Acta fytotechn. zootechn., 18, 2015(2): 36–43

Original Paper

Fungi associated with a decline of *Pinus nigra* in urban greenery

Helena Ivanová

Institute of Forest Ecology of the Slovak Academy of Sciences, Zvolen, Slovak Republic

During the years 2013–2014, complex mycoflora of Austrian pine trees (Pinus nigra Arnold) was monitored within a survey on health state of trees growing in urban environment. Nine species of microscopic fungi were isolated and identified from samples collected from different part of Pinus nigra needles during the study period. This study reports the occurrence of the fungi *Asterosporium asterospermum*, *Beltrania rhombica*, *Camarosporium pini*, *Coleosporium* sp., *Cyclaneusma niveum*, *Lophodermium seditiosum*, *Nigrospora* sp., *Phomopsis* sp., *Trichothecium roseum* of *P. nigra*. In Austrian pine the greatest damage has been caused by the fungi *Lophodermium seditiosum* and *Cyclaneusma niveum*. Damage caused by rust fungus (*Coleosporium* sp.) and by fungi *Asterosporium asterospermum* and *Beltrania rhombica* occurs less frequently.

Keywords: Austrian pine, disease symptoms, health state, parasitic microscopic fungi

1. Introduction

In recent years, among urban trees Austrian pine also called European black pine (*Pinus nigra* Arnold) belongs to the particularly affected tree species. At the same time with regard to the expected global climate change, this change may cause the pathogens to adapt to the previously resistant species of woody plants (Kolářík et al., 2005). 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 (Sinclair et al., 1987).

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 (Sinclair et al., 1987). Among pathogenic fungi, pathogens identified from the dead pine tissues include also *Asterosporium asterospermum* (Pers.) S. Hughes, *Beltrania rhombica* Penz., *Camarosporium pini* (Westend.) Sacc., *Coleosporium* sp., *Cyclaneusma niveum* (Persoon ex Fr.) DiCosmo, Peredo & Minter, *Lophodermium seditiosum* Minter, Staley & Millar, *Nigrospora* sp., *Phomopsis* sp. and *Trichothecium roseum* (Pers.) Link.

The occurrence of causal pathogens causes withering of branches and tree thinning in the lower parts of the tree crown as a beginning of woody plants weakening by the low temperatures in winter period and by the drought from the spring to summer season (Grove, 1922; James, 1984; Manoharachary et al., 2003; Karadžić and Milijašević, 2008).

The aim of this study was to research distribution, disease symptoms, some important characteristics in pure culture and distinctive morphological features of the most important parasitic fungi in Austrian pine. 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.

2. Material and methods

From spring to autumn 2013–2014 needles of *Pinus nigra* with blight symptoms were collected at several locations from plants growing in private gardens and public greenery of the town of Nitra (Nitra-Zobor, Nitra-Chrenová, Nitra-Sihoť, Nitra-Kynek). Altogether 20 trees were studied. The age of evaluated trees was between 35–40 years. Samples were taken from some sections of trees with damaged needles from every locality. Every sample was cultivated on 30 Petri dishes (PD) with 3% PDA (potato-dextrose agar – complete name of medium, when first use) medium. 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. Classical phytopathological approaches were used to isolate and obtain pure hyphal cultures. Fungi were isolated from the needles first

***Correspodence:** Helena Ivanová, Institute of Forest Ecology of the Slovak Academy of Sciences Zvolen, Branch for Woody Plants Biology, Akademická 2, 949 01 Nitra, Slovak Republic, helena.ivanova@savzv.sk

Faculty of Agrobiology and Food Resources, 2015
http://www.fapz.uniag.sk/
DOI: 10.15414/afz.2015.18.02.36–43

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 medium, potato-dextrose agar (PDA). Petri dishes with the medium 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 Minter et al. (1978), Woodward (2001), Tanaka et al. (2010), Luo et al. (2010) or morphological studies in Botella et al. (2010). All the isolates were examined from time to time and the rate of colony development was observed, as well as the fructifications in the culture, etc. The fungi were identified based on the sporulation in the culture, the appearance of hyphae, rate of growth, etc. Visual characteristics of symptomatic needles were examined with a stereomicroscope SZ51 (Olympus) and fungal structures observations were accomplished with a microscope BX41 (Olympus) under a 400× magnification.

