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Species diversity of microscopic fungi on Austrian pines growing in urban greenery of Nitra town

Helena Ivanová[1], Slávka Bernadovičová[2]

Branch for Woody Plants Biology, Institute of Forest Ecology of the Slovak Academy of Sciences, Akademická 2, 949 01 Nitra, Slovak Republic,
[1]E-mail: helena.ivanova@savzv.sk, [2]E-mail: slavka.bernadovicova@savzv.sk

Abstract

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During the year 2009, complex mycoflora of Austrian pine trees (*Pinus nigra* Arnold) was monitored within a survey on health state of trees growing in urban environment. Four species of microscopic fungi were isolated and identified from samples collected from different part of Nitra town during the study period. This study reports the occurrence of the fungi *Camarosporium pini* (Westend.) Sacc., *Fusarium* sp. and *Mycosphaerella dearnessii* M.E. Barr on needles and *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton on needles and cones of *P. nigra*. Disease symptoms, some important characteristics in pure culture and distinctive morphological features are described. The presence of these fungi in host tissue of symptomatic trees has been described using classical phytopathological approaches and microscopical identification based on morphological keys. *Sphaeropsis sapinea* was identified on pine needles and cones in all examined samples with high frequency. The occurrence of *Fusarium* and *Camarosporium* fungi was relatively common. *Mycosphaerella dearnessii* was found as a fungal pathogen with sporadical incidence, its presence was noticed only in two cases from the all examined samples.

Key words

Camarosporium pini, Fusarium sp., microscopical characteristics, *Mycosphaerella dearnessii, Pinus nigra, Sphaeropsis sapinea*

Introduction

Recently, with regard to the expected global climate change, there is an increased risk of a shift of climatic barrier in Europe preventing penetration of many diseases and pests so far. At the same time, this change may cause the pathogens to adapt to the previously resistant species of woody plants (Kolářík et al., 2005). In recent years, among urban trees Austrian pine also called European black pine (*Pinus nigra* Arnold) belongs to the particularly affected tree species. Austrian pine is a bold-textured and urban-tolerant pine having a broad-pyramidal growth habit with ascending branches and showy spring candles and often developing a flat-topped crown and ornamental bark with age. This evergreen tree in pine family is used either as a single specimen or in group or mass plantings as visual screens or windbreaks.

Numerous fungal diseases affect pine trees, including Austrian pine. Some of them are managed easily by applying chemical substances or biological means, the other spread rapidly through pine populations, as there have been no known means for controlling the fungal spread yet. Among pathogenic fungi, pathogens identified from the dead pine tissues include also *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton, *Camarosporium pini* (Westend.) Sacc., *Fusarium* sp. and *Mycosphaerella dearnessii* M.E. Barr (Sinclair et al., 1987). In connection with the occurrence of causal pathogens withering of branches and tree thinning was recorded in tree individuals in the lower parts of the tree crown as a beginning of woody plants weakening.

One of the most common and widely distributed pathogens of conifers worldwide, including European black pine is *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton, syn. *Diplodia pinea* (Desm.) J. Kickx f. (teleomorph in *Botryosphaeria*). It is a cosmopolitan fungus in Coelomycetes (Botryosphaeriales) identified in more than 50 countries of the world, on all continents but it is primarily the species of warm lands. The last decade is characterised by the fast movement of this pathogen from southern Europe to the north (Hanso and Drenkhan, 2009). This southern fungus moves north during drought periods: it is known that *D. pinea* can be released from its quiescent stage in the host by host water stress (Stanosz et al., 2001; Paoletti et al., 2001). Hard drought in last period encouraged *D. pinea* to become epidemic in Central Europe (Blaschke and Cech, 2007). Sphaeropsis tip blight, also called Diplodia blight is considered to be a "disfiguring disease" that attacks pine trees growing under stressful conditions. The fungus does not typically kill the tree but will significantly disfigure the tree if not properly cared for or controlled. The most endangered and frequent host coniferous plants are species in Pinaceae family – it occurs on 48 pine species, among which the most susceptible are *Pinus nigra* Arnold, *P. radiata* D. Don*, P. sylvestris* L., *P. ponderosa* Dougl. ex C. Lawson, *P. resinosa* Sol. ex Aiton, *P. mugo* Turra, *P. pinaster* Aiton and *P. elliotti* Engelm. (Milijašević, 2002). *Pinus nigra* is the most susceptible to *Diplodia* infection in spring when shoots are just elongating and not yet lignified. After the fungus has killed the host tissue, it can produce pycnidia which overwinter and can be a source of inoculum the following spring (Hartman, 2009). The first symptom may be exudation of a drop of resin from a small lesion, which enlarge quickly and infected buds or shoots cease growth before or during needle elongation. Tissues in lesions are resinsoaked and discolored dark reddish brown, and they often exude resin, which in time crystallizes, making the dead shoot hard and brittle (Sutton, 1980). The most conspicuous symptoms of this disease – brown, stunted new shoots with short, brown needles first appear on the newly elongating shoots in late April to early May. In late May the diseased tips are noticably necrotic and stunted. Progression of the fungus can lead to branch dieback and eventually death of the tree. Most trees escape infection for the first 15 to 20 years of life, only to develop symptoms after they reach maturity and begin to develop cones (Gregorová et al., 2006).

