegetation in agricultural landscape is an important contribution to water balance regime as was described in Nete catchment of Belgium (Staes and Meire, 2007). Decreasing of forest cover, scattered woody vegetation and permanent grassland in landscape has had negative effect on erosion processes and deposition of floated sediments to the water basins (Yoo et al., 2014). Permanent vegetation formations and scattered forest remains as a complex of green infrastructure caused an increase in landscape connectivity, ecological stability and sustainability of agriculture through supporting and regulating services and inputs to the crop yields (Leal, 2004; Plieninger et al., 2006; Reyers et al., 2009; Tóth et al., 2016; Zhang et al., 2007).

Cultural and recreational services of permanent vegetation in different regions of agricultural landscape and its transmission zones are described by Pröpper and Haupts (2014), and Cieszewska et al. (2010). There is an evidence of aesthetic, cultural, historical and production functions of vineyard landscape through permanent fruit trees and vine plants (Supuka et al., 2011; Štefunková et al., 2011; Verešová and Supuka, 2012). Elements of scattered vegetation are also assessed as important features supporting an ecological and sustainable agriculture (Šarapatka et al., 2010).

## Material and methods

For assessment of scattered vegetation in the landscape structure, the study territory of Oponice cadastre in the south-western part of Slovakia was chosen. In the first part of our contribution, we have focused on categorisation and content structure of scattered permanent vegetation as a result of continual land-use forms and features of current landscape structure (Supuka et al., 2013). The subsequent part is devoted to functions and service characteristics of permanent scattered vegetation and its penetration to the sustainable agriculture. For this chapter, published sources and our theoretical and practice accomplishment have been used (e.g. Reyers et al., 2009; Supuka et al., 2008; Supuka et al., 2013; Swinton et al., 2007; Zachar, 1982).

The last chapter is devoted to a survey and assessment of scattered woody vegetation in the study territory with an emphasis on landscape structure development and the share of permanent vegetation in this structure, space distribution and woody species composition. In this chapter, we describe the development and share of landscape elements within three time periods (1869, 1949, and 2010) with an accent on permanent vegetation at the studied territory of Oponice cadastre with a total areal cover of 1,232.55 ha. The location and boundary of the mentioned studied area are shown on maps (Figs 1, 2) and statistical area values of landscape elements are stated in Table 1. Landscape structure in the historical period of 1869 has been elaborated from the map document of the 2nd military mapping of the former Austrian-Hungarian Empire. Later periods were elaborated using the cadastral database and the aerial photo of 1949, next by using of remote sensing orthophoto map of 2010 (Figs 1, 2). Scattered woody vegetation was assessed with regard to external and internal structure and context. The internal characteristics involve areal and spatial landmarks (areal, linear, group and solitaire types), categories according to origin (natural, planted, mixed), and according to functions and land distribution (e.g. grove, orchard, windbreak, accompanying vegetation of roads and streams, tree alley, shrub hedge, etc.). Internal features of scattered vegetation represent the layer structure, area, density, average height and width, spatial composition, share, gene-pool and heritage values (Supuka et al, 2013; Tóth and Feriancová, 2014).

In the conclusion, functional importances of scattered vegetation for ecological stability and sustainable agriculture are described.

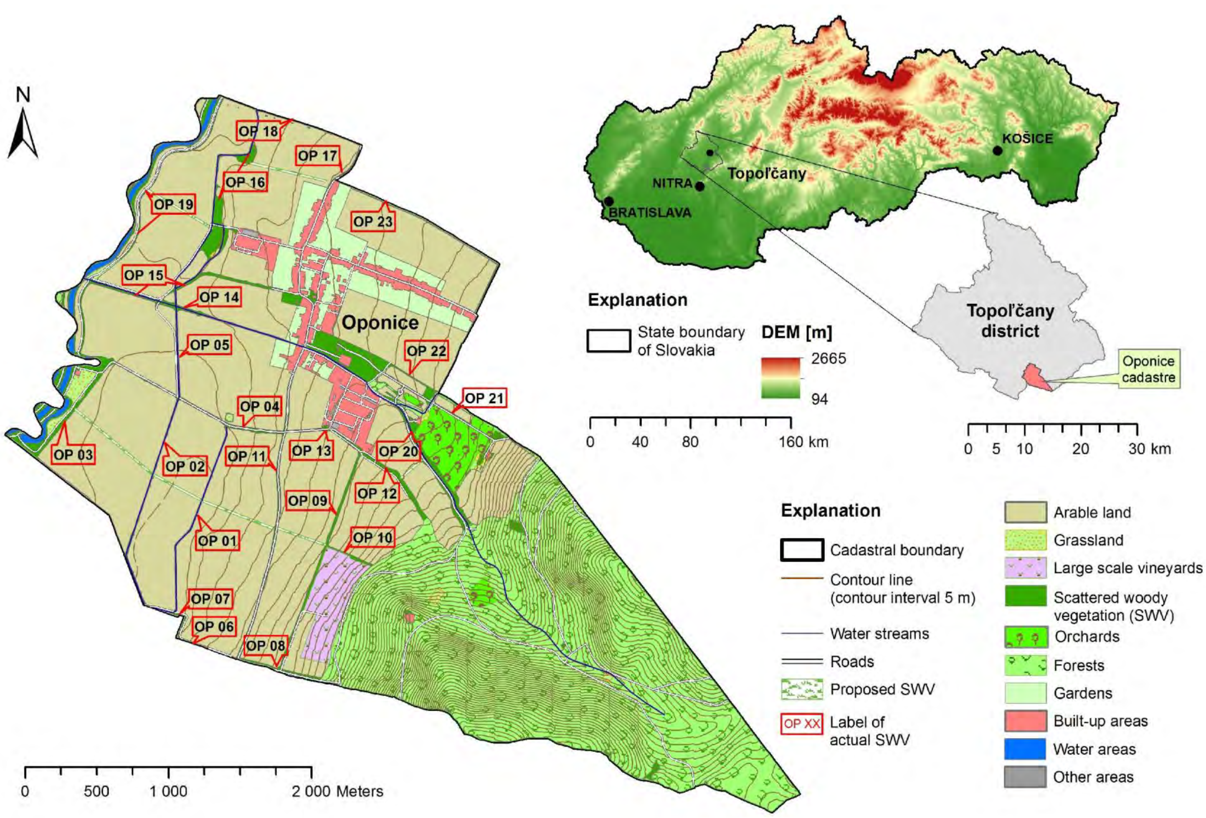


Fig. 1. Secondary landscape structure of Oponice cadastre in 2010 with regard to distribution of scattered woody vegetation, state and proposal (elaborated by Karol Šinka and Ján Supuka, 2014).


Fig. 2. Comparison of landscape structure of the Oponice cadastral territory by aeral photos in different time periods of a) –1949, b) – 2010 with regard to distribution of scattered woody vegetation (elaborated by Karol Šinka, 2013).

Table 1. Area of Oponice cadastral territory in historical land-use

| Landscape element | Area in ha according to years | | | |
|---|---|---|---|---|
| | 1869 | 1949 | 2010 | Proposal |
| Arable land | 665.82 | 685.60 | 647.92 | 642.97 |
| Grassland | 115.24 | 80.91 | 10.51 | 10.51 |
| Orchads | 0.00 | 0.00 | 20.15 | 20.15 |
| Vineyards | 0.00 | 15.66 | 18.82 | 18.82 |
| Scattered woody vegetation | 38.94 | 36.73 | 64.24 | +4.95<br>69.19 |
| Forests | 362.52 | 334.94 | 362.23 | 362.23 |
| Water streams and areas | 11.25 | 21.58 | 13.96 | 13.96 |
| Gardens | 19.68 | 26.17 | 45.23 | 45.23 |
| Built-up areas | 14.41 | 22.60 | 43.50 | 43.50 |
| Roads | 4.69 | 8.36 | 9.49 | 9.49 |
| Other areas | 0.00 | 0.00 | 1.45 | 1.45 |
| Sum | 1,232.55 | 1,232.55 | 1,232.55 | 1,232.55 |

## Results

### Categories of scattered woody vegetation in the landscape

Scattered woody vegetation in the agricultural landscape should be categorised according to origin, spatial and areal composition, main functions, utility values and stand interior characteristics. Basically, they are divided to following categories.

*Natural vegetation* – this has been developed by natural successive ways on uncultivated land, extreme relief and dry or wet soil hydrology conditions without direct man intervention and management. In many cases, their character is similar to the potential natural vegetation with native woody plant species composition and herbs in stand undergrowth. They have group, areal and linear composition consisting of permanent grassland, shrubs and trees in vertical layers. In terms of ecosystem, they represent, more or less fragments of original biotopes with high ecostability and wild life biodiversity effectivness.

*Semi-natural and synanthropic vegetation* – most often grown on anthropogenic relief and land arranged areas as are terraces on boundary lines, along roads, bank side of water streams and basins, on dumping and disposal sites. This category also includes extensive and abandoned orchards, vineyards and woody plantations. Concerning the woody plant species composition, they consist of native and planted cultural species, varieties and sorts as mixed biotopes and plant communities. Former grasslands as part of woodlands have been regularly cut and represent therefore a very rich herb species composition with aesthetic perception qualities. They represent very effective biotopes, which keep and support wild animal biodiversity (birds and mammals), thanks to rich fruits as food sources and suitable habitats for wildlife and reproduction.