3. Results and discussion

Asterosporium asterospermum (Pers.) S. Hughes, syn. *Stilbospora asterosperma* Pers. is fungus associated with a severe needle blight of ponderosa and lodgepole pines (Hunt, 1985) or scots pine (Kowalski and Kehr, 1992). In our experiments the fungus was isolated from needles of Austrian pine. Conidia stellate, brown composed with three arms were 15–17–25 × 10 μm, with two septa (Figure 1a). Number and length of arms are important distinquising criterion (Prášil and Réblová, 1995). Fruting bodies 1.5 mm in diameter were subepidermal at first, splitting irregularly to expose the dark spore mass later. Conidiogenous cells cylindrical, hyaline. Mature conidia massive, dark brown, smooth in pulvinate acervuli on twigs. Conidia appear as star shape that consisting of 4 arms at 90° to each other. The length of conidia was 20–55 μm from tip to tip of the branches which were 10–25 μm thick at the base. Conidia are formed solitary at the apex of coniodiophores (Boujari et al., 2012). According to Kobayashi and Kubano (1986) acervuli 800–930 μm in diam. occurred on dead beech bark are black, scattered, at first immersed within the epidermal layer, then erumpent. Conidiophores are slender, simple, hyalin to pale brown, 121.5–80 μm long. Dark brown, terminal, holoblastic conidia are smooth and consisting of 4 arms at 90° in angle to each other, connected with conidiophore at the center cell, 30–50 μm long from the tip of one arm to that of the opposite one: arms 15–25 × 7.5 × 12.5 μm with 3–4 septa. *Asterosporium asterospermum* isolated from Fagales trees with typical flattened acervuli with a wide opening. Conidiomata were pycnidial with a wide ostiole of more than 100 μm diameter. Conidial septation was euseptate. Central cell was indistinct. Conidia stellate, brown, composed of 4 equally developed arms, 30.5–55 (–61) μm between the widest points, arms 17–28 (–30) × 8–9.5 (–11) μm (Tanaka et al., 2010).

The hyphomycetous fungus *Beltrania rhombica* Penz. which occurs on dead leaves of many trees and shrubs caused foliar damage and reduced the ornamental value of host trees (Manoharachary et al., 2003). Fungus was dominant between endophytic fungi on *Pinus thunbergii* (Rambelli et al., 2008; Kim et al., 2012). On PDA produced gray, later dark red-brown, floccose, effuse colonies. Conidiophores simple, or sometimes branched at the base, gently flexuous, clear or light brown, cylindrical, smooth, septate. Conidiogenous cells integrated, terminal, polyblastic, sympodial, denticulate, sub-clavate; separating cells oval, swollen, 9.5 × 8 μm. Conidia solitary, biconic, appendiculate-spicate, 0-septate, smooth, dark reddish-brown, with hyaline transverse band in the widest part of the conidium, 21–25 × 9–12 μm (Rambelli et al., 2008) or conidia unequally biconic, unicellular, dark brown with a pale brown band, 26–31 × 8.5–12 μm (Mulas et al., 1993). In our experiments from *Pinus nigra* needles this fungus formed on PDA medium red-brown mycelium. Conidia were light brown, biconic, 20–28 × 10–14 μm, solitary with double external wall and dark brown transverse septum in widest part of the cell (Figures 1b, c). Comparable results achieved Shi et al. (2012) by the study of popular ornamental plant *Tibouchina semidecanta* Cogn. which is damaged by the fungus *Beltrania rhombica*. The fungus formed on the leaves round spot with a brown center surrounded by a reddish brown border and on PDA medium gray, floccose colonies with light brown, cylindrical, simple or sometimes branched at the base conidiophores 105–202 × 3–5 μm in size and unequally biconic, unicellular, dark brown with a pale brown or subhyaline band just above the widest part conidia 26–31 × 8.5–12 μm in size.

Fungus *Camarosporium pini* (Westend.) Sacc., syn. *Hendersonia pini* Westend., causes severe infection that can result in significant growth reduction. The fungus 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 black spots which represent pycnidia of 180–300 μm in diameter arranged linearly and in parallel to venation were observed. 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. Through the pycnidium pore there come out numerous oval brown

Faculty of Agrobiology and Food Resources, 2015
http://www.fapz.uniag.sk/
DOI: 10.15414/afz.2015.18.02.36–43