The genus *Fusarium* (Hyphomycetes, Hypocreales), (teleomorph in genus *Gibberella*, *Nectria*) contains many species that attack numerous hosts, including pine trees of all ages. Among the diseases caused by *Fusarium* sp. are root rots, blight and damping-off. Under optimum conditions, e.g. abundant fungal inoculum, high humidity and warm weather, susceptible pine species are likely to top blight (Affeltranger, 1983). The *Fusarium* species cause foliage diseases of pine trees. Symptoms of *Fusarium*-induced top blight often start at the growing tip of the plant, killing needles from the base upward. Under wet conditions the disease progresses laterally. *Fusarium* survives as resting or survival spores (chlamydospores) in organic matter and recently killed host tissues, particularly root pieces. Pathogen is spreading locally by the wind or insects. At greater distances it can be expanded through the contaminated seeds and seedlings. Branches and bark where the fungal spores are able to survive can also be the source of infection. Fungi in *Fusarium* attack the vegetative and generative plant organs and disease symptoms can occur in any season. *Fusarium* colonizes root system causing brown discoloration and decomposition of the surface layers of roots. The symptoms are not noticeable on tree aboveground part unless the fungus reaches the root collar and surrounds the tree stem. In the next phase there is a change in color of needles (yellowing to brown). Infection does not usually result in tree death, but deformed, slow-growing trees can be economically or aesthetically problematic.

The fungus *Mycosphaerella dearnessii* M.E. Barr (Ascomycetes, Dothideales), syn. *Scirrhia acicola* (Dearn.) Sigg., anamorph: *Lecanosticta acicola* (Thüm.) Syd., syn. *Septoria acicola* (Thüm.) Sacc. causes the disease called brown-spot needle blight. The fungus kills the foliage and retards the growth of many pine species. This fungus tends to infect pines from seedlings to eight years of age. Warm wet weather encourages infection progress. Disease usually takes several years to reach epidemic conditions. Thus allows enough time to prevent an epidemic outbreak. Brown-spot needle blight disease causes severe defoliation of pine trees where only last years needles remain on the branches that can be half dead from the late summer months. The disease is most evident on this year needles in summer period when the green needles are brown-spotted with yellow borders diffusing in brown, yellow-bordered stripes. Brown-spot needle blight is in Europe reported from Austria (Brandstetter and Cech, 2003; Kirisits and Cech, 2006), France (Chandelier et al., 1994), Italy (La Porta and Capretti, 2000), Germany (Pehl, 1995), Switzerland (Holdenrieder and Sieber, 1995), Bulgaria and formerly Yugoslavia (Holdenrieder and Sieber, 1995), Croatia (Novak-Agbaba and Halambek, 1997). Some new records origin from Slovenia (Jurc and Jurc, 2009) and Czech Republic (Jankovský et al., 2009).

Needle and shoot blights of pines caused by fungi in *Camarosporium* genus are doing more damage to coniferous foliage in Europa than any other group of fungi although literary records on these pathogens are rather insufficient. Within the diseases, fungus *Camarosporium pini* (Westend.) Sacc. (Coelomycetes, Pleosporales), syn. *Hendersonia pini* Westend. (teleomorph unknown) induces severe infection that can result in significant growth reduction. The micromycete parasitizes the needles of *Pinus nigra* weakened by the low

temperatures in winter period and by the drought from the spring to summer season. On the dry needles, little back spots (pycnidia) arranged linearly and in parallel with the nervure can be noticed (Grove, 1922).

This study aims to describe the characteristic symptoms of fungal diseases and based on examination of growth and morphological attributes, the distinctive morphological features of studied microscopic fungi in *Sphaeropsis*, *Fusarium*, *Camarosporium* and *Mycosphaerella* genus are causative agents involved to a different degree in health state degradation of *Pinus nigra* Arnold in urbanized settings. For this purpose, some important characteristics as growth in culture, conidial formation and size differences in microscopical structures were studied in hyphal cultures of the examined pathogens isolated from symptomatic Austrian pine trees.

Material and methods

In the late summer and autumn 2009, damaged twigs with needles and cones had been taken from symptomatic Austrian pines growing in different parts of Nitra town. The material was collected at several locations from the diseased pine trees, in the areas of Nitra – Kynek, Nitra – Chrenová (Agrokomplex, Lidl, SPU – park, UKF – park) and Nitra – Zobor (private gardens). Altogether 128 trees were studied. The age of evaluated trees was between 15 and 70 years. The samples of material have been deposed at the Institute of Forest Ecology of the Slovak Academy of Sciences, Branch for Woody Plants Biology in Nitra. The fungi were identified by microscopic analyses based on the appearance of the fruiting bodies, spore bearing organs (asci), reproduction organs (conidia and ascospores) and the appearance of the fungi in pure cultures. Classical phytopathological approaches were used to isolate and obtain pure hyphal cultures. Fungi were isolated from the needles first immersed for one minute into 70% alcohol and then for 15 minutes into sodium hypochlorite (1% available chlorine). After that, the needles were washed twice in sterilized distilled water and cut to fragments of 2–5 mm which were placed on the nutritive media, potato-dextrose agar (PDA) and Czapek-Dox agar (CzD). Petri dishes with the media and host fragments were incubated at 24 ± 1 °C and 45% humidity in dark in a versatile environmental test chamber MLR-351H (Sanyo). Pure fungal cultures were obtained using multiple purifications. The identification was performed using morphological keys according to Arx (1957, 1970), Hitchcock and Cole (1980), Nelson et al. (1983), Sutton (1980), Woodward (2001) and Tello et al. (2000). The fungi were identified based on colony development, sporulation and fructification in cultures, hyphal appearance, growth rate etc. Visual characteristics of symptomatic needles were examined with a stereomicroscope SZ51 (Olympus) and fungal structures observations were accomplished with a clinical microscope BX41 (Olympus) under a 400× magnification.