*Cultivated cultural vegetation* – arranged composed and planted woody vegetation, permanently or occasionally maintained. Regarding woody species composition, they consist of plants of native and alien, fruit, forest and landscaping importance. This category includes cultivated orchards, vineyards, biomass and energetic production plantations, windbreaks, infiltration forest belts on slopes, road and water body bank side vegetation, tree alleys as component of historical landscape structure, groves, rural parks, small groups and tree solitaires in the landscape, composed vegetation at farm yards, agritourism and recreation centres, gardens at cottages and village family houses and others.

In different geographical conditions, with different land-use forms, diverse formations of scattered vegetation and its combinations should be inventoried through historical periods. Very often, the occurred elements have a group form, diverse and mosaic form, with dominat solitaire trees, with dominat linear forms and or dominant areal composition. A special attention should be dedicated to old, oversized and rare species, as well as valuable utility trees occurring in the countryside and having an importance as gene pool and cultural heritage elements as well as recreation usage.

### Functions and services of scattered vegetation in relation to sustainability of agriculture

In traditional classification approaches, the positive influences and importance of scattered vegetation in the agricultural landscape were defined as functions. Basically, they are divided to two categories as are natural and anthropogenic. The first group includes positive influences on abiotic landscape elements related to bedrock, soil, water and climate characteristics. Woody vegetation has improving effectiveness to quality and quantity properties, conservation and counterbalance of mentioned elements. Regarding biotic elements, scattered vegetation supports and keeps the plant, animal and microbial biodiversity at the natural (wildlife) and cultural level (as cultural biodiversity). The second anthropogenic group includes economic functions related to agriculture, forestry, water, hunting etc. management and improving their production and yields. In the sphere of social anthropogenic importance, there are effective cultural, educational, researches, spatial, compositional and aesthetic functions.

Many scientists have been interested in research and consequent categorisation and definition of vegetation functions in the landscape. In a wider and general view, the activities in this field were focused on describing and defining ecosystem services, where the category of ecosystems and realised groups of services should be derived. Wider approaches regarding functions and services of vegetation are described in the so called Netherland Model, founded on nature function contributions for human society. Following functions are categorised in this schematic model:

*Production functions* – takes most important position founded on biomass and energy flow from nature resources to human society.

*Carrier functions* – ecosystem components including vegetation formations have been granted spaces for setting, dwelling, recreation etc.

*Information services* – related to information needs of human society as are spatial orientation and identification, research and education, culture and historical values.

*Regulating services* – consist of ability of natural and cultural ecosystems to regulate principal ecological processes and life supporting systems.

A similar approach to classification of ecosystem services with an emphasis on forest components has been described in the document of MEA (Millenium Ecosystem Assessment 2005) where they were divided to four groups. We have paid higher attention to these functional categories and service characteristics because scattered woody vegetation is a spatial fragment of forests having important effectiveness in landscape ecological stability and sustainable agriculture.

*Supporting* – support sustainable production features of internal and adjacent ecosystems and living spaces through photosynthesis and oxygen production, water and nutrient cycle, soil fertility and production potential as basic assumption for biology and agriculture production.

*Provisioning* – represent biological products and natural sources obtained from ecosystems, e.g. forest and agriculture, as are cultural crops, fruits, farm animal breeding and fishing, representing food production in a broad sense, energy and technical biomass, pharmacy plants, gene pool resources, etc.

*Regulating* – there are benefits from internal self keeping ecosystem ability and self regulation processes led into air and water quality, climate characteristics regulation, erosion, pests and diseases control, biodiversity regulation, insect pollination supporting and reproduction, habitats functioning and conservation.

*Cultural* – represents spiritual values and features reflected in human perceiving process. They involve spatial composition, aesthetic, cultural, historical and educational features, natural and cultural (tree) heritage, genius loci space or element, recreation supply, etc.

Regarding scattered woody vegetation, it represents a compositional component of the agricultural landscape ecosystem with long life span, supporting ecological stability, biodiversity, cultural visual landmarks and sustainable production potential. When describing positive effectiveness and contribution of scattered vegetation to sustainability of agriculture, we have to analyse functions and services according to certain criteria. We need to take into account a specific position of scattered vegetation in the landscape. It is not an independent landscape ecosystem but an important biological component in the entire agricultural ecosystem. On the other hand, an agricultural ecosystem has a mixed character composed by anthropogenic and natural or semi-natural elements. The agricultural landscape is under a continual management and maintenance involving subsidiary energy regime, alternating crop rotation resulting to land-use changes trough growing seasons, predominantly in arable land. Other biological, natural and cultural elements considered as permanent biotopes have been supporting and counterbalancing effectiveness in relation to annual crops and their growing environment. There are involved all categories of permanent grasslands and woody vegetation in different formations, original and managed in different intensity. They are relatively stable in internal meaning and have also stabilizing influences in course to the external space and to neighbouring cultivated crops and plants. Those are principal reasons why scattered woody vegetation is considered an important contribution to agricultural sustainability and landscape ecological stability.

General characteristics of functions and services of permanent woody vegetation are the basis for understanding its participation in agricultural sustainability of rural landscape ecosystems.

In this paragraph, we try to define main features of agricultural sustainability: a) rational and thrifty utilisation of natural resources; b) avoidance of soil, water and air pollution, their degradation under loading capacity and healthy limits; c) keeping and supporting biotic values, fertile capacity and production potential of soil; d) application of progressive technologies in land management, such as precision agriculture, organic and other alternative forms including integrated pest and disease control; e) organisation of optimal land use forms with equable share of landscape structure elements; f) increase in the share of ecology stabilising elements in the landscape as are scattered woody vegetation, permanent grassland and water network elements; g) sustaining landscape diversity, gene pool and biodiversity with an emphasis on nature protection, specific habitats and biotopes of wild life; h) sustaining and supporting cultural and historical values, cultural biodiversity, aesthetic landmarks, objects and localities of cultural heritage; i) increase in the recreation potential, dwelling and life quality of the countryside; j) increase in food production and achieving food safety for people.

**Assessment of landscape changes in the study territory with an emphasis on scattered vegetation and agriculture sustainability**

The study territory within the period from 1869 to 1949 is characterised by small plots of agricultural land use farmed by individual peasants with simple technologies and animal power utility (see Fig. 2a). The southern part of the cadastral territory was arranged to larger size blocks of arable land, because that land section was owned by the lordship of the Aponyi family. Regarding landscape structure changes within the assessed period, the forest covered nearly 27%, and then this proportion has slightly decreased. A similar situation is shown in scattered woody vegetation, which in the first period covered 3.16%, during next period slightly decreased by 0.18%. Arable land as the dominant land use covered almost 54% and slightly increased by 1.60% (Table 1). The built up areas of the village have been under progressive development and also adjacent gardens as part of family houses. The western part where the River Nitra passes through the cadastral boundary was covered by permanent meadows and pastures and annually flooded by river. There is a visible a rapid decrease in the share of meadows decreasing from 9.35% to 6.56% during the surveyed periods.

Regarding ecological stability of the assessed cadastre, the representing landscape elements of this feature have been balanced and covered 41.93% (forests, grasslands and scattered woody vegetation). Small size plots in the northern part of the cadastral territory provided mosaic field structure and supported ecological stability partially. Oak-beech forest in the eastern part and flooding willow-poplar forest in the west, along the Nitra River as well as meadows and scattered vegetation contributed to ecological stability and it should be declared that also agriculture fulfilled almost all mentioned criteria of sustainability. No chemistry was used in agriculture, just simple technologies, hard work, low crop yields and low life quality, therefore at the turn of the 19th and 20th century, many people emigrated overseas to the "New World". The criteria of food safety and sufficiency, as well as social aspects of rural people were not fulfilled in that time.

Within the assessed period of 1949 and 2010, remarkable changes in the landscape structure arose. There was a considerable decrease in land cover of permanent grassland (6.56% in 1949 / 0.85% in 2010), partially also arable land (55.62% / 52.17%). The area of vineyards has slightly increased (1.27% / 1.53%), forest area has increased due to afforestation of shallow and unfertile soils (27.17% / 29.39%), a new large-scale orchard has occurred (0% / 1.63%). Built-up areas of Oponice village have increased rapidly (1.83% / 3.53%) and adjacent family house gardens (2.12% / 3.67%) as well as land cover of scattered woody vegetation due to planting new windbreaks and shelterbelts (2.98% / 5.21%) (Area values in hectares are stated in Table 1).

As reflection of agriculture intensification processes mentioned in the introduction of this article, the small size plots have been altered by large blocks of arable land (these achieved 50–150 ha), meadows have been drained up-ploughed, many linear-form vegetation along plot boundaries have been removed. On the other hand, new windbreaks and shelterbelts were planted according to the model of foreign countries. In spite of that, ecological stability and wildlife biodiversity have decreased, the cultural and visual aspects have changed and new landmarks and values have occurred. Progressive technologies and new techniques have influenced soil, water and air characteristics but rather in negative ways. On the other hand, the crop yield, food safety, and life quality of rural people have substantially increased.