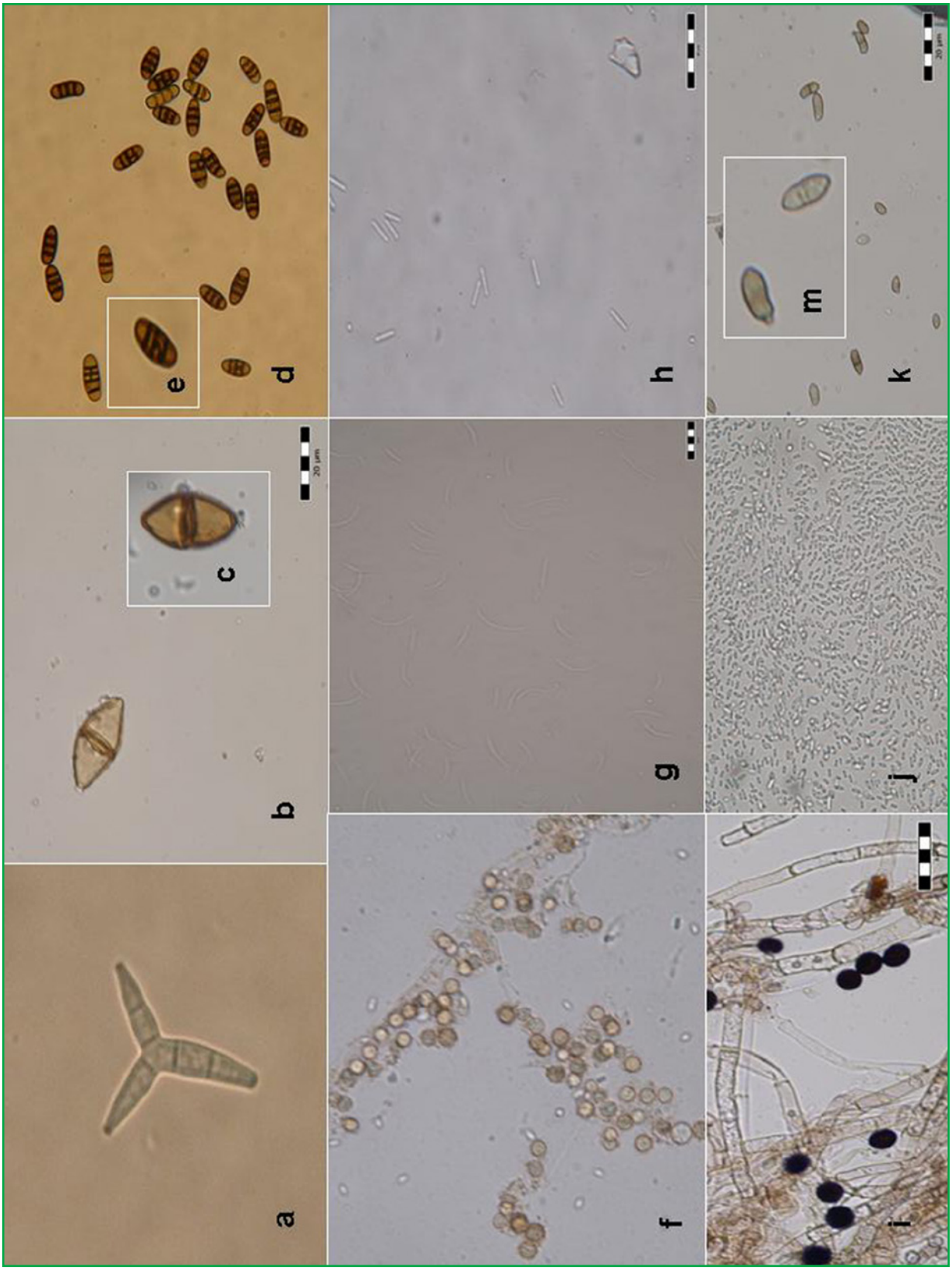

Figure 1a–m **a** – three arms of *Asterosporium asterospermum* conidium; **b** – biconic conidia of *Beltrania rhombica*; **c** – conidia of *Beltrania rhombica* – detail; **d** – thin walled conidia of *Camarosporium pini*; **e** – conidium of *Camarosporium pini* – detail; **f** – yellow oranges uredinospores of *Coleosporium* sp.; **g** – sickle-shaped conidia of *Cyclaneusma niveum*; **h** – rod-shaped, smoll conidia of *Lophodermium seditiosum*; **i** – ovoid, brown or black conidia of *Nigrospora sp.*; **j** – α conidia of *Phomopsis* sp.; **k** – bicellular, often pear-form conidia of *Trichothecium roseum*; **m** – conidium of *Trichothecium roseum* – detail; scale bars **b**, **g**, **h**, **k** = 20 µm, **i** = 50 µm

Faculty of Agrobiology and Food Resources, 2015
http://www.fapz.uniag.sk/
DOI: 10.15414/afz.2015.18.02.36–43

conidia with three transversal walls and 1–2 vertical walls of sizes ranging between 18–20 × 9–10 μm. Conidia oblong, rounded at both ends, often slightly curved, 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). According Dennis (1964) spores elongate-oval, brown, 3–6-septate (or even 8-septate) and muriform, not constricted, 15–25 × 7–9 μm, sporophores indistinct. 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. 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 were pale brown, thin-walled, with three transversal walls and 1, sometimes 2 vertical walls, smooth fusiform to fusiform-elliptical, straight, apex subobtuse, base truncate 20–22 × 6–8 μm (Figures 1d, e). Results of comparable parameters achieved James (1984) on *Pinus contorta*. Spores were fusoid-elliptical, 4-celled, reddish brown and slightly constricted 15.5–22.0 × 5.5–6.8 μm in size. Pycnidia were immersed in host mesophyll tissue. Symptoms of damage on the lover crown side were observed.

Over 20 species of *Coleosporium* cause rust diseases on pine. The first sign is small yellow spots in spring or early summer. *Coleosporium asterum* (Dietel) Syd. & P. Syd. is the best known of the group and infects 2- and 3-needle pines including Austrian, red, and Scote (Kunca and Leontovyč, 2013). The rust *Coleosporium tussilaginis* cause pine needle rust disease on *Pinus sylvestris* and *Pinus nigra* (Jones, 2006; Jurc, 2007). These needle rusts complete their life cycles in about one year and may begin on pine in later summer or early Autumn when it is infected by basidiospores produced on the alternate host. The fungus grows into and overwinters in the needles. Yellow spots develop on them the following spring. Fruiting bodies – spermagonia develop beneath these spots initially. Tongue-like fruiting bodies – aecia grow from these spots and when they burst, they release bright orange spores that will reinfect the alternate host (Pirone, 1978). In late summer develop telia on the margins of the uredinia (Nicholls et al., 1968; Nicholls and Anderson, 1978). Urediniospores in mass were first yellow orange, later white and in our experiments were subgloboid 18 μm, hyaline, with thick walls Close-up of urediniospores showing echinulate outer surface (Figure 1f). According to Gjærum et al. (2008) individual urediniospores were subgloboid or ellipsoid, 18–28 × 16–22 μm, walls 1 μm thick, hyaline, and densely verrucose. Basidiospores which develop from the telia, will infect pine needles. A few species of *Coleosporium* will survive for more than one year as mycelium in the living tissue of the pine host (Karadžić and Milijašević, 2008).