Results and discussion

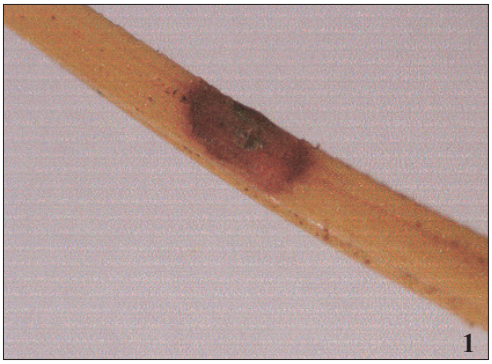
The fungus *Sphaeropsis sapinea* is worldwide in distribution and importance on a great number of coniferous, most often on pine species. It kills the pine shoots and needles, individual branches, tree tops or the entire plants, and also it causes dying of the young plants in nurseries. On *Pinus* species, the fungus *S. sapinea* is a typical parasite and it colonises the current-year shoots and needles. The study of morphological characteristics of the fungus on these host plants and the comparative analysis with the morphological characteristics of the same fungus on *Pinus* sp. shows a difference between them (Milijašević, 2003).

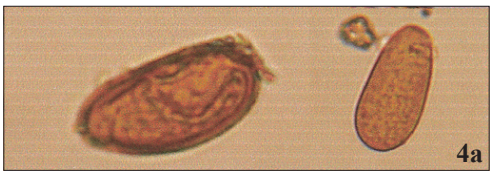
In our study, *Sphaeropsis sapinea* was identified on Austrian pine needles and cones from all examined samples with high frequency. The infected needles manifested presence of tiny black fruiting structures – pycnidia about 200 µm in diameter (Fig. 1). Pycnidia break throught the surfaces of killed needles, cone scales and the bark of twigs beginning in late summer in the year of infection and continuing the next spring. In cylindric-clavate, bitunicate 4–6(–8)-spored asci are formed reproduction organs (ascospores), brown, oblong to ovate, without septa, moderately thick-walled with smooth surface (Figs 2a, 2b). The fungus also produces spores in fruiting structures that develop on the second-year female cones (Fig. 3). Because most of spores are produced on the mature cones, tip blight often does not show up untill the trees are 15 to 20 years old and produce the large cones. Spreading through the whole tree organism can cause the eventual death of all infected branches and ornamental value of affected tree is significantly diminished (Engelbrecht, 2005). Pycnidia contain spores dispersed by rain splash and windblown rain. The spores require a high relative humidity to germinate and infect needles and shoots. A wet period of 12 hours at 12 °C to 36 °C is enough for spore germination and infection. In warm, moist weather symptoms appear in about three to four days. The *Sphaeropsis* conidia are generally dark brown and large, oblong to clavate, thick-walled, 0–1-septate. In our experiments conidia were 30–42 × 14–16 µm, were brown, oblong to clavate, thick-walled. Most conidia are unicellular (Fig. 4a) but some also two-celled (Fig. 4b).

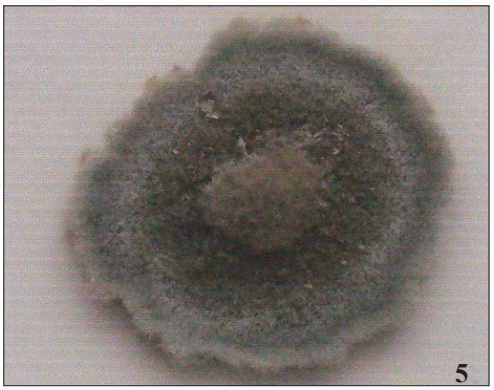
According to Palmer and Nicholls (1983), the fungus grows rapidly on standard mycological media, such as malt or potato-dextrose agar possible for isolation of *Sphaeropsis* and incubation in dark or light (at 20–25 °C for 2 or 4 days). According to Milijašević (2002), the optimal temperature for mycelial growth on the media PDA and MEA is 28 °C. Palmer

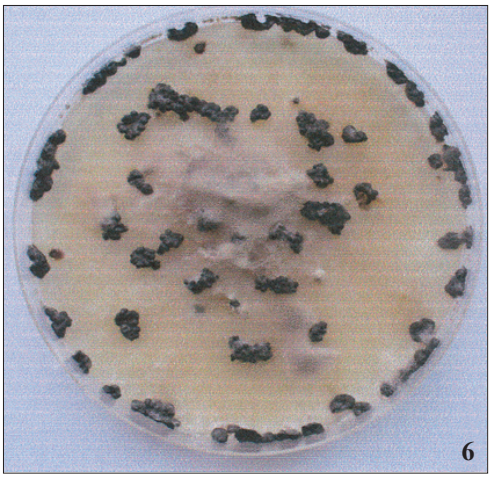
and NICHOLLS (1983) observed that the fungus formed gray-green fluffy mycelium. Cultures of this fungus are first white, later then darken with age. *Diplodia* does not sporulate readily in culture. For positive identification authors suggest to place sterile pine needles over actively growing cultures and incubate them in light. Pycnidia and spores are usually produced in about 1 week. In our experiments potato-dextrose agar (PDA 3%) and Czapek-Dox agar (CzD 3%) were used for cultivation. Rapid growth rates we obtained by incubation in dark at 24 ± 1 °C for 5 days. The fungus formed white mycelium, becoming darker and gray-green with age (Fig. 5). The formation of fruiting bodies in the culture is in dark for two weeks (Fig. 6).