At the studied area of Oponice cadastral territory, we have surveyed all categories of scattered woody vegetation and their woody species composition in 2012. There were inventoried 23 vegetation formations, of these 20 are in linear forms and the other are in areal ones. In terms of their origin, the 3 formations have been considered natural, 11 mixed and 9 of cultural character. At tree layer, 21 woody species were identified from those 6 alien and 5 fruit tree species. In the shrubby layer, 15 woody species were inventoried, from these 6 alien and 2 fruit shrub species. In the shrubby layer there also surveyed 10 woody species which belong to tree growth formations but achieved under 3 m height only according to inventory methods. The dominant tree species in the floodplain forest and at river banks were *Populus alba* L., *P. nigra* L., *Salix alba* L., *S. fragilis* L., *Alnus glutinosa* (L.) Gaertn. In windbreaks, the most common inventoried species were *Robinia pseudoacacia* L. (dominated in 15 line form woody vegetation), *Populus x canadensis* Moench., *Fraxinus excelsior* L., *Tilia cordata* Mill., *T. platyphyllos* Scop., *Ulmus leavis* Pall., *Acer platanoides* L., *A. pseudoplatanus* L., *A. campestre* L., *Negundo aceroides* Moench., *Cerasus avium* L. and other. In floodplain biotopes some bank sides of river streams are covered by remains of flooded forest with old and oversize trees of poplar (*Populus alba* L., *P. nigra* L.) and willow (*Salix alba* L., *S. fragilis* L.) species as important gene pool elements. Particular overview of woody plants occurrence according to non-forest woody vegetation formations from OP 1 till OP 23 (see Fig. 1) is given in Table 2 and Table 3 for tree and shrubby woody species separately.

Table 2. List of tree species and their frequency occurrence according to formations of the non-forest woody vegetation in Oponice cadastral territory

| Tree species | Occurrence in formation of the non-forest woody vegetation (NFWV) according to map Fig. 1 = OP1– OP23 | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NFWV OP1 – OP 23 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | *FO |
| *Acer campestre* L. | | + | | | | | + | + | | + | | + | | + | + | + | | | + | + | | | | 10 |
| *Acer platanoides* L. | | | | | | | | | | | | | + | | | | | | | + | | + | | 3 |
| *Alnus glutinosa* (L.) Gaernt. | | + | | | | | | | | | | | | | | | | | + | | | | | 2 |
| *Cerasus avium* (L.) Moench. | | | | | | | | + | + | + | | | | + | + | | | | + | + | + | + | + | 10 |
| *Fraxinus excelsior* L. | + | | + | + | | + | + | + | | + | | + | | + | + | + | | + | | + | | + | + | 15 |
| *Juglans regia* L. | + | | | + | | | | | + | + | + | | + | + | + | + | + | + | + | | | + | + | 14 |
| *Malus domestica* Borkh. | | | | | | | | | | | | | | + | | | | | + | | | + | + | 4 |
| *Morus alba* L. | | | | | | | | | | | | | | | + | | | | | + | | | | 2 |
| *Negundo aceroides* Moench. | | | + | + | | | + | | | | | | | + | | | | | + | + | | | | 6 |
| *Populus alba* L. | | | + | | | | | | | | | | | | | | | | + | + | | | | 3 |
| *Populus nigra* L. | | | | | | | | | | | | | + | | | | | | + | + | | | | 3 |
| *Populus tremula* L. | | + | | + | | | | | | | | | | | | | | | | | | | | 2 |
| *Populus x canadensis* Moench. | | | + | | | | | | | | | | | | | | | | | | | | | 1 |
| *Prunus cerasifera* Ehrh. | + | | | + | + | | | | | | | | | + | | + | | | | | | | + | 6 |
| *Prunus domestica* L. | + | | | + | + | | | | | + | | | + | + | | | | | | | | + | + | 8 |
| *Prunus padus* L. | | | | | | | + | | | + | | | | | + | + | | + | + | | | | | 6 |
| *Robinia pseudoacacia* L. | | + | + | + | + | + | + | + | + | + | | + | + | | + | | | | | + | | + | + | 15 |
| *Salix alba* L. | | | | | | | | | | | | | | | | + | | | + | | | | | 2 |
| *Salix fragilis* L. | | + | | | + | | | | | | | | | | + | + | | | + | + | | | | 6 |
| *Tilia cordata* Mill. | | + | | + | | | | | | | | | | | | | | | | + | | | | 3 |
| *Ulmus laevis* Pall. | | + | + | | + | | + | + | | | | | | | | | | | | + | | | | 6 |
| *FO | 4 | 7 | 6 | 8 | 5 | 2 | 6 | 5 | 3 | 7 | 1 | 3 | 5 | 8 | 8 | 7 | 1 | 3 | 11 | 12 | 1 | 7 | 7 | 127 |

*FO, frequency of occurrence in NFWV formations.

Table 3. List of shrubby woody species and their frequency occurence according to formations of the  non-forest woody vegetation in Oponice cadastral territory

| Shrubby woody species | Occurence in formation of the non-forest woody vegetation (NFWV) according to map Fig.1 = OP1–OP23 | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NFWV OP1–OP 23 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | *FO |
| *Clematis vitalba* L. | | | | | | | | + | + | + | | | | | + | | | | | + | | + | | 6 |
| *Crataegus monogyua* Jacq. | + | + | | | + | | + | + | + | + | | + | + | + | + | + | + | + | + | + | | + | + | 18 |
| *Cornus mas* L. | | | | | | | | + | | | | | | | | | | | | + | | | | 2 |
| *Enonymus europaeus* L. | + | + | + | | + | + | + | + | + | + | | | + | | | + | | | | + | | + | | 13 |
| *Ligustrum vulgare* L. | + | | + | | + | + | | | | | | | | | + | + | | | | + | | | | 7 |
| *Prunus spinosa* L. | + | + | + | + | | + | + | + | + | + | | | + | | | + | | | | + | | | + | 13 |
| *Rhamnus catharticus* L. | | + | | | | | | | | | | | | | | | | | | + | | | | 2 |
| *Rosa canina* L. | + | + | | | + | + | + | + | + | + | | + | + | + | + | + | + | + | + | + | | + | + | 19 |
| *Rubus fruticosus* L. | | | | | | | | + | | + | | | + | + | + | + | | + | + | | | | | 8 |
| *Sambucus nigra* L. | + | + | + | + | + | + | + | + | + | + | | + | + | | + | + | | + | + | + | | + | | 18 |
| *Swida sanguinea* (L.) Opiz | + | + | | | | | | | | + | | | + | | | | | | | + | | + | | 6 |
| *Symphoricarpus albus* L. | | | | | | | | | | | | | | | | | | | | + | | | | 1 |
| *Syringa vulgaris* L. | | | | | | | | + | | | | | | | | | | | | | | | | 1 |
| *Ulmus minor* Mill. | | | | | | | | + | | | | | | | | | | | | | | | | 1 |
| *Viburnum opulus* L. | | + | | | | | | | | | | | | | | | | | | | | | | 1 |
| *FO | 7 | 8 | 4 | 2 | 5 | 5 | 5 | 10 | 6 | 8 | 0 | 3 | 7 | 3 | 6 | 7 | 2 | 4 | 4 | 11 | 0 | 6 | 3 | 116 |

*FO – Frequency of occurrence in NFWV formations.

From the point of view of current woody species composition, it should be considered a very rich contribution to higher level of biodiversity, habitat conservation in plant and animal groups. A rich and variable biodiversity has a positive impact on insect pollination processes, regulation of pest and diseases and finally crop yields. Beside these positive landmarks there are many problems with *Robinia pseudoacacia* L. (black locust) because of its invasive growth manifestation, which is in conflict with principles of nature protection. Therefore, we propose to successively replace them by native forest trees and to add also more fruit trees to windbreaks for supporting animal wildlife. Spatial distribution of scattered woody vegetation contributes to an improvement of the water regime, mitigation of extreme climatic characteristics, as well as erosion control. In order to improve biotic, abiotic and social services of woody vegetation in the agricultural landscape, we have proposed to increase its share by 5.0 ha in order to reach near 6% and divide large-size arable blocks to smaller plots. This proposal should be also reflected by an enhancement of the cultural and aesthetic values and qualities of the countryside.

The described spatial distribution of scattered woody vegetation and species composition richness in the studied territory can be perceived as supporting landmarks of sustainable agriculture theory and practice.

## Discussion

Many branches of science may contribute to solution of real issues which have been cumulated during land use development in the countryside. At the present time, particular functions and services of terrestrial ecosystems have been described, but each landscape is covered by different and specific biotopes and plant communities including competent animal world. Agricultural landscape represents one of the most important ecosystem categories, where nature resources and individual landscape elements are being overlapped by a wide spectrum of social economy activities, e.g. setting, building, industry, transport, water network, energy supply, forestry and food production as dominant phenomena. Within the tertiary landscape structure, there are cultural and spiritual values, as well as ineterests of nature and landscape protection. It is difficult to find a balanced consensus between particular activities, interests, potentials and services. On the other hand, a human population has wishes to get chance for sufficient amount of accessible and healthy foods. Therefore, scientists are looking for gentle agriculture technologies with minimizing impact on natural resources and environment and to achieve food safety (Anderson et al., 2004; Plieninger et al., 2006; Reyers et al, 2009; and others). This complex approach is defined in the theory of sustainable agriculture which has a wide scale of individual criterias and subdefinitions (Leal, 2004; Šarapatka et al., 2010 and others) as we also described in this article. Scattered woody vegetation has been long ago a structural and compositional part of agricultural landscapes when farmers completely used the wide spectrum of their benefits and services. This is confirmed by our studied territory and also in other historical landscape structures (Špulerová et al., 2011) as well as in modern approach of windbreaks and hedges establishment and management (Kurz et al., 2011; Soltner, 1991) or in ecological restoration of biotope remains (Jongepierová et al., 2012).

Concerning woody plant species composition in scattered vegetation, the more species the more biodiversity and space for topical and food chain services. But invasive alien species have a destructive effect on natural gene pool and plant communities. Variable space formations of scattered vegetation and variable species proportion increase cultural and visual values and landscape recreational potential (Cieszewska et al., 2010). Scattered woody vegetation fulfils a wide spectrum of described services in the landscape. Agriculture in the mentioned sustainable theory and practice actions should act as provider and recipient of ecosystem services, including services of scattered vegetation and reflecting in agricultural economy parameters (Swinton et al., 2007).