Cyclaneusma niveum (Persoon ex Fr.) DiCosmo, Peredo & Minter, syn. *Naemacyclus niveus* (Pers.ex Fr.) Fuck. is secondary pathogen which occurs on the old needles, and never on one year old needles of many trees. According to some authors fungus parasite causes chlorosis and cast on the needles (Butin, 1973; Glavaš, 1981; Giordano and Gonthier, 2011) and the second opinion is that this fungus is only saprophyte. This fungus is very common in some regions of the world. First symptoms of this fungus are not typical. Symptoms for diagnosis of this pathogen can be found on two years old and older needles and sure sign for determination are apothecia which color is whitish and cream-like (Minter and Dudka, 1996). Ascomata apothecial, scattered, subepidermal, elliptical when fully open, reddish brown when young, becoming concolorous with the needle surface, 0.3–1.0 × 0.15–0.4 mm. Asci are subcylindrical, 110–130 × 12–15 μm. Ascospores are filiform, 0–2-septate, 75–120 × 3–4 μm, hyaline (Gadgil et al., 2005). *Cyclaneusma niveum* has larger ascomata, asci and ascospores than *Cyclaneusma minus*, though only in ascomatal length are the two species well differentiated (Minter and Dudka, 1996). In our experiment with old needles of *Pinus nigra* we achieved ascospores 80–100 × 2.5–3 μm with 2 septa and hyaline, thick-walled conidia 16–20 × 1 μm, 0-septate (Figure 1g). Conidia are also longer in *Cyclaneusma niveum* (9–21 μm) than in *Cyclaneusma minus* (5–11.5 μm); there is some tendency for the two species to occupy different pine species; *Cyclaneusma niveum* tends to have a bulkier appearance than *Cyclaneusma minus* (Minter and Dudka, 1996). According to Gadgil et al. (2005) conidiomata were pycnidial, scattered, immersed, globose to subglobose, 0.1–0.2 mm in diameter. Conidia were sickle-shaped, hyaline, 0-septate, 9–22 × 1–1.5 μm.

Lophodermium needle cast disease is caused by the fungus *Lophodermium seditiosum* Minter, Staley & Millar which infects needles of Austrian (*Pinus nigra*), mugo (*P. mugo*), red pine (*P. resinosa*) and Scotch pine (*P. sylvestris*) and can kill young trees within a year (Karadžič and Milijaševič, 2008; Rajkovic et al., 2013). This fungus overwinters as vegetative mycelium in needles infected the previous season. Fungal fruiting pustules develop on fallen needles over the summer. Windborne spores during wet periods (from August to October) are released, which infect the current year's needles, resulting in a small

Faculty of Agrobiology and Food Resources, 2015
http://www.fapz.uniag.sk/
DOI: 10.15414/afz.2015.18.02.36–43

brown spot, often with a yellow halo. The spots on needle later coalesced to give blighter appearance and resulted in premature defoliation. Reproductive structures of the fungus can often be seen on dead needles. On native pines needlecast is most evident in late spring to early summer when needles shen. Examination of blighter needles showed the presence of small pycnidia which erupt through the epidermis of infected needles. Conidia were 8.34 × 1.60 μm in size (Ahanger et al., 2011). In our experiments this fungus on 2% PDA fast growing and formed small pycnidia with rod-shaped, small conidia about 7–8 × 1 μm in size (Figrue 1h). According to Minter et al. (1978), cultures of *Lophodermium seditiosum* on 2% malt agar initially fast-growing. Growth invariably terminating before the edge of the Petri dish is reached. The fungus formed pycnidial, subepidermal, often coalescing, 300–500 μm long conidiomata, and bacillar, 6–8 μm long conidia. Similar results achieved Rajkovic et al. (2013) by experiments with fungus *Lophodermium seditiosum* on some pine trees includes Austrian pine. Conidia 7×0.7 μm were smaller, rod-shaped, cylindrical, ends rounded, straight or slightly curved to a side, hyaline and aseptate.