Comparison of biometric characteristics and morphological features of *Sphaeropsis sapinea* on *Pinus*









Sphaeropsis sapinea on *Pinus nigra:*
Fig. 1. The tiny black pycnidia on affected needle.
Fig. 2. Asci with ascospores (2a), detail of ascus (2b).
Fig. 3. Conidiomata on the cone scales of *P. nigra.*
Fig. 4. Unicellular (4a) and two-celled conidia (4b).
Fig. 5. Gray-green mycelium of fungus formed l days after inoculation on potato-dextrose agar (PDA).
Fig. 6. Fruiting bodies formation for 2 weeks on PDA.

Table 1. Comparison of biometric characteristics and morphological features of *Sphaeropsis sapinea* on genus *Pinus* sp. reported by other authors and examined material from Slovakia

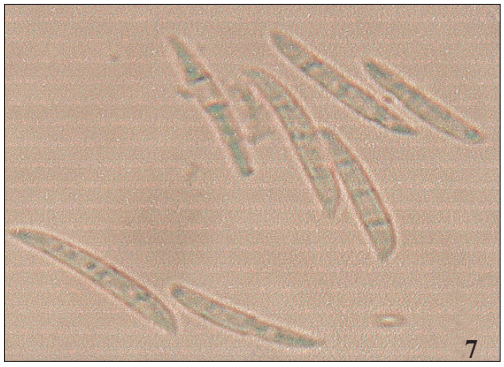
Author(s)	Host	Size of conidia (µm)	Conidia
Peterson (1977, 1978)	*P. sylvestris* *P. resinosa* *P. ponderosa* *P. radiata* *P. nigra*	absent	Conidia brown, one septate or withouth any cross walls (septa)
Sutton (1980)	*P. nigra* *P. ponderosa* *P. sylvestris*	30–45 × 10–16	Conidia dark brown, most unicellular, but a few are two-celled and large
Palmer, Nicholls (1983)	*P. sylvestris* *P. nigra* *P. resinosa* *P. banksiana*	22.5–44 × 9.5–18.5	Conidia brown, sometimes with one septation, rough-walled and elliptical in shape
Peterson, Nicholls (1989)	*P. nigra* *P. mugo* *P. resinosa* *P. ponderosa* *P. sylvestris* *P. radiata*	30–45 × 10–15	Spores are brown at maturity, ellipsoid, usually one-celled
Crous et al. (1990)	*Pinus sp.*	30–45 × 10–16	Conidia yelowisch to brown, oblong to clavate, rounted at apex with bunt basal end, 0–1-septate
Koltay (2001)	*P. nigra* *P. sylvestris*	35–40 × 15–20	–
Milijašević (2002)	*Pinus sp.*	14.8–51.4 × 9.7–20.2	–
Jankovský, Palovčíková (2003)	*Pinus sp.*	25–40 × 10–15	Conidia dark, 1 and 2-celled
Adamčíková, Juhásová (2005)	*P. nigra*	35–40 × 15–20	–
Phillips et al. (2005)	*Pinus sp.*	30–45 × 10–16	Conidia wall dark brown, internally ruoghened, sometimes with 1 septum, oblong to clavate, straight, thick-walled, ornamented on the inner surface of the wall
Juhásová et al. (2006)	*P. nigra*	33.2–41.2 × 16.6	Conidia dark, ovoid, elongate, 1-celled, mature 2-celled
Hanso, Drenkhan (2009)	*P. nigra*	30.6–44.7 (47.1) × (11.8) 14.1–16.8	Conidia brown
Examined material (2009)	*P. nigra*	30–42 × 14–16	Conidia brown, oblong to clavate, thick-walled 0–1-septate

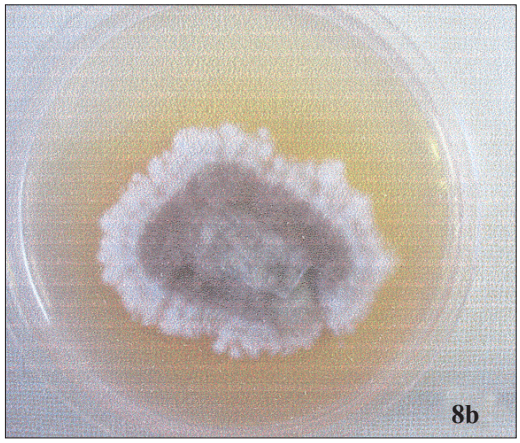
species reported by other authors and examined material from Slovakia is shown in Table 1.

The occurrence of *Fusarium* and *Camarosporium* fungi in our survey was relatively common. Considerable damage to pine trees, especially *Pinus nigra* caused by fungi *Fusarium* isolated from damaged tissue of needles sampled during evaluation of the trees in urban greenery was noticed. Presence of typical macroconidia with 1–3 septa of *Fusarium* with dimensions 26–45 × 4–6 µm on damaged needles was observed (Fig. 7). Pure cultures with colonies of aerial, white (Fig. 8a) or slightly violet fungal mycelium (Fig. 8b) formed 10 days after inoculation on PDA medium.