## Conclusions

The agricultural landscape has been since long-ago impacted by permanent human interventions with a dominant interest to produce sufficient amount of foods. These actvivities are connected with landscape structure changes, which increase or decrease landscape values and characteristics. Current fields of natural and agricultural sciences have defined criteria and properties of ecosystem services including agricultural ecosystems. To achieve a balanced production of healthy food for people, it needs a new biology and technology approaches defined in theory and practice of sustainable agriculture.

Scattered woody vegetation should be one of the most important elements supporting landscape ecological stability through described services and functions. There are external positive influences leading to modification and mitigation of extreme values of abiotic environmental elements and also land conditions of cultivated crops and fruit orchards. On the other hand, scattered vegetation has kept internal space of plant and animal biodiversity and rare gene pool resource conservation. Achieved and presented results confirm this characteristics and services. From the social aspect, scattered vegetation is a landscape design element with cultural aesthetic values. The main conclusion of this paper is the proposition to increase the share of scattered vegetation up to 6% by new added linear forms in order to divide the land of the studied territory and decrease the area size of plots and increase its multifunctional services in context of sustainable agriculture enhancement.

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**References**

Anderson, E., Baldock, D., Bennet, H., Beanfoy, G., Bignal, E.M., Browner, F., Elbersen, E., Eiden, G., Godeshalk, F., Gwyn, J., McCracken, D., Nieuwenhuizen, W., van Eupen, M., Hennekens, S., Zervas, G., 2004. *Developing a high nature value farming area indicator. Final Report.* Copenhagen: European Environment Agency. 75 p.

Cieszewska, A., Drexler, D., Bihuňová, M**.,** Kalincsák, P., Belova, O., Procházka, J., 2010. Eastern Region. Chapter 6. In *Management of recreation and nature based tourism in European forests*. Berlin Heidelberg: Springer-Verlag, p. 115–139.

Forman, R.T.T., Godron, M., 1993. *Krajinná ekologie* [Landscape ecology]. Praha: Academia. 584 p.

Grešová, L., Streďanský, J., 2011. *Veterná erózia v krajine – súčasné trendy, metódy a spôsoby výpočtov* [Wind erosion in the landscape – current trends and methods of assessment]. Nitra: Slovenská poľnohospodárska univerzita. 108 p.

Hreško, J., Guldanová, H., 2012. Analýza zmien druhotnej krajinnej štruktúry v oblasti chráneného vtáčieho územia Uľanská mokraď (Analysis of the secondary landscape structure changes in the protected birds area of the Úľanská mokraď wetland). *Životné Prostredie,* 46: 28–33.

Jongepierová, I., Pešout P., Jongepier, J.W., Prach, K., 2012. *Ecological restoration in the Czech Republic*. Prague: Nature Conservation Agency of the Czech Republic. 148 p.

Kurz, P., Machatschek, M., Iglhauser, B., 2011. *Hecken. Geschichte und Ökologie, Anlage, Erhaltung und Nutzung.* Graz, Stuttgart: Leopold Stocker Verlag. 440 p.

Leal, W., 2004. Ecological agriculture and rural development in CEE countries in the context of EU enlargement. In Leal, W. (ed. *Ecological agriculture and rural development in Central and Eastern European countries*. Amsterdam: IOS Press, p. 1–13.

Miština, T., 2009. Vplyv pôdoochranných pestovateľských technológií na pôdu a prostredie. (Influence of soil protection cultivation technologies on the soil and environmental conditions). *Životné Prostredie,* 43: 355–357.

Plieninger, T., Hochtl, F., Spek, T., 2006. Traditional land-use and nature conservation in European rural landscapes. *Environmental Science and Policy,* 9: 317–321.

Pröpper, M., Haupts, F., 2014. The culturality of ecosystem services. Emphasizing process and transformation. *Ecological Economics*, 108: 28–35.

Pucherová, Z., 2004. *Vývoj využitia krajiny na rozhraní Zobora a Žitavskej pahorkatiny* [Land use form development on the boundary of Zobor and Žitava highland]. Nitra: Univerzita Konštantína Filozofa. 147 p.

Reyers, B., O´Farell, P., Cowling, R.M., Egoh, D., Le Maitre, D.C., Vlok, J.H.J., 2009. Ecosystem services, land-cover change and stake holders: finding of sustainable foodhold for semiarid biodiversity hotspot. *Ecology and Society*, 14: 38.

Soltner, D., 1991. *L´arbre et la haie*. Angers: Collection Science et Techniques Agricoles. 203 p.

Staes, J., Meire, P., 2007. A tool for participatory land-use planning and river basin management. In Mäkinen, H. (ed.). *Enhancing training on collaborative* p*lanning of natural resources management*. Reports of Finish Environment Institute, 27. Helsinki: Edita Prima, p. 74–87.

Supuka, J., Feriancová, Ľ., Jančura, P., Schlampová, T., 2008. *Krajinárska tvorba* (Landscape design). Nitra: Slovenská poľnohospodárska univerzita. 256 p.

Supuka, J., Verešová, M., Šinka, K., 2011. Development of vineyards landscape structure values. *Ekológia (Bratislava),* 30: 229–238.

Supuka, J., Šinka, K., Pucherová, Z., Verešová, M., Feriancová, Ľ., Bihuňová, M., Kuczman, G., 2013. *Landscape structure and biodiversity of woody plants in the agricultural landscape*. Folia Universitatis Agriculturae et Silviculturae Mendelianae Brunensis. Monographic Series, vol. 6, no. 9. Brno: Mendel University in Brno. 187 p.

Swinton, M.S., Lupi, F., Robertson, G.P., Hamilton, S.K., 2007. Ecosystem services and agriculture: cultivating agricultural ecosystems for diverse benefits. *Ecological Economics,* 64: 245–252.

Šarapatka, B., Abrahámová, M,. Čížková, S. Dotlačil, L., Hluchý, M., Kren, J., Kuras, T., Laštůvka, Z., Lososová, Z., Pokorný, E., Pokorný, J., Pokorný, R., Salašova, A., Tkadlec, E., Tuf, I., Vácha, M., Zámečník, V., Zeidler, M., Žalud, Z., 2010. *Agroekologie, východiska pro udržitelné zemědělské hospodáření* [Agroecology, basis for sustainable agriculture]. Olomouc: Bioinstitute. 440 p.

Špulerová, J., Dobrovodská, M., Lieskovský, J., Bača, A., Halabuk, A., Kohút, F., Mojses, M., Kenderessy, P., Piscová, V., Barančok, P., Gerhátová, K., Krajčí, J., Boltižiar, M., 2011. Inventory and classification of historical structures of the agricultural landscape in Slovakia. *Ekológia (Bratislava),* 30: 157–170.

Štefunková, D., Dobrovodská, M., Kanka, R., Krnáčová, Z., 2011. *Atraktivita malokarpatskej vinohradníckej krajiny s dôrazom na historické agrárne štruktúry a biodiverzitu* [Atractivity of Small Carpathian vineyard landscape with

emphasise to historical agrarian structure and biodiversity]. Bratislava: Ústav krajinnej ekológie SAV. 163 p. + attachments.
TÓTH, A., FERIANCOVÁ, Ľ., 2014. By improvement of the green infrastructure towards Sustainable Landscapes and Resilient Environments. In *G20 Youth Forum 2014, Garmisch-Partenkirchen, Germany*. Genève: G8&G20 Alumni Association, p. 405–410.
TÓTH, A., KUCZMAN, G., FERIANCOVÁ, Ľ., 2016. Species composition and diversity of non-forest woody vegetation along roads in the agricultural landscape. *Lesnícky Časopis - Forestry Journal,* 62: 56–66.
VARGA, L., BODO, T., VÁCLAV, J., 1999. Optimalisation of woody plant composition in the forest protection belts and riparian stands. In VARGA, L. (ed.). *Les a dreviny v inntenzívne obhospodarovanej poľnohospodárskej krajine.* Zvolen: Lesnícky výskumný ústav, p. 71–80.
VEREŠOVÁ, M., SUPUKA, J., 2012. *Kultúrno-historické a vizuálno-estetické hodnoty vinohradníckej krajiny* [Cultural, historical and visual aesthetic values of the vineyards landscape]. Nitra: Slovenská poľnohospodárska univerzita. 112 p.
YOO, J., SIMONIT, S., CONNORS, J.P., KINZIG, A.P., PERRINGS, CH., 2014. The valuation of off-site ecosystem service flows: deforestation, erosion and the amenity value of lakes in Prescott, Arizona. *Ecological Economics*, 97: 74–83.
ZACHAR, D., 1982. *Les v krajine* [Forest in landscape]. Bratislava: Príroda. 237 p.
ZACHAR, D., TEŠLIAR, J., 1989. História vetrolamov na Slovensku (History of windbreaks in Slovakia). In ZACHAR, D. (ed.). *Polyfunkčná zeleň v poľnohospodárskej krajine.* Bratislava: Veda, p. 20–27.
ZHANG, W., RICKETTS, H., KREMEN, C., CARNEY, K., SWINTON, S.M., 2007. Ecosystem services and dis-services of agriculture. *Ecological Economics*, 64: 253–260.

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**Short communication**

# Spruce tree fighting back – study of honey fungus infection

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**Abstract**

Buza, A. K., Divos, F., 2016. Spruce tree fighting back – study of honey fungus infection. *Folia Oecologica*, 42: 204–207.