Members of *Nigrospora* (Ascomycota, Sordariomycetes) are widespread and mainly distributed in tropical and subtropical countries but also in temperate regions. They can be isolated from air, soil, foodstuffs or dead plant debris. It is rapidly growing fungus that produces at first a white to gray or black cottony colony of mycelium with a black reverse, within ten days. The three common species are separated by conidial size: *N. oryzae* (10–) 12–14 (–16) μm; *N. sphaerica* (14–) 16–18 (–20) μm; *N. sacchari* (16.5–) 20–22 (–24) μm. Species of anamorphic fungi separated on the basis of spore diameter can vary from 10 to 30 μm (Webster, 1952; Wilson et al., 1986). This evidence supports Kavosi et al. (2013) with dark grey colonies of *Nigrospora gossypii* Jack on PDA mediu. On simple, transparent conidiophores with length 10–12.5 μm were formed black, unicellular and semi spherical and partially elliptical conidia with even surface and flat section that their size was 11–15 μm. In our experiments with *Pinus nigra* needles we obtained conidia solitary, unicellular, aseptate, ovoid or ellipsoidal, at the beginning hyaline late brown or black with conidial size 8– (10) 12–14 (–16) μm. Hyphae are 5–7 μm in diameter, septate, thick-walled (Figure 1i). According to Ellis (1971) hyphae are 3.4–7.5 μm in diameter, septate, thick-walled, dematiaceous. Conidiophores 3.5–6.2 μm thick, thick-walled, hyaline to brown, very short, little differentiated from hyphae. Conidiogenous cells 7.2–8.4 μm in diameter, thick-walled, hyaline, flask-shaped. Blastoconidia 11–13.7 μm in diameter, subglobose to slightly elliptical, not round, thick-walled, smooth, from hyaline to brown when first develops to black when mature. Conidia solitary, aseptate, unicellular, black, smooth walled, ovoid or broadly ellipsoidal, horizontally flattened often with an equatorial colorless line or germ slit.

Fungus *Phomopsis* causing cankers, needle loss, branch death and often tree death of mature trees. *Phomopsis* shoot blight causes young needles and shoots to turn brown and curl downward. Symptoms of *Phomopsis* leaf spot are small irregular, or round shaped, pale green-to-yellow spots with dark centers. Dark irregular shaped lesions develop on infected shoots (Nita et al., 2003; Karadžič and Milijaševič, 2008). Strees from environmental conditions have played a role in the development of *Phomopsis* decline. Moisture is an important factor in disease spread and severity. Small black fruiting bodies – pycnidia with two types of conidia: alpha and beta are formed on this dead tissue (Woodward, 2001). α-conidia are hyaline, nonseptate oblongelliptic in shape with a size of 6.3–11.2 × 1.7–2.8 μm, and β-conidia are hyaline, nonseptate, filiform and curved in shape with a size of 20–25 × 1 μm (Nita et al., 2003). According to some literature data size of spores occurs from 5 to 18 × 2–4 μm, α-conidia often 7–10 × 2.5 μm. In our experiment with *Pinus nigra* needles we obtained formation only α-conidia, which are fusiform, 10 × 2–2.5 μm. On both tapering edges we occurr one oil drop (Figure 1j). Conidia are produced in these structures and when moisture is present masses of conidia will ooze out to form tendrils or horns. The conidia are spread by rain and various other means such as mechanical disruption and insects. The fungus enters the plant through wounds as well as unbroken tissue. The browning of the needles and twigs begins at the tips and progresses downward toward the stem.

Trichothecium roseum (Pers.) Link syn. *Cephalothecium roseum* Corda, *Sphaeria rosea* Pers., *Trichoderma roseum* Pers. is a filamentous mitosporic fungus word-widely distributed, which reduced germination. The fungus is mostly saprophytic or weakly parasitic (Barnett and Hunter, 1972). It was found as laboratory contaminant, was previously recorded on felled trunks and fallen branches of *Acer*, *Corylus*, *Fagus*, *Prunus*, *Quercus* and *Ulmus* (Ellis and Ellis, 1985), or of many conifers (Mason and Arsdel, 1978; Karadžič and Milijaševič, 2008). Our survey has resulted in finding that *T. roseum* is the rare pathogenic fungus isolated from inspected leaf tissues in connection with attacked cherry laurel trees (Bernadovičová and Ivanová, 2011). Colonies of *Trichothecium* on PDA mediu grow rapidly, are flat, granular and powdery. From the front, the colour is white initially and becomes pale pink to peach-coloured, reverse is pale. The conidiophores are more or less rough-walled, long, erect and unbranched. Conidia in our experiments are bicellular, smooth, elliptical or pear form with one septum, each with

Faculty of Agrobiology and Food Resources, 2015
http://www.fapz.uniag.sk/
DOI: 10.15414/afz.2015.18.02.36–43

Table 1 Comparison of biometric characteristics of asexual spores all fungi determinated on genus *Pinus* sp. reported by other authors with examined material from Slovakia

Asterosporium asterospermum in µm	*Beltrania rhombica* in µm	*Camarosporium pini* in µm	*Coleosporium* sp. in µm	*Cyclaneusma niveum* in µm	*Lophodermium seditiosum* in µm	*Nigrospora* sp. in m	*Phomopsis* sp. in µm	*Trichothecium roseum* in µm
Our experiments 15–17–25 × 10	Our experiments 20–28 × 10–14	Our experiments 20–22 × 6–8	Our experiments 18 × 18	Our experiments 16–20 × 1	Our experiments 7–8 × 1	Our experiments 8–(10)12–14(–16)	Our experiments α: 10 × 2–2.5 β: –	Our experiments 16–20 × 5–7
Boujari et al., 2012 20–55 × 10–25	Rambelli et al., 2008 21–25 × 9–12	Grove, 1922 15–18 × 7–8	Gjærum et al., 2008 18–28 × 16–22	Gadgil et al., 2005 9–22 × 1–1.5	Ahanger et al., 2011 8.34 × 1.60	Webster, 1952 10–30	Nita et al., 2003 α: 6.3–11.2 × 1.7–2.8 β: 20–25 × 1 µm	Shamsi and Sultana, 2008 12–18 × 8–10 or 13.5–27 × 8–11
Kobayashi and Kubano, 1986 25 × 7.5 × 12.5	Mulas et al., 1993 26–31 × 8.5–12	Dennis, 1964 15–25 × 7–9	Karadžić and Milijašević, 2008 25 × 20	Minter and Dudka, 1996 9–21	Minter et al., 1978 6–8	Wilson et al., 1986 10–30	–	Wright et al., 2007 12–23 × 8–10
Tanaka et al., 2010 17–28(–30) × 8–9.5(11)	Shi et al., 2012 26–31 × 8.5–12	James, 1984 15.5–22.0 × 5.5–6.8	–	–	Rajkovic et al., 2013 7 × 0.7	Ellis, 1971 11–13.7	–	–