Carlucci et al. (2007) noticed numerous trees of genus *Pinus* (*P. halepensis* Mill., *P. pinea* L.) in urban parks and gardens in Apulia showing crown decline as a consequence of dieback of twigs and branches and withering of needles. The needles of affected twigs and branches wilted, faded, turned yellow, then red, and were discarded. The species of *Fusarium* was consistently isolated from all infected tissues. Pure cultures were obtained by single hyphal tip transfers on PDA and synthetic nutrient agar medium. Colonies were incubated at 22 ± 3 °C for 7 to 10 days. The species was identified as *Fusarium circinatum* Nirenberg & O'Donnell on the basis of morphological and cultural characteristics. According to Britz et al. (2002) branches and stems of pine trees of any age may be infected. Infection usually begins as a canker and dieback of small branches. Needles wilt above the infection site (becoming chlorotic, then red and brown), and resin accumulates on the branch surface. Repetition of these symptoms throughthout the canopy may lead to extensive dieback. The trunk and larger branches may be in due course infected, producing copious amounts of resin, and accelerating the decline of the tree. Girdling of the main stem may lead to death of the tree. Zad and Koshnevice (2001) in study of damping-off collected samples from the roots of *Pinus nigra* seedlings from nurseries in the south of Iran. After disinfecting, standard media like PDA, MA and CLA were used and the following fungi were identified: *Fusarium solani* (Mart.) Sacc., *Fusarium oxysporum* (Schltdl.), *Fusarium sambucinum* Fuckel and others. Infection of *P. nigra* with *F. solani* and *F. oxysporum* was extensive. In Europe, disease caused by *F. circinatum* previously has been reported only from Spain on *P. radiata* and *P. pinaster* (Landeras et al., 2005). In Serbia, presence of *Fusarium* on Austrian pine needle litter was confirmed by Karadžić and Milijašević (2008). Our results are comparable with studies carried out for *Fusarium* on *Pinus* species in several other countries (Table 2).

Other damage to pine needles and shoots is caused by the fungus *Camarosporium pini* (Westend.) Sacc. Literary records on *Camarosporium* pathogens on conifers are rather insufficient. The fungi of genus *Camarosporium* cause more damage to deciduous





Fusarium sp. on *Pinus nigra*:

Fig. 7. Characteristic macroconidia of *Fusarium* (1–3 septate) isolated from infected tissues of Austrian pine needles.

Fig. 8. Hyphal cultures with aerial white (8a) or later sligtly violet (8b) fungal mycelium 10 days after inoculation on potato-dextrose agar.

trees in Europa. For example, the fungus *Camarosporium elaeagni* Potebnia with rather small, densely gregarious pycnidia was noticed on *Eleagnus angustifolia* L. On *Spirea callosa* Lindl. & Paxton, the fungus *Camarosporium spiraeae* Cooke with large pycnidia covered with elevated epidermis, and on *Salix alba* L. and *S. fragilis* L. *Camarosporium salicinum* (Vize) Grove with densely scattered, roundish pycnidia with a protruding ostiole occurred. On dead branches of *Robinia pseudoacacia* L. the fungus *Camarosporium robiniae* (Westend.) Sacc. was noticed (Juhásová et al., 2004).

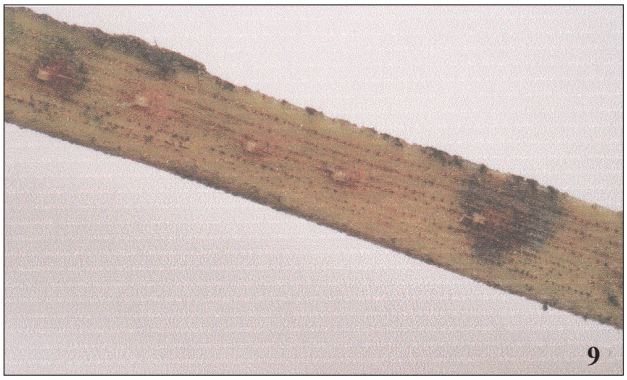
The micromycete *Camarosporium pini* parasitizes the needles of *Pinus nigra* weakened by low temperatures in winter and by drought in spring and summer. On the dry needles little black spots arranged linearly and in parallel to venation were observed (Fig. 9). These spots represent pycnidia of 180–300 μm in diameter, black, spherical swelling of the epidermis with pustules. Through the pycnidium pore there come out numerous oval brown conidia with three transversal walls and 1–2 vertical walls of sizes ranging between 18–20 × 9–10 μm. Pycnidia rather crowded, up to 500 μm diameter, very convex, roundish or elongated, black, covered by the epidermis, then bursting it irregularly or by a slit, texture thick and dark, indistinct, paler inwards. Spores oblong, rounded at both ends, often slightly curved, at first continuous, at length 3-septate, not or hardly constricted, with frequently one or two longitudinal divisions, 15–18 × 7–8 μm, cells uniformely brown, the central cells often shorter than the terminal ones, sporophores short and indistinct (Grove, 1922). Pycnidia were immersed in the bark, at first scattered, than arranged in line, up to 700 μm diameter, subglobose, brown, perforating the epidermis, ostiole subpapilliform. Spores elongate-oval, brown, 3–6-septate (or even 8-septate) and muriform, not constricted, 15–25 × 7–9 μm, sporophores indistinct (Dennis, 1964). Comparable results with the frequent fungi in *Camarosporium* genus occuring on fallen cones on Austrian and Scots pine were obtained by Karadžić and Milijašević (2008) in Serbia.