Fungi infection is a common disease, problem for trees. Becoming fearful of the presence of fungi is obvious, although the effects of the infection are variable widely. Honey fungus (*Armillaria mellea*) is considered to be dangerous species weakening the trees. A honey fungus attacked spruce could be measured repeatedly for three years with acoustic tomography while pulling tests were performed as well. The research goes on, although the results interestingly show us the tree "fighting back". The safety and stability of the examined spruce is very good, the conditions of the root system are – surprisingly – getting better.

**Keywords**

acoustic tomography, fungi attack, honey fungus, pulling test, urban tree

## Introduction

Honey fungi including *Armillaria mellea* infect often both forest and agricultural crops (Cruickshank et al., 2011), while the fungi are spread on all continents except Antarctica (Westwood et al., 2012; DeLong, 1995). These are "white rot" fungi, killing the root system and often moving upward into the lower parts of the trunk (Glaeser, 2008). The tree may answer such a common infection with decline, extensive basal decay or premature withdraw (LaBonte et al., 2015; Williams et al., 1986). The attacked trees can become very dangerous and hazardous.

Researches on how fungi attacks a living tree are available numerous, for instance Bieker and colleagues (2009) examined *Trametes versicolor* infection on *Fraxinus excelsior*, Deflorio and his team (2008) measured several tree-fungi combinations to test different sonic tomography's ability to catch the infection, while Cruichshank and colleagues (2011) studied non-lethal root infections associated with *Armillaria* root disease. The mentioned tests were performed in laboratories, or at least somehow disturbed condition, and focus of the measurement was on the beginning of the infection.

Our research begins with a tree which has already been decayed in about 63% of the cross section at 30 cm height. (The date of the infection is unknown.) As well the tree was left in its original position; no disturbance was made except the measurements themselves.

## Material and methods

### The tested tree

A spruce (*Picea albies*) in the botanical garden of the University of West Hungary was selected for the measurements. Honey fungus (*Armillaria mellea* s.l.) inflection was noticed on the tree, as the reason to start the research. In 2013 at the beginning of the tests the tree was 92 years old, with 20 m high, while the crown area was 30.4 m$^2$, the crown centre was at 12.7 m, and the trunk had 1° incline. (Fig 1.)

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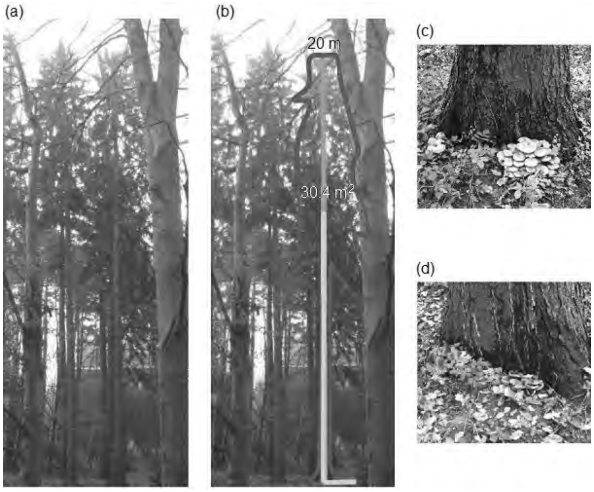


Fig. 1. The measured tree is in the botanical garden of the University of West Hungary (a) and the tree with the data (height, crown area, height of crown center, b). The fruiting bodies of honey fungus were seen in different years (c and d).

## Acoustic tomography

Acoustic tomography for studying decays inside living trees is a technique used for decades (Wang, 2013). Several tests proved the correlation of tomograms with the trunks' real conditions. (Divos and Szalai, 2003; Divos et al., 2008; Wang et al., 2009; Li et al., 2011; Feng et al., 2014; Li et al., 2014).

The basic idea of the measure is the change in signal detection time in the presence of decay. As seen in Fig. 2, in healthy tree, in non-decayed wood the acoustic signal can travel directly through the trunk. Decays change the path of the signal and increase the time of the detection.

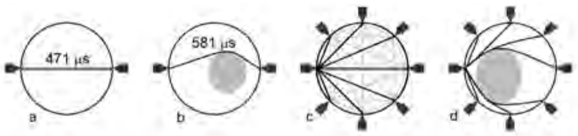


Fig. 2.
a) Acoustic or ultrasound time-of-flight (TOF) measurement on a healthy trunk.
b) TOF measurement on a decayed trunk.
c) The schematic image of a tomography with 8 receivers with the measuring paths.
d) Tomography on a decayed trunk. The travelling paths become longer. With the help of the collectable data a tomogram of the trunk can be prepared, the decay can be localized and safety estimations can be done.

(When the acoustic signal reaches the edge of wood, the end of continuous material it slows down during the penetration to the air. This is why the signal "in a roundabout way" will be the first reaching the detector. It also leads to one limit of this technique. Using acoustic tomography the measured data have information about only the healthy, the continuous part. Acoustic tomography does not "see" inside the decay (Nicolotti et al., 2003)).

The size of the decay is another limiting factor for this technique. Incipient decays like the beginning of a fungi attack (2 months after artificial infection) cannot be seen in tomograms (Deflorio et al., 2008).

However in our case the decay could be seen, measured and quantified clearly.

ArborSonic 3D Acoustic Tomograph was used for the measurements and the program ArborSonic3D 5.2. was used to estimate safety factors for the trunk's levels.

## Pulling test

The pulling or winching test is well-known method to investigate the root-soil system, the root anchorage, estimate safety of trees, and evaluate stem conditions, while it can serve as calibration for measurements in real wind situations as well (Bell et al. 1991; Wessolly 1991; Peltola et al. 2000).

An inclino-type pulling test (measuring inclinations and force) developed by the University of West Hungary was used during the test, which is a tool to get information about the roots' condition. The wind load is simulated by a cable. During the test the pulling force and the inclination of the trunk at ground level are measured. As seen on Fig 3.

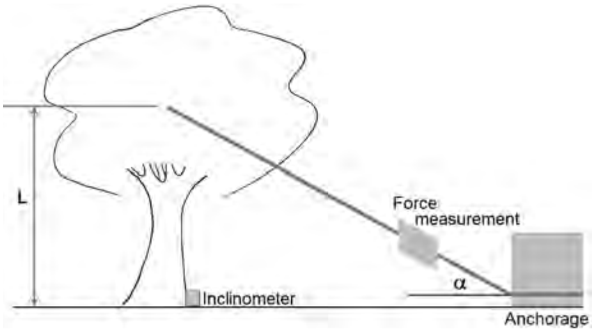


Fig. 3. The schematic picture of a pulling test. A cable is on the measured tree as close to the crown center as it is possible. The height of the cable is L.

For pulling tests which evaluates safety, the cable should be as close to the crown centre as it can be. While the inclinometer should be as close to the ground as it is possible. The anchorage should bear the force (Neild and Wood, 1999; Peltola et al., 2000).

During the measurement the inclination of the measured tree was under 0.2°. The force and the inclination were measured continuously with 1 data sec$^{-1}$. The used program was PullingCollect which calculated safety factors for the root system as well (Brudi and Wassenaer, 2002).

## Results

Acoustic measurements were performed during the springs of 2013, 2014 and 2015. Pulling tests were achieved in 2013 and 2015 spring.

Tomograms were made in three levels, at 30, 100 and 170 cm above ground level. (Fig 4.)

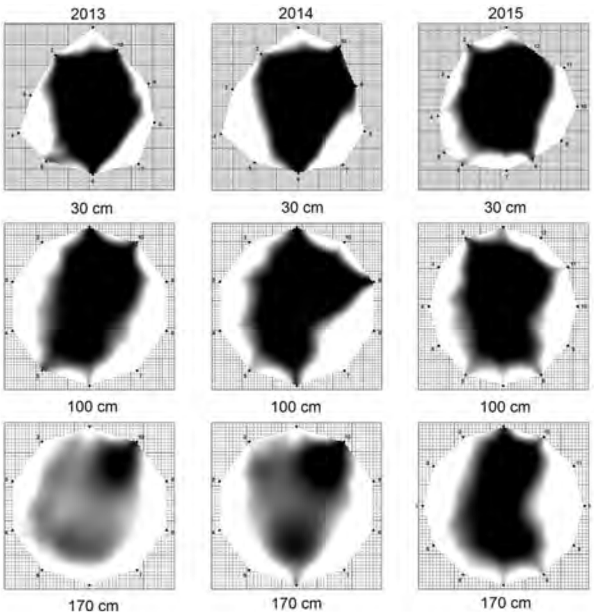


Fig. 4. Acoustic tomograms (white color refers to healthy wood while grey and black refer to decayed areas).

The measurements showed the spruce was "fighting" against the infection. The tree grew in each year, the change in circumference was between 3 and 7 cm, while the diameter widened with 0.5 to 2 cm.

At 30 cm height the decay decreased and the safety increased during the years. (The stem growth was faster than the extent of the decay). At 100 cm height the change in the decay was small while the safety factor rose. At 170 cm height the decay spread and the safety dropped off. The data of the measurements are summarized in Table 1. The tree is still in good conditions. (Safety factors are calculated for 120 km $h^{-1}$ wind gusts. Usually 150% safety factor is accepted for a tree to be safe).

High produce of resin could also be observed on the tree.

## Discussion

Three years are long time – even in the life of a tree – while three years do not let us make final conclusions. The examined tree "defends" itself against the infection; compartmentalization of decay could be observed (Shigo, 1984). The change seems to be slow at 30 cm while the decay doubled at 170 cm height.