Faculty of Agrobiology and Food Resources, 2015
http://www.fapz.uniag.sk/
DOI: 10.15414/afz.2015.18.02.36–43

a flattened protuberance at the base, hyaline or bright, slightly thick-walled, formed an elongated clusters at apex of conidiophore, upper cell usually langer than basal cell, 16–20 × 5–7 μm in size (Figures 1k, m).

Biometric characteristics of asexual spores all obtained fungi are in Table 1. Comparison of biometric characteristics and morphological features of other fungi also isolated and determinated before on genus *Pinus* sp. are reported in Ivanová and Bernadovičová (2010), Pastirčáková et al. (2014), Ivanová and Ondrušková (in press).

4. Conclusions

Complementarity of *Pinus nigra* Arnold mycoflora growing in urban environment includes the nine species of microscopic fungi (*Asterosporium asterospermum*, *Beltrania rhombica*, *Camarosporium pini*, *Coleosporium* sp., *Cyclaneusma niveum*, *Lophodermium seditiosum*, *Nigrospora* sp., *Phomopsis* sp., *Trichothecium roseum*) which were isolated and identified from samples collected from different part of *Austrian pine* needles. The fungi *Lophodermium seditiosum* and *Cyclaneusma niveum* has been caused the greatest damage. Fungi *Coleosporium* sp., *Asterosporium asterospermum* and *Beltrania rhombica* occurs less frequently.

5. Acnowledgements

This study was carried out with support from the scientific projects VEGA of the Slovak Academy of Sciences No 2/0069/14 and 2/0071/14.

6. References

AHANGER, F.A., DAR, G.H., BEIG, M.A. AND SOFI, T.A. (2011) First report of needle blight on blue pine (*Pinus wallichiana*) and *Aleppo pine* (*P. halepensis*), caused by *Lophodermium maccii*, from Asia. In *Plant Pathology Journal*, vol. 10, pp. 181–186. DOI: http://dx.doi.org/10.3923/ppj.2011.181.186

BARNETT, H.L. and HUNTER, B.B. (1972) *Illustrated genera of Imperfect Fungi*. 3rd edition. Burgess Publishing Company, Minneapolis, Minnesota. 241 pp.

BERNADOVIČOVÁ, S. and IVANOVÁ, H. (2011) Fungi of the Hyphomycetes and Coelomycetes isolated from affected leaves and twigs of cherry laurel. In *Folia oecol.*, vol. 38, no. 2, pp. 137–145.

BOTELLA, L., SANTAMARÍA, O. and DIEZ, J.J. (2010) Fungi associated with the decline of *Pinus halepensis* in Spain. In *Fungal Diversity*, vol. 40, pp. 1–11.
DOI: http://dx.doi.org/10.1007/s13225-010-0025-5

BOUJARI, M., ABBASI, F. and ADEL, F. (2012) Introducing of *Asterosporium asterospermum* (Coelomycetes) as a new member for Iranian mycobiota. In *Appl. Ent. Phytopath.,* vol. 80, pp. 95–96.

BUTIN, H. (1973) Morphologische und taxonomische Untersuchungen an *Naemacyclus niveus* (Pers. ex Fr.) Fuck. ex Sacc. und verwandten Arten. In *Eur. J. Fort. Path.*, vol. 3, pp. 146–163.

DENNIS, R.W.G. (1964) The fungi of the Isle of Rhum. In *Kew Bull.*, vol. 19, pp. 77–127.

ELLIS, M. B. (1971) *Dematiaceous Hyphomycetes*. Commonw. Mycol. Inst., Kew.

ELLIS, M.B. and ELLIS, J.P. (1985) *Microfungi on land plants:* An Identification Handbook. Croom Helm, London and Sydney, 818 pp. ISBN 07099-0950-0.
DOI: http://dx.doi.org/10.1016/s0007-1528(85)80077-8

GADGIL, P.D., DICK, M.A., HOOD, T.A. and PENNYCOOK, S.R. (2005) Fungi on trees and shrubs in New Zealand. [Online] Available from: http://virtualmycota.landcareresearch.co.nz/webforms/vM_Species_Detail.aspx? [Accessed: 2013-11-25].