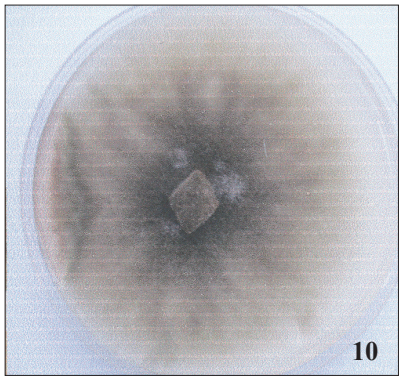
Cultures obtained during our cultivation from injured needles of *Pinus nigra* on PDA medium were initially white with abundant aerial mycelium, gradually becoming grey to dark grey (Fig. 10). The reverse side of the colonies is at first white, but after 2–3 days becoming dark green to olive green from the centre. This coloration gradually spreads to the edge and becomes darker from the centre until the entire underside of the colony is black. Conidia are pale brown, thin-walled, smooth, fusiform to fusiform-elliptical, straight, apex subobtuse, base truncate, 20–22 × 6–8 μm (Figs 11a, 11b).

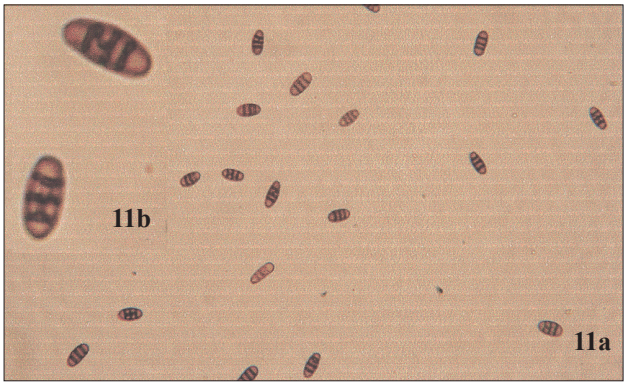
Table 2. Comparison of biometric characteristics and morphological features of *Fusarium* sp. on genus *Pinus* sp. reported by other authors and examined material from Slovakia

Author(s)	Hosts / pathogen	Size of conidia [μm]	Conidia, mycelium
Zad, Koshnevice (2001)	*P. nigra / F. solani*	M: 27–52 × 4.4–6.8	–
		m: 8–16 × 2–4	
Britz et al. (2002)	*Pinus sp. / Fusarium sp.*	M: 32–48 × 3.3–3.8	M: typically 3-septate, with slightly curved walls
		M: absent	m: typically single-celled, ovoid (or nearly oval or allanoid), are borne in false heads on aerial polyphialides
			Mycelium: aerial on which they are borne, gives a distinctive colony, morphology. Aerial mycelium is white, or slightly violet. Colonies are frequently sectored
Landeras et al. (2005)	*P. radiate*	Absent	–
	P. pinaster / F. circinatum		
Carluci et al. (2007)	*P. halepensis* *P. pinea / F. circinatum*	Absent	M: typically 3-septate with slightly curved walls
			m: single-celled
			Mycelium: white aerial, violet pigment
Karadžić, Milijašević (2008)	*P. nigra / Fusarium sp.*	Absent	–
Examined material (2009)	*P. nigra / Fusarium sp.*	M: 26–45 x 4–6	M: 1–3-septate
		m: absent	Mycelium: aerial, white or slightly violet

M, macroconidia; m, microconidia.







Camarosporium pini on *Pinus nigra*:

Fig. 9. Fig . 9. Little black spots of the fungus arranged linearly and parallelly to the venation of damaged Austrian pine needle.

Fig. 10. Cultura of *C. pini* on potato-dextrose agar with abudant aerial mycelium gradually becoming gray.

Fig. 11. Thick-walled, brown, elliptical, straight conidia of *C. pini* (11a), detail of conidia (11b).

During the study period, the needle cast *Mycosphaerella dearnessii* was found as a fungal pathogen with sporadical incidence, its presence was noticed only in two cases from all examined samples. The first report of *M. dearnessii*, the causal agent of brown-spot needle blight was observed on the southern slopes of the Alps and in Italy on the *Pinus mugo* about 50 years old and 2.0 to 2.5 m high by La Porta and Capretti (2000). *P. mugo* exhibited extensive necroses and defoliation of the crown starting from the bottom upward especially on the shaded portions of infected trees. Symptomatic needles were confined to the 2–3 years old internodes. Infected needles had several dark to purplish-brown spots surrounded by green tissue and usually had dead tips. Pycnidia and conidia of *Lecanosticta acicola* were observed.

Dead or blighted one-year-old needles of *P. sylvestris* and *P. mugo* growing in Slovenia affected by *Lecanosticta acicola* (*Mycosphaerella dearnessii*) were found in 2008 and 2009. The results obtained by Jurc and Jurc (2009) represent a first report about this fungus in Slovenia. Recently, brown-spot needle blight associated with *Mycosphaerella dearnessii* on 10–40 years old *Pinus rotundata* in the Czech Republic has been recorded by Jankovský et al. (2009). The symptoms begin with the appearance of yellow and brown spots, later becoming bands on first-year needles that decline from the tip. Acervuli erupt from needles incubated for two to three days in a moisture chamber. After three weeks incubation of conidia at 21 °C on malt extract agar, dark green olive stromatic mycelial colonies produced conidia identical to those detected on incubated needles.