As honey fungus usually attacks from the soil and the roots (Glaeser, 2008), it is not surprising that the lowest level lost the most material while the defense worked for more time. The defense may be so effective in the living tissues to stop the infection in radial direction. The observed situation might be a balance but there were not enough measurements to decide it.

The longitudinal direction forecasts are not very encouraging. The infection could grow faster than the trunk. The safety decreased. The tree is in good condition at the moment but a break at this level or even higher may happen in a few years.

The measure will continue, the conditions will be monitored. The practical advice in this situation is to measure more often, in every half or quarter year.

Table 1. The measured decays and the calculated safety factors (SF) with their change to the data from 2013. The decay decreased and the safety increased at 30 cm high. The decay increased a little while the safety increased also at 100 cm high. At 170 cm the decay increase was followed by decrease in safety. For the root system the pulling test showed increasing safety. Note: the growth of the trunk's diameter was 0.5–2 cm $year^{-1}$.

| | 2013 | 2014 | 2015 |
|---|---|---|---|
| Acoustic tomography | | | |
| 30 cm | | | |
| Decayed area (%) | 63 | 61.00 | 61.00 |
| Change from 2013 (%) | 0 | –3.17 | –3.17 |
| SF for trunk level (%) | 586 | 616.00 | 617.00 |
| Change from 2013 (%) | 0 | 5.12 | 5.29 |
| 100 cm | | | |
| Decayed area (%) | 52 | 55.00 | 53.00 |
| Change from 2013 (%) | 0 | 5.77 | 1.92 |
| SF for trunk level (%) | 392 | 483.00 | 501.00 |
| Change from 2013 (%) | 0 | 23.21 | 27.81 |
| 170 cm | | | |
| Decayed area (%) | 23 | 42.00 | 46.00 |
| Change from 2013 (%) | 0 | 82.61 | 100.00 |
| SF for trunk level (%) | 488 | 475.00 | 411.00 |
| Change from 2013 (%) | 0 | –2.66 | –15.78 |
| Pulling test | | | |
| SF for root system (%) | 302 | | 347.00 |
| Change from 2013 (%) | 0 | | 14.90 |

## Conclusion

A honey fungus infected spruce was examined for three years. Even as the fungus is considered to be a dangerous one the tree is still in fair condition both for trunk breaking and tipping.

Acoustic tomography and pulling tests were done in the springs of 2013, 2014 and 2015. The acoustic tomogram shows 5–30% change in the decay at 30 and 100 cm from ground while the decayed area doubled at 170 cm height.

The pulling test showed the root system to stay in good condition, the safety increased.

The tree is in good condition to let it stay at the botanical garden. The measure will go on for more years with more often examinations.

**References**


Bell, H.J., Dawson, A.R., Baker, C.J., Wright, C.J., 1991. Tree stability. In Hodge, S.J (ed.). *Research for Practical Arboriculture*. Forestry Commission bulletin, 97. London, University of York: HMSO, p. 94–101.

Bieker, D., Kehr, R., Weber, G., Rust, S., 2009. Non-destructive monitoring of early stages of white rot by Trametes versicolor in Fraxinus excelsior. *Annals of Forest Science*, 67: 210p1–p7.

Brudi, E., van Wassenaer, P.J., 2002. Trees and statics: nondestructive failure analysis. In *Tree structure and mechanics conference proceedings: how trees stand up and fall down. Savannah, Georgia, U.S., October 2001*. Champaign, Illinois, USA: International Society of Arboriculture, p. 53–70.

Cruickshank, M.G., Morrison, D.J., Lalumiere, A., 2011. Site, plot, and individual tree yield reduction of interior Douglas-fir associated with non-lethal infection by Armillaria root disease in southern British Columbia. *Forest Ecology and Management*, 261: 297–307.

Deflorio, G., Fink, S., Schwarze, F.W.M.R., 2008. Detection of incipient decay in tree stems with sonic tomography after wounding and fungal inoculation. *Wood Science and Technology*, 42: 117–132.

DeLong, D.L., 1995. *Geographic distribution of Armillaria species*. Research summary (Nelson Forest Region (B.C.)), RS-015.; Microlog, 106-05490. Nelson, B. C.: Forest Service, British Columbia [cit. 2015-09-30]. http://www.for.gov.bc.ca/hfd/pubs/RSI/FSP/Nelson/RSN015/RSN015.htm

Divos, F., Divos, P., Divos, G., 2007. Acoustic techniques: from seedling to wood structures. In Ross, R.J. (ed.). *Proceedings of the 15th International symposium on nondestructive testing of wood, Sept. 10–12, 2007*. Madison, Wisconsin: Forest Products Society, p. 3–12.

Divos, F., Szalai, L., 2002. Tree evaluation by acoustic tomography. In Beall, F.C. (ed.). *Proceedings of the 13th International symposium on nondestructive testing of wood. University of California, Berkeley Campus, Berkeley, California, USA, August 19–20, 2002*. Madison, Wisconsin: Forest Products Society, p. 251–256.

Feng, H., Li, G., Fu, S., Wang, X., 2014. Tomographic image reconstruction using an interpolation method for tree decay detection. *BioResources,* 9 (2): 3248–3263.

Glaeser, J.A.M., 2007. Wood decay and discoloration. In Ross, R.J. (ed.). *Proceedings of the 15th International symposium on nondestructive testing of wood, Sept. 10–12, 2007*. Madison, Wisconsin: Forest Products Society, p. 59–63.

LaBonte, R.N., Ostry, M.E., Ross-Davis, A., Woeste K.E., 2015. Estimating heritability of disease resistance and factors that contribute to long-term survival in butternut (Juglans cinerea L.). *Tree Genetics and Genomes,* 11: 63, 12 p. DOI 10.1007/s11295-015-0884-8.

Li, G., Wang, X., Feng, H., Wiedenbeck J., Ross, R.J., 2014. Analysis of wave velocity patterns in black cherry trees and its effect on internal decay detection. *Computers and Electronics in Agriculture,* 104: 32–39.

Li, L., Wang, X., Wang, L., Allison, R.B., 2011. Acoustic tomography in relation to 2D ultrasonic velocity and hardness mappings. *Wood Science and Technology,* 46: 551–561.

Neild, S. A., Wood, C. J., 1999. Estimation stem and root-anchorage flexibility in trees. *Tree Physiology*, 19: 141–151.

Nicolotti, G., Socco, L.V., Martinis, R., Godio, A., Sambuelli, L., 2003. Application and comparison of three tomographic techniques for detection of decay in trees. *Journal of Arboriculture,* 29: 66–78.

Peltola, H., Kellomaki, S., Hassinen, A., Granander, M., 2000. Mechanical stability of Scots pine, Norway spruce and birch: an analysis of tree-pulling experiments in Finland. *Forest Ecology and Management*, 135 (1–3): 143–153.

Shigo, A.L., 1984. Compartmentalization: a conceptual framework for understanding how trees grow and defend themselves. *Annual Review of Phytopathology,* 22: 189–214.

Wang, X., 2013. Acoustic measurements on trees and logs: a review and analysis. *Wood Science and Technology,* 47: 965–975.

Wang, X., Wiedenbeck, J., Liang, S., 2009. Acoustic tomography for decay detection in black cherry trees. *Wood and Fiber Science,* 41 (2): 127–137.

Wessolly, L., 1991. Verfahren zur Bestimmung der Stand- und Bruchsicherheit von Bäumen. *Holz als Roh- und Werkstoff*, 49: 99–104.

Westwood, A.R., Conciatori, F., Tardif, J.C, Knowles, K., 2012. Effects of Armillaria root disease on the growth of Picea mariana trees in the boreal plains of central Canada. *Forest Ecology and Management*, 266: 1–10.

Williams, R.E., Shaw III, C.G.,Wargo, P.M., Sites, W.H., 1986. *Armillaria root disease.* U.S. Department of Agriculture Forest Service, Forest Insect and Disease Leaflet 78 [cit. 2015-09-26]. http://www.na.fs.fed.us/spfo/pubs/fidls/armillaria/armillaria.htm



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**Short communication**

# State of Carabid assemblages in forest ecosystem previously affected by high levels of heavy metals Cu and Pb

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**Abstract**
Machava, J., Timko, Ľ., Šustek, Z., Bublinec, E., 2016 State of Carabid assemblages in forest ecosystem previously affected by high levels of heavy metals Cu and Pb. *Folia Oecologica*, 43: 208–212.

Human activities in industrial areas have released into the atmosphere solids of different nature, including heavy metals. The center of the Spiš region belongs to the foremost polluted areas in Slovakia. The Copper Smelting Plant Krompachy contaminated surrounding areas around this town by metals mainly by copper and lead. Despite significant improvement of environmental situation in the 1990s, resistant pollutant load from the mentioned pollution source continues to negatively impact on the surrounding countryside. The residual pollutant load has persisted for more than 20 years. Species diversity and cumulative abundance of Carabids in all stands in the contaminated environment was extremely low due to synergic effect of three factors, acidity of substrate, southern exposition and discontinuity of the vegetation coverage that resulted in forming of two assemblages. The open stands were inhabited by heliophilous species of genera *Cicindela*, *Harpalus* and *Microlestes*. The characteristic forest species did not occur there or penetrated here only rarely. In the closed stand, only three mesohygrohilous forest species *Carabus violaceus*, *Carabus hortensis* and *Pterostichus oblongopunctatus* were recorded.