GIORDANO, L. and GONTHIER, P. (2011) An outbreak of *Cyclaneusma minus* needle cast on swiss mountain pine (*Pinus uncinata*) in Italy. In *J. Plant Pathol.*, vol. 93, no. 4, pp. 63–89.

GLAVAŠ, M. (1981) Prilog poznavanju proširenosti gljive *Naemacyclus niveus* Fuck, ex Sacc. na borovim iglicama u SR Hrvatskoj. In *Zaštita bilja*, vol. 32, no. 2, pp. 155–159.

GJÆRUM, H.B., SEMB, L. and TALGØ, V. (2008) *Coleosporium telekiae*, a new member of the Norwegian rust flora.In *Plant Health Progress*.
DOI: http://dx.doi.org/10.1094/PHP-2008-1006-01-BR

GROVE, W.B. (1922) New or noteworthy fungi: VIII. In *Journal of Botany, British and foreign*, vol. 60, pp. 81–86.

HUNT, R.S. (1985) *Leptomelanconium pinicola* comb. nov. and associated needle blight of pines. In *Can. J. Bot.*, vol. 63, no. 6, pp. 1157–1159. DOI: http://dx.doi.org/10.1139/b85-159

IVANOVÁ, H. and BERNADOVIČOVÁ, S. (2010) Species diversity of microscopic fungi on Austrian pines growing in urban greenery of Nitra town. In *Folia oecol.*, vol. 3, no. 2, pp. 168–181.

IVANOVÁ, H. and ONDRUŠKOVÁ, E. Comparison of two *Coniochaeta* species (*C. ligniaria* and *C. malacotricha*) with a new pathogen of black pine needles – *Sordaria macrospora* (in press).

JAMES, R.L. (1984) *Hendersonia* blight of lodgepole pine in Idaho. In *Nursey Disease Notes*, vol. 9, pp. 2–8.

JONES, D.J. (2006) Pest Risk Analysis for *Coleosporium asterum* on cut flowers imported from contries outside the EU. [Online] Available from: https://secure.fera.defra.gov.uk/phiw/riskRegister/plant-health/documents/coleosp.pdf. [Accessed: 2015-01-20].

JURC, D. (2007) Pines – *Pinus* spp.: Diseases of needles *Lophodermium seditiosum*, *Mycosphaerella pini*, *Mycosphaerella dearnessii*, *Cyclaneusma minus*. In *Gozdarski vestnik*, vol. 65, no. 7–8, pp. 393–408.

KARADŽIČ, D. and MILIJAŠEVIČ, T. (2008) The most important parasitic and saprophytic fungi on austrian pine and scots pine plantations in Serbia. In *Bulletin of the Faculty of Forestry*, vol. 97, pp. 147–170.

KAVOSI, M.R., FARIDI, F. and HAJIZADEH, G. (2013) Fungal species isolated from *Quercus castaneifolia* in Hyrcanian Forests, North of Iran. In *Biodiversitas*, vol.14, no. 2, pp. 61–66.
DOI: http://dx.doi.org/10.13057/biodiv/d140202

KIM, CH.K., EO, J.K. and EOM, A.H. (2012) Molecular Identification of Endophytic Fungi Isolated from Needle Leaves of *Pinus thunbergii*. In *Korean journal of mycology*, vol. 40, no. 4, pp. 183–186. DOI: http://dx.doi.org/10.4489/KJM.2012.40.4.183

KOBAYASHI, T. and KUBANO, T. (1986) Materials for the fungus flora of Japan. In *Trans. Mycol. Soc. Japan*, vol. 27, pp. 29–32.

KOLAŘÍK, J., HORÁČEK, P., BURIAN, S., REŠ, B. and PEJCHAR, M. (2005) *Care for woody plants out of forest II*. Vlašim: ČSOP, 720 p. (in Czech).

Faculty of Agrobiology and Food Resources, 2015
http://www.fapz.uniag.sk/
DOI: 10.15414/afz.2015.18.02.36–43

KOWALSKI, T. and KEHR, R.D. (1992) Endophytic fungal colonization of branch bases in several forest tree species. In *Sydowia*, vol. 44, no. 2, pp. 137–168.

KUNCA, A. and LEONTOVYČ, R. (2013) Laboratory experiments with growth potential of *Cenangium ferruginosum* tested on natural nutrition soils. In *Lesnícky časopis : Forestry Journal*, vol. 59, no. 1, pp. 44–49.

LUO, J.T., LIN, Y.R., SHI, G.K. and HOU, Ch.L. (2010) *Lophodermium* on needles of conifers from Yunnan Province, China. In *Mycol Progress*, vol. 9, pp. 235–244.
DOI: http://dx.doi.org/10.3923/ppj.2011.181.186

MANOHARACHARY, C., AGARWAL, D.K. and KRISHNA, R.N. (2003) Beltraniomyces a new genus of Dematiaceous Hyphomycetes from India. In *Indian Phytopathol.*, vol. 56, no. 4, pp. 418–421.

MASON, G.N. and van ARSDEL, E.P. (1978) Fungi associated with *Pinus* taeda seed development. In *Plant Dis. Rep.*, vol. 62, pp. 864–867.