A presumptive indication of the presence of *M. dearnessii* in pine needles from Nitra town conditions is from early September where yellow, resin-soaked spots later becoming brown in the centre appeared (Fig. 12). Eventually, the needles turn all brown from the tip to the base. Some have a yellow patches or brown infected tissue (Figs 13a, 13b) and may fall off. Lower branches are more likely to be infected first due to less air circulation, and the infected needles fall on the ground. Infected needles droop and fall by September of the next year. If the infection is severe, whole needles are killed and drop, leaving bare branches. The disease can affect trees of any age but seedlings are most susceptible. On the brown-coloured dead parts of the needles, the black stromata 0.2–0.4 × 0.1–0.3 mm in size develop under the epidermis visible as round black spots. The oval-shaped fruit bodies are arranged parallelly to long axis of the needles. Under damp conditions, conidial masses protrude from both sides of the conidiomata (Fig. 14), and break the epidermis through opening by a 1–2 longitudinal slit. The conidia are olive-coloured, straight to curved, thick-walled, 1–3-septate, fusiform to cylindrical, with verrucose structure, 22–40 × 3.5–6 µm in size (Fig. 15). The fungus should be isolated from the affected needles with brown dead tissue (brown spots, dead parts with black stroma spots). After an incubation period of 2–3 weeks at 22 °C in dark, isolates can be examined. Slow-growing mycelia should be subcultured onto fresh PDA media. The fungus grows very slowly

Table 3. Comparison of biometric characteristics and morphological features of *Mycosphaerella dearnessii* on *Pinus* sp. reported by other authors and examined material from Slovakia

Author(s)	Host	Size of conidia [µm]	Conidia
Laut et al. (1966)	*P. concorta* var. *latifolia* *P. banksiana*	21–44.5 × 2.5–3.5	Conidia (0–)3(–4)
Sutton (1980)	*Pinus* sp.	15–35 × 3–4	1–3-septate, multicellular, olive-coloured spores
Jewell (1983)	*P. palustris*	Absent	Conidia have one to three walls or with or withouth several indistinst walls, rarely were mature conidia of less than four cells observed
Evans (1984)	*Pinus* sp.	(10)–12–45(–55) × 2–4.5	Conidia 1–5-septate, extremely variable in form, subhyaline to dark-brown, echinulate to verrucose or tuberculate, thick-walled
Li et al. (1986)	*P. elliottii* *P. massoniana* *P. thunbergii*	24.5–51 × 3.4–6.3	Conidia with 1–6 septa
Holdenrieder, Sieber (1995)	*P. mugo* *P. uncinata*	28–54 × 2.9–4.9	Conidia with 0–3 septa
Pehl (1995)	*P. mugo*	19.2–48 × 2.5–5.0	Conidia with 0–5 septa
Suto, Ougi (1998a, 1988b)	*P. thunbergii*	20–53 × 3.3–5.0	Conidia subhyaline to dark brown, verrucose, thick walled, straight to curved, 1–5-septate, fusiform to cylindrical with a round apex and truncate base
La Porta, Capretti (2000)	*P. mugo*	20–30 × 3–4	Conidia 4-celled, curved, pointed at one end and blunt at the other, pale olive-brown
Jurc, Jurc (2009)	*P. sylvestris* *P. mugo*	30 (16–42) × 4 (2–5)	Conidia are brown, strieght or curved, with a rounded apex and truncate base, thick and verrucose wall, and 0–6 septa
Jankovský et al. (2009)	*P. rotundata*	3–5 × 21–44	Conidia subhyaline, thick-walled, straight to curved, fusiform to cylindrical, with rounded apex and truncate base, with 1–5 septa, occasionally slightly constricted at the septa. The surface have small warts or spines
Examined material (2009)	*P. nigra*	22–40 × 3.5–6	Conidia olive-coloured, straight to curved, thick walled, 1–3-septate, fusiform to cylindrical

in culture, forming grey, brown or greenish-black stromatic colonies (Fig. 16) with slimy masses of pink-grey or greenish conidia. Some biometric characteristics and morphological features of *Mycosphaerella dearnessii* on pine trees noticed by several authors are in Table 3.

The presented results indicate that the health condition of Austrian pine trees in urban environment may be negatively affected by microscopic fungal pathogens which can cause individual tree subjects degrade to different degrees. In this study, the concerned fungi were identified with using morphological keys. However, characteristics of necrotic and chlorotic needles with fruiting bodies were examined with a stereo-microscope, and identification of isolates and fungal structures was made based on microscopical differentiation. This preliminary identification, however, needs using methods of molecular biology for confirmation, since the morphological characteristics alone may not be fully reliable for this purpose. The planned molecular analysis based on large subunit nuclear ribosomal DNA sequences is required for detailed study of the discussed pathogens.