**Key words**
Carabids, contamination, forest ecosystems, heavy metals, soil

The worst polluted areas affected by air pollutants in Slovakia include also the center of Spiš region, where the largest exhalation resources have comprised metallurgical and mining-processing units of iron mines (Želba) in Rudňany town and the Copper Smelting Plant Krompachy (Banásová and Lackovičová, 2004; Hronec et al., 2008; Takáč et al., 2008). In the vicinity of these resources increased concentrations of all heavy metals (HM) except for Mn (Lackovičová et al., 1994; Hančuľák et al., 2006) were found.

Increased concentrations of risk elements in forest ecosystems deteriorated the health state of forest tree species and other ecosystem components. This critical situation was not improved even after the closure of the plant in Rudňany town in 1993.

The effect of residual components of elevated concentrations of HM on ecosystems in the central Spiš region was studied after elimination or a significant reduction of emissions from those exhalation sources (Hronec and Szabová, 1995; Konôpka and Paulenka, 1996; Konôpka et al., 1997). After 10 years, the heavy metals contamination in the soil around Rudňany was not noticeable reduced (Machava, 2002, 2003).

Environmental pollution has diverse effects on different living organisms, including insects. It relates also to the ground beetles (*Coleoptera, Carabidae*) closely

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bound to the litter and ground surface. Some of the especially important chemical and physical properties of the soil which might exert an influence on the distribution of Carabids include humidity, shadowing by vegetation, pH value, calcium content, and pollutant load. Carabids can be considered as bioindicators of human practices, including pollution sensitive species (Basedow, 1990). Environmental change can cause different kinds of effect in the indicator species.

Increase or decrease of species number or abundance might be caused directly by changes in abiotic and/or biotic factors (Blake et al., 1996). Carabids represent a component of the ground-dwelling fauna strongly linked to soil characteristics. They are a well-studied group, frequently discussed in studies concerning the effects of environmental changes (Avgin and Luff, 2010).

Carabids play an important role in trophic chains, nutrition cycling and energy flow. They contribute to maintaining balance and stability of ecosystems. Anthropogenic actions lead to changes in soil properties, and are reflected by changes species diversity and abundance of individual species. Literary reviews of soil organisms lay emphasis on bioindicative ability of *Carabidae* in various forest habitats differently changed by human actions (Ranio and Niemela, 2003; Avgin and Luff, 2010; Koivula, 2011).

In this work the environmental situation on the research plot of Dubie was investigated after the 20-year period after the ceasing of high pollutant load. The total concentrations of heavy metals were determined and the occurrence of Carabids was observed in the studied environment strongly affected by high levels of Cu and Pb.

In April 2014, A open, B and C partly-open and D closed habitats close to the top of Dubie hill (around 562 m asl) near Krompachy town were established. The stands are located north of emission source of the Copper Smelting Plant Krompachy, at a distance of 1 km, at the relative altitude 180 m above the pollutants source, located just below the top hill on a southerly exposed slope with a 5% inclination. The herbaceous cover is formed mainly by *Calamagrostis epigeios* (a generating wooden vegetation of about 20 years old; A), *Pleurozium schreberi*, *Vaccinium vitis–idaea, Calamagrostis epigeios* and *Vaccinium myrtillus* (only herbs and shrub floor; B and C), *Acetosella vulgaris*, *Vaccinium myrtillus*, *Milium effusum, Luzula luzuloides* and *Convallaria majalis* (a forest habitat of 80 years old, D).

Six pitfall traps were installed in all sites at distances of 10 m. This number of traps is considered to be sufficient to record about 70% of species living in a habitat (Obrtel, 1971; Niemelä et. al., 1990). On the stand D, traps placement respected the patchy structure of vegetation. The traps were exposed from 1st April to 30th June and from 1st July to 23rd September in 2014. The species were identified by the keys by Hůrka (1996) and Trautner and Geigenmueler (1987). The data on species ecology were taken from Burmeister (1939) and Lindroth (1949).

In the autumn 2014, two soil profiles were dug up on the stands A and D, from which soil samples were taken to determine the actual elevated concentrations of heavy metals (Cu and Pb). Soil samples were collected from individual horizons of the prepared soil profiles (Societas pedologica Slovaca, 2014), dried at a laboratory temperature, ground, sieved through a sieve of 2 mm mesh to prepare a fine earth. The basic pedologic characteristics of the soil were measured. Soil pH was determined by a potentiometric method using the multifunction equipment 340i WTW. Total contents Cu and Pb were determined by atomic absorption spectroscopy using the GBC SensAA spectrometer. Prior to elements determination, soil samples were processed – 0.5 g of fine earth was digested with 5 ml of concentrated $HNO_3$, p.a. in the microwave oven Speedwave MWS-2, co. Berghof. The cumulative abundance of Carabid species and concentrations of Cu and Pb were evaluated by Pearson correlation coefficient *r* using the statistical package SPSS. An independent variable has a certain relationship with dependent variables when coefficient *r* is greater than 0.3.

The concentrations of Cu and Pb on the open stand A in the soil pit 1 were extremely high. Total Cu levels recorded in Oh and Ao horizons were around 1,900 mg $kg^{-1}$ (Fig. 1), which was approximately 30 times higher than the limit value for very acid Cambisol. Concentrations of Pb in Oh and A horizons have exceeded the limit value more than 13 times.

Cu and Pb concentrations in Ol horizon in the second soil pit excavated in the closed stand D were comparable with those of the first soil pit (Fig. 1). However, the levels of both elements in the A horizon were higher about 1,000 mg $kg^{-1}$, which is a clear evidence of the higher previous pollutant load. This place was more influenced by the west-eastern wind flow and that is why higher levels of examined elements were found in the soil there. Soil pH values fluctuated between 4.33 (Bv1) to 4.49 (A), only the Oh horizon was acidic (pH = 4.74). This acidic environment resulted in higher mobility of extremely high Cu and Pb contents which negatively influenced the behaviour of Carabidae and massively reduced their abundance. No significant correlation was observed between the cumulative abundance of Carabids and total Cu and Pb concentration in the soil.

In all studied stands 52 individuals of Carabids belonging to 10 species were recorded. In individual stands, number of species ranged from 3 to 6, while number of individuals from 5 to 21. All catches from June were 2–5-times richer than those from September (Fig 2.). The strongest decline of number of individuals, to about on half or third of the values recorded in June, was observed especially in *Cicindela sylvicola*. The observed decline in number of individuals corresponds with the typical course of seasonal dynamics of Carabids that always culminates approximately in June to July and considerably declines in August and September (Novák 1973; Thiele 1977). In the most abundant *Cicindela sylvicola* the decline of number of individuals in September also might result from the rainy weather in that month that inhibited activity of this heliophilous species.

In spite of very low number of species and individuals, the community structure perfectly reflects the state of the canopy in each stand and its distance from sites with closer canopy and less insolated and dried soil surface. Thus, in the larger open site A only the heliophilous species (*Cicindela sylvicola, Cicindela germanica* and *Harpalus luteicornis*) were recorded.

Among them the all *Cicindela* species even require discontinuous herbage vegetation for their enormous flight activity. In the stand B forming a strip between two closed patches of wooden vegetation, only the heliophilous species form the catch from June, while a typical forest species *Pterostichus oblongopunctatus* co-occurred there in September, when the humidity and temperature gradient between the open and closer stand was not so steep. In the less open stand C, the heliophilous species (*Cicindela sylvicola*, *Cicindela campestris, Harpalus luteicornis* and the xerophilous species *Microlestes minutulus*) still predominated, but out of *Pterostichus oblongopunctatus* the community was enriched by further forests species *Carabus hortensis* in the June sample. Unlike the open or half-open stands, in the almost completely closed stand D there only three mesohygrophilous forests species occurred such as *Carabus hortensis, Carabus violaceus* and *Pterostichus oblongopunctatus* being accompanied by the eurytopic *Notiophilus biguttatus* in the sample from September. The same relationship is also illustrated by representation of flying species that are mostly characteristic for open habitats or for disintegrated forests and by wingless, non-flying species predominating in closed continuous forests.

The highest species diversity was observed in the open or partly open stands (A, B and C), where well-flying predatory tiger-beetles *Cicindela germanica, Cicindela sylvicola* and *Cicindela campestris* predominated. Their characteristic habitats are open landscape with discontinuous herbage vegetation or in *Cicindela sylvicola* also sparse well insolated forests (Hůrka, 1996). They were accompanied by *Harpalus*

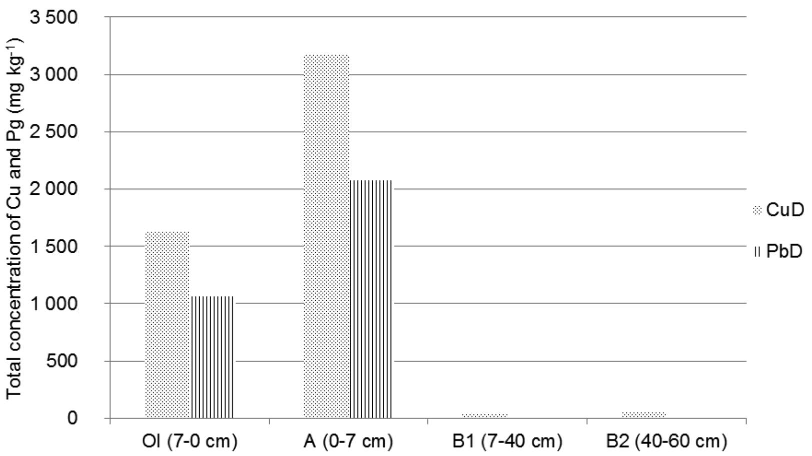


Fig. 1. Total concentrations of Cu and Pb in the stand D (forested area).