MINTER, D.W., STALEY, J.M. and MILLAR, C.S. (1978) Four species of *Lophodermium* on *Pinus sylvestris*. In *Trans. Br. Mycol. Soc.*, vol. 71, no. 2, pp. 295–301.
DOI: http://dx.doi.org/10.1016/S0007-1536(78)80110-7

MINTER, D.W. and DUDKA, I.O. (1996) *Fungi of Ukraine. A Preliminary Checklist*. pp. 361. Egham, UK, International Mycological Institute & Kiev, Ukraine, M.G. Kholodny Institute of Botany.

MULAS, B., PASQUALETTI, M. and RAMBELLI, A. (1993) Influence of the substratum on the morphology of *Endophragmiella boewei* and *Beltrania rhombica*. In *Fl. Medit.*, vol. 3, pp. 233–238.

NICHOLLS, T.H. and ANDERSON, R.L. (1978) *How to identify and control pine needle rust disease*. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 8 pp.

NICHOLLS, T.H., PATTON, R.F. and VAN ARSDEL, E.P. (1968) Life cycle and seasonal development of *Coleosporium* pine needle rust in Wisconsin. In *Phytopathology*, vol. 58, no. 6, pp. 822–829.

NITA, M., MADDEN, L.V., WILSON, L.L. and ELLIS, M.A. (2003) Evaluation of a disease prediction system for *Phomopsis* cane and leaf spot of grape. In *Phytopathology*, vol. 93, 65 pp.

PASTIRČÁKOVÁ, K., IVANOVÁ, H. and PASTIRČÁK, M. (2014) Species diversity of fungi on *Pinus* in urban and extra-urban vegetation. In *Dendrological days in Arborétum Mlyňany 2014, 18.9.2014. Vieska nad Žitavou: Arborétum Mlyňany SAV*. pp. 150–157 (in Slovak).

PIRONE, P.P. (1978) *Diseases and Pests of Ornamental Plants*. USA, 5th. Ed. 575 pp.

PRÁŠIL, K. and RÉBLOVÁ, M. (1995) *Asterosporium asterospermum* (Coelomycetes) in artificial culture. In *Novit. Bot. Univ. Carol.*, vol. 9, pp. 29–33.

RAJKOVIC, S., MARKOVIC, M. and RAKONJAC, L. (2013) Incubation Methods for Forecasting the Occurrence and Development of *Lophodermium seditiosum* Minter, Staley & Millar on Pine. In *Fungicides – Showcases of Integrated Plant Disease Management from Around the World*. pp. 153–182. DOI:http://dx.doi.org/10.5772/51233

RAMBELLI, A., VENTURELLA, G. and CICCARONE, C. (2008) Dematiaceous Hyphomycetes from *Pantelleria mediterranean* maquis litter. In *Flora Mediterranea*, vol. 18, pp. 441-467.

SHAMSI, S. and SULTANA, R. (2008) *Trichothecium roseum* – a new record of hyphomycetous fungus for Bangladesh. In *Bangladesh J. Plant Taxon*, vol. 15, pp. 77–80.
DOI: http://dx.doi.org/10.3329/bjpt.v15i1.932

SHI, Z.R., XIANG, M.M., ZHANG, Y.X. and HUANG, J.H. (2012) First report of leaf spot on *Tibouchina semidescandra* caused by *Beltrania rhombica* in China. *In Plant Disease,* vol. 96, no. 9, pp. 1380–1382.
DOI: http://dx.doi.org/10.1094/PDIS-04-12-0363-PDN

SINCLAIR, W.A., LYON, M.H. and JOHNSON, W.T. (1987) *Diseases of trees and shrubs*. Ithaca, NY: Cornell Univ. Press. 575 pp.

TANAKA, K., MELNIK, V.A., KAMIYAMA, M., HIRAYAMA, K. and SHIROUZU, T. (2010) Molecular phylogeny of two coelomycetous genera with star-shaped conidia, *Prosthemium* and *Asterosporium* on *Fagales* trees. In *Botany*, vol. 88, pp. 1057–1071. DOI: http://dx.doi.org/10.1139/B10-078

WEBSTER, J. (1952) Spore projection in the hyphomycete *Nigrospora sphaerica*. In *The New Phytologist*, vol. 5, no. 2, pp. 229–235.
DOI: http://dx.doi.org/10.1111/j.1469-8137.1952.tb06129.x

WILSON, M.E., DAVIS, N.D. and DIENER, U.L. (1986) A toxic metabolite of *Nigrospora oryzae* (Berk and Br.) Petch. In *Mycopathologia*, vol. 95, no. 3, pp. 133–138.
DOI: http://dx.doi.org/10.1007/BF00437116

WOODWARD, J.W. (2001) Simplified fungi identification key. In *Special Bull.*, vol. 37. [Online] Available from: http://plantpath.caes.uga.edu/extension/documents/fungikey.pdf. [Accessed: 2015-01-20].

WRIGHT, E.R., PIZZINGRILLI, P. and CALIGARIS, M.V. (2007) Rose dieback caused by *Trichothecium roseum* in Argentina. In *Plant Disease,* vol. 9, pp. 631–634.
DOI: http://dx.doi.org/10.1094/PDIS-91-5-0631C

Faculty of Agrobiology and Food Resources, 2015
http://www.fapz.uniag.sk/
DOI: 10.15414/afz.2015.18.02.36–43