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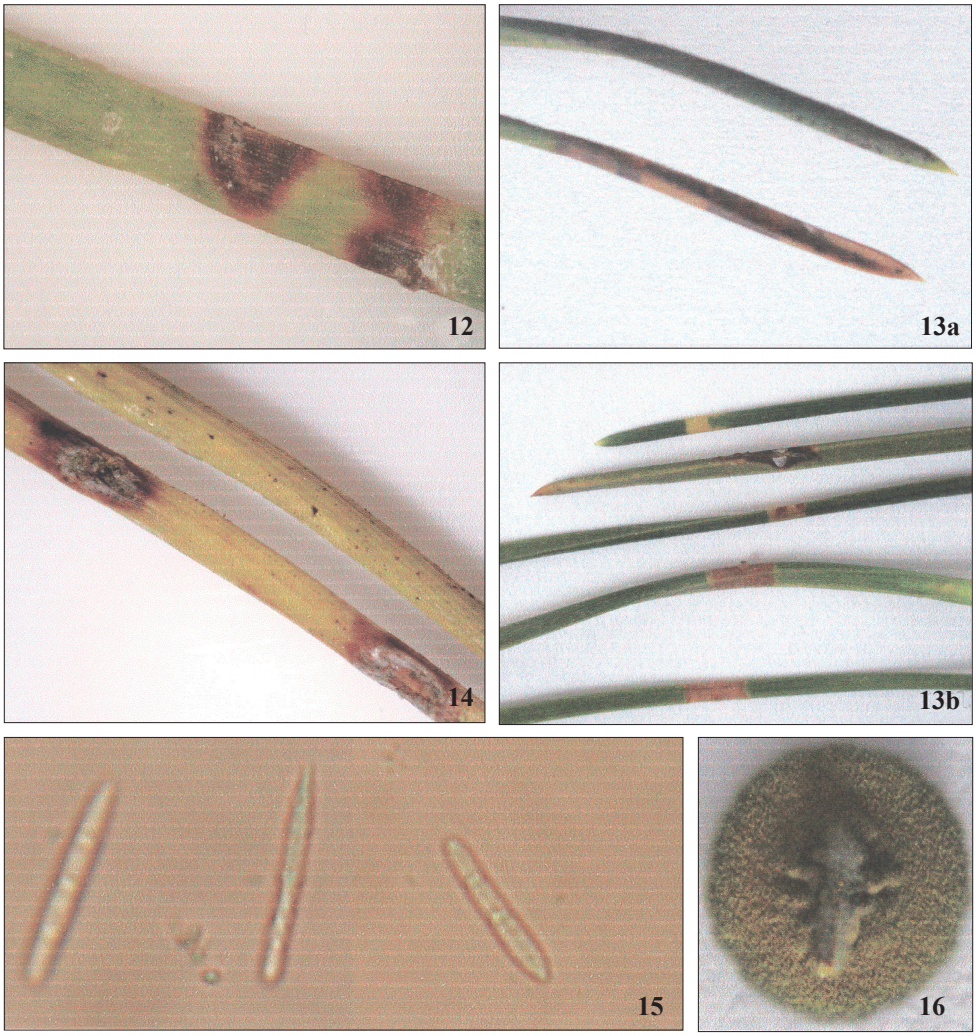

Mycosphaerella dearnessii on *Pinus nigra*:

Fig. 12. Detail of yellow to brown resin-soaked spots on needle affected with brown-spot needle blight.
Fig. 13. Dead tips of damaged needles (13a), yellow patches or browning of infected tissue (13b).
Fig. 14. Conidial masses protruding from both sides of the conidiomata under damp conditions.
Fig. 15. Light brown conidia of the fungus with verrucose structure.
Fig. 16. Greenish-ochre stromatic cultura of *M. dearnesii* on potato-dextrose agar.

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Druhová diverzita mikroskopických húb borovice čiernej rastúcej v urbanizovanom prostredí Nitry

Súhrn

Príspevok prezentuje výsledky štúdia druhovej diverzity mikroskopických patogénov, ktorí sú pôvodcami hubových chorôb *Pinus nigra* Arnold rastúcej ako súčasť sídelnej vegetácie mesta Nitra. V priebehu vegetačného obdobia 2009 boli za účelom štúdia mykoflóry borovice čiernej v rámci výskumu zdravotného stavu drevín v urbanizovanom prostredí zaznamenané charakteristické symptómy chorôb, pôvodcami ktorých sú viaceré druhy mikroskopických húb. Zo vzoriek symptomatických stromov sme izolovali a mikroskopicky identifikovali huby *Camarosporium pini* (Westend.) Sacc., *Mycosphaerella dearnessii* M.E. Barr a *Fusarium* sp. na ihliciach a *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton na ihliciach a šiškách borovice čiernej. Huba *Sphaeropsis sapinea* bola identifikovaná ako patogén s najvyššou frekvenciou. Pomerne bežný bol výskyt druhov rodu *Fusarium* a *Camarosporium*. Huba *Mycosphaerella dearnessii* bola nájdená sporadicky. Práca na základe štúdia kulturálnych a morfologických vlastností skúmaných patogénov popisuje rozlišujúce morfologické znaky húb, ich anamorfných (acervuly, pyknidy, konídie) a teleomorfných (vrecká, askospóry) štádií a rastové charakteristiky na živnom médiu (rast v kultúre, vzhľad kultúry). Výsledky poukazujú na možnosť oslabenia zdravotného stavu borovice čiernej pôsobením patogénnych druhov mikroskopických húb podieľajúcich sa rozličnou mierou na poškodení hostiteľa, prítomnosť ktorých môže predstavovať zvýšené riziko šírenia hubových chorôb hlavne v kompaktnejších výsadbách druhov rodu *Pinus*.

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