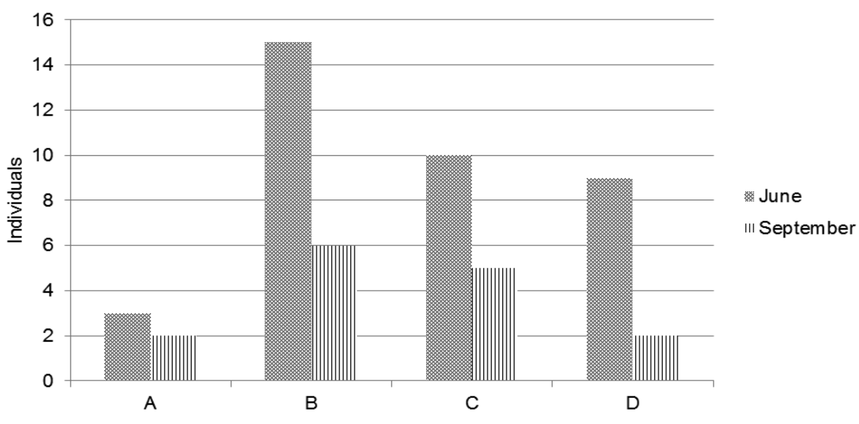


Fig. 2. Cumulative abundance of Carabids in June (J) and September (S) in four study sites A–D

*luteicornis* and *Harpalus affinis* representing characteristic component of faunal of arable land, meadows. All these species are well flying, are able to migrate on large distances and easily occupy the new sites, even in the city centers (Šustek, 1999).

The results obtained illustrate the procedure of gradual species turnover, which flexibly follows the ecological state of the habitat segments and the subsequent changes in them. On an ecologically clean meadow habitat, there at least 10–12 species of Carabids should be found (Koivula, 2011), but usually the number of species exceeds 40–60 (Louda 1973; Obertel 1968), more than on our investigated stand.

The results serve as an example that anthropogenic deterioration of environment can lead not only to serious damaging of plant and animal communities, but can simultaneously create conditions for highly specialized and even rare species like tiger-beetles in this case.

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**References**

Avgin, S. S., Luff, M. L., 2010. Ground beetles (Coleoptera: Carabidae) as bioindicators of human impact. *Munis Entomology and Zoology*, 5 (1): 209–215.

Banásová, V., Lackovičová, A., 2004. Degradácia travinných porastov v blízkosti huty na spracovanie medi v Krompachoch (Slovenské Rudohorie) [The degradation of grassland vegetation near The Copper Smelting Plant Krompachy (Slovenské Rudohorie Mts)]. *Bulletin Slovenskej botanickej spoločnosti,* 26: 153-161.

Basedow, T., 1990. Effects of insecticides on Carabidae and the significance of these effects for agriculture and species number. In Stork, E. (ed.). *The role of ground beetles in ecological and environmental studies*. Andover, Hampshire: Intercept, p. 115–125.

Blake, S., Foster, G.N., Fischer, G.E.J., Ligertwood, G.L., 1996. Effects of management practices on the ground beetle faunas of newly established wildflower meadows in southern Scotland. *Annales Zoologici Fennici*, 33: 139–147.

Burmeister, F., 1939. *Biologie,* Ökologie *und Verbreitung der europäischen Käfer auf systematischer Grundlage. I. Band., Adephaga I., Familiengruppe Caraboidea*. Krefeld: Hans Goecke Verlag. 307 p.

Hančuľák, J., Bobro, M., Šestinová, O., Brehuv, J., Slančo, P., 2006. Ortuť v prostredí starých banských záťaží Rudnian a Merníka [Mercury in environs of old mining loads of Rudnany and Merník]. *Acta Montanistica Slovaca,* 11*,* 2: 295–299.

Hronec, O., Szabová, T., 1995. Ortuť, olovo, kadmium, chróm a arzén v rastlinách stredného Spiša [Mercury, lead, cadmium, chromium, and arsenic in plants of the Middle Spiš region]. *Poľnohospodárstvo,* 41: 749–757.

Hronec, O., Vilček, J., Tóth, T., Andrejovský, P., Adamišin, P., Andrejovská, A., Daňová, M., Huttmanová, E., Vilimová, M., Škultéty, P., Juhászová, M., 2008. Ťažké kovy v pôdach a rastlinách v Rudniansko-gelnickej zaťaženej oblasti [Heavy metals in soils and plants in the Rudnany-Gelnica-loaded area]. *Acta Regionalia et Environmentalica*, 1: 24–28.

Hůrka, K., 1996. *Carabidae of the Czech and Slovak Republics. Carabidae* České *a Slovenské republiky*. Zlín: Kabourek. 565 p.

Koivula, M. J., 2011. Useful model organisms, indicators, or both? Ground beetles (Coleoptera, Carabidae) reflecting environmental conditions. *Zookeys*, 100: 287–317.

Konôpka, B., Paulenka, J. 1996. Poškodzovanie lesov v oblasti Spiša [Forest degradation in the Spiš region]. *Les,* 52 (11): 18–19.

Konôpka, B., Paulenka, J., Konôpka, J., 1997. Poškodenie ihličnatých porastov na Spiši [Damage to coniferous stands in the Spiš region]. *Lesnictví - Forestry*, 43, 9: 381–388.

Lackovičová, A., Martiny, E., Pišút, I., Streško, V., 1994. Element content of the lichen Hypogamniaphysodes and spruce needles in the industrial area of Rudňany and Krompachy. *Ekológia (Bratislava)*, 13: 415–423.

Lindroth, C. H., 1949. *Die fennoskandischen Carabidae I. Spezieler Teil*. Göteborg: Wettergren and Kerbers Verlag. 710 p.

Louda, J., 1973. Die Laufkäfer des Wiesenbestandes in Vorgebirge des Böhmisch-Mährnischen Höhenzuges. *Acta Entomologica Bohemoslovaca*, 70: 390–399.

Machava, J., 2002. *Vplyv zvýšených koncentrácií* ťažkých *kovov na zdravotný stav smreka* a *buka* v *modelových podmienkach* a *prírodnom prostredí* [The influence of enhanced heavy metal concentrations on a health state of spruce and beech in model conditions and natural environment]. PhD thesis. Zvolen: Technical University in Zvolen, Faculty of Forestry. 159 p.

Machava, J., 2003. Relationships between soil and spruce after many years of heavy metal pollutant load in forest ecosystem. In Sobocká, J., Jambor, P. (eds). *Druhé pôdoznalecké dni* v SR. *Zborník* z *konferencie. 16. – 18. 6. 2003, Stará Lesná*. Bratislava: Societas pedologica slovaca, Výskumný ústav pôdoznalectva a ochrany pôdy, p. 217–225.

Niemelä, J., Haila, Y., Halme, E., Pajunen, T., Punttila, P., 1990. Diversity variation in carabid beetle assemblages in the southern Finnish taiga. *Pedobiologia*, 34:1–10.

Novák, B., 1973. Jahreszeitliche Dynamik der diurnalen Aktivität bei Carabiden in einem Waldbiotop (Col. Carabidae). *Acta Universitatis Palackianae Olomucensis, Facultas Rerum Naturalium*, 43: 251–280.

Obrtel, R., 1968. Carabidae und Staphylinidae occurring on soil surface in Lucerne fields (Coleoptera). *Acta Entomologica Bohemoslovaca*, 66: 5–20.

OBRTEL, R., 1971. Number of pitfall traps in relation to the structure of catch of soil surface. *Acta Entomologica Bohemoslovaca*, 68: 300–309.

RAINIO, J., NIEMELA, J., 2003. Ground beetles (Coleoptera: Carabidae) as bioindicators. *Biodiversity and Conservation*, 12: 487–506.

SOCIETAS PEDOLOGICA SLOVACA, 2014. *Morfogenetický klasifikačný systém pôd Slovenska. Bazálna referenčná taxonómia* [Morphogenetic classification system of Slovakia. Baseline reference taxonomy]. VÚPOP Bratislava, 76 s.

ŠUSTEK, Z., 1999. Light attraction of Carabid beetles and their survival in the city center. *Biologia, Bratislava*, 54: 539–551.

TAKÁČ, P., KOZÁKOVÁ, Ľ., VAĽKOVÁ, M., ZELEŇÁK, F., 2008. Ťažké kovy v pôdach stredného Spiša [Heavy metals in the soils of the Middle Spiš region]. *Acta Montanistica Slovaca*, 13, 1: 82–86.

THIELE, H. U., 1977. *Carabid beetles in their environment*. Berlin-Heidelberg-New York: Springer-Verlag. 369 p.

TRAUTNER, J., GEIGENMUELER, K., 1987. *Tiger beetles, ground beetles. Illustrated key to the Cicindelidae and Carabidae of Europe*. Germany, Aichtal: J. Margaf Publishing. 487 p.

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***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. *Environmental Entomology*, 24: 1239−1244.

Eiberle, K., Nigg, H., 1984. Zur Ermittlung und Beurteilung der Verbissbelastung. *Forstwissenschaftliches Centralblatt*, 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.

***Book series***

NYBOM, H., RUMPUNEN, K. (eds), 2005. *Proceedings of the 1st international rose hip conference. Gümüşhane, Turkey, September 7–10, 2004*. Acta Horticulturae, 690. Leuven: International Society for Horticultural Science. 301 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.

***Web source***

WOODWARD, J.L.W., 1999. *Effect of fungicide treatment to control shot-hole disease of cherry laurel*. Centre for Applied Nursery Research [cit. 2011-10-12]. http://www.canr.org/98013.pdf

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