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

Slávka Bernadovičová[1], Helena Ivanová[2]

[1]Gabčíkova 8, 8941 05 Bratislava 4, Slovak Republic,
E-mail: slavka.bernadovicova@savzv.sk,
[2]Branch for Woody Plants Biology, Institute of Forest Ecology of the Slovak Academy of Sciences,
Akademická 2, 949 01 Nitra, Slovak Republic,
E-mail: helena.ivanova@savzv.sk

Abstract

Bernadovičová, S., Ivanová, H. 2011. Hyphomycetes and Coelomycetes fungi isolated from affected leaves and twigs of cherry laurel trees. *Folia oecol.*, 38: 137–145.

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

Key words

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

Introduction

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

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

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

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

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

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

Material and methods

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

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

The colonies of fungi were identified using various keys for identification: Andersen et al. (2002), Arx (1970, 1981), Burgess et al. (1988), Ellis and Ellis (1987), Hanlin (1973), Mordue (1971), Samson et al. (2002), Simmons and Roberts (1993) and Sutton (1980), based on micro- and macroscopic symptoms of isolates. The samples of material have been deposed at the Institute of Forest Ecology of the Slovak Academy of Sciences, Branch for Woody Plants Biology in Nitra.

Results and discussion

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

Class Hyphomycetes

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

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

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

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

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

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

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

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

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

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

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

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

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

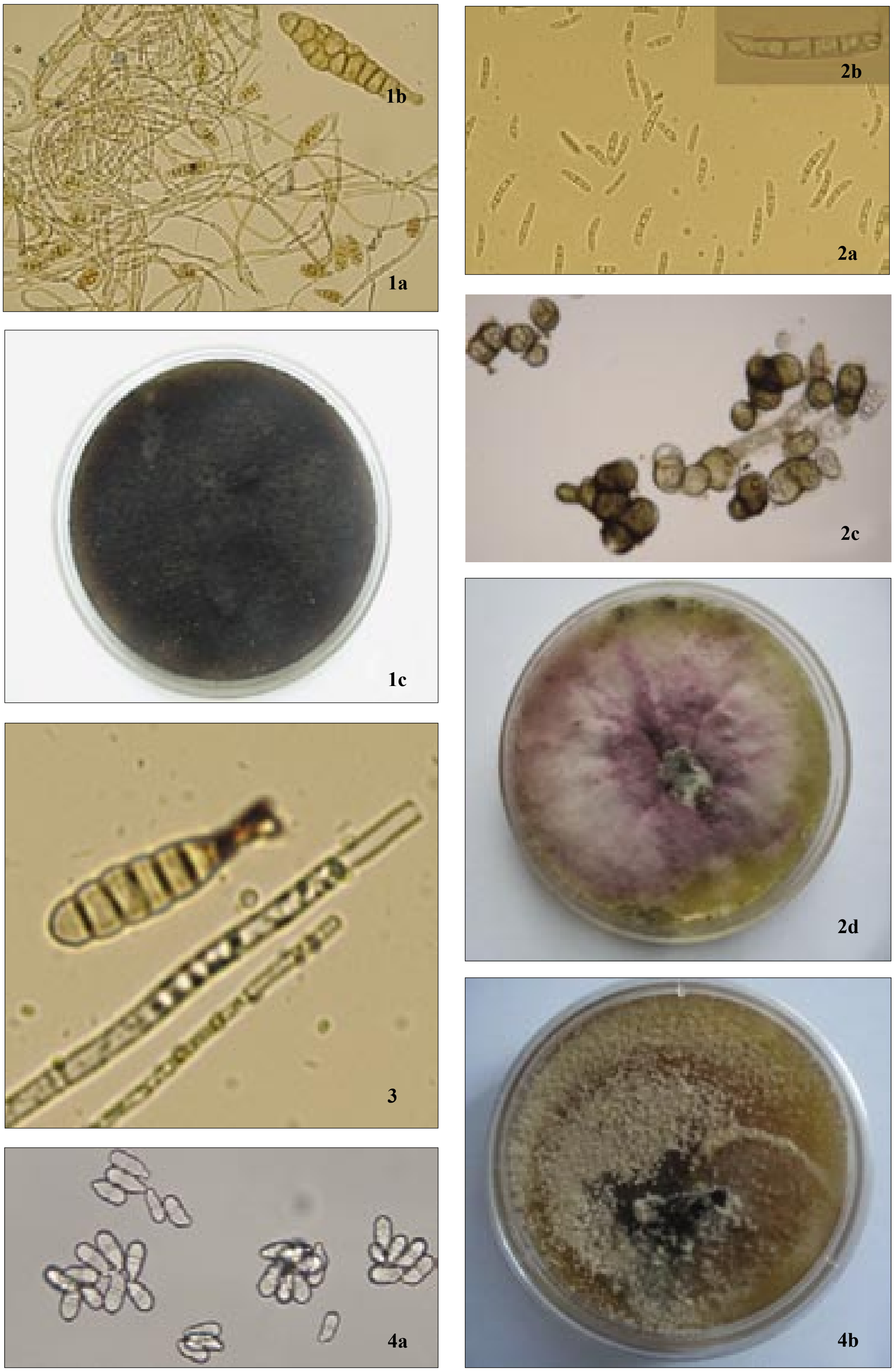
Colonies of *Trichothecium* grow rapidly. At 25 °C and on potato-dextrose agar, colonies are flat, granular and powdery. From the front, the colour is white initially and becomes pale pink to peach-coloured, reverse is pale. The conidiophores are indistinguishable from the septate hyaline vegetative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

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

Class Coelomycetes

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

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



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

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

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

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

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

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

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

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

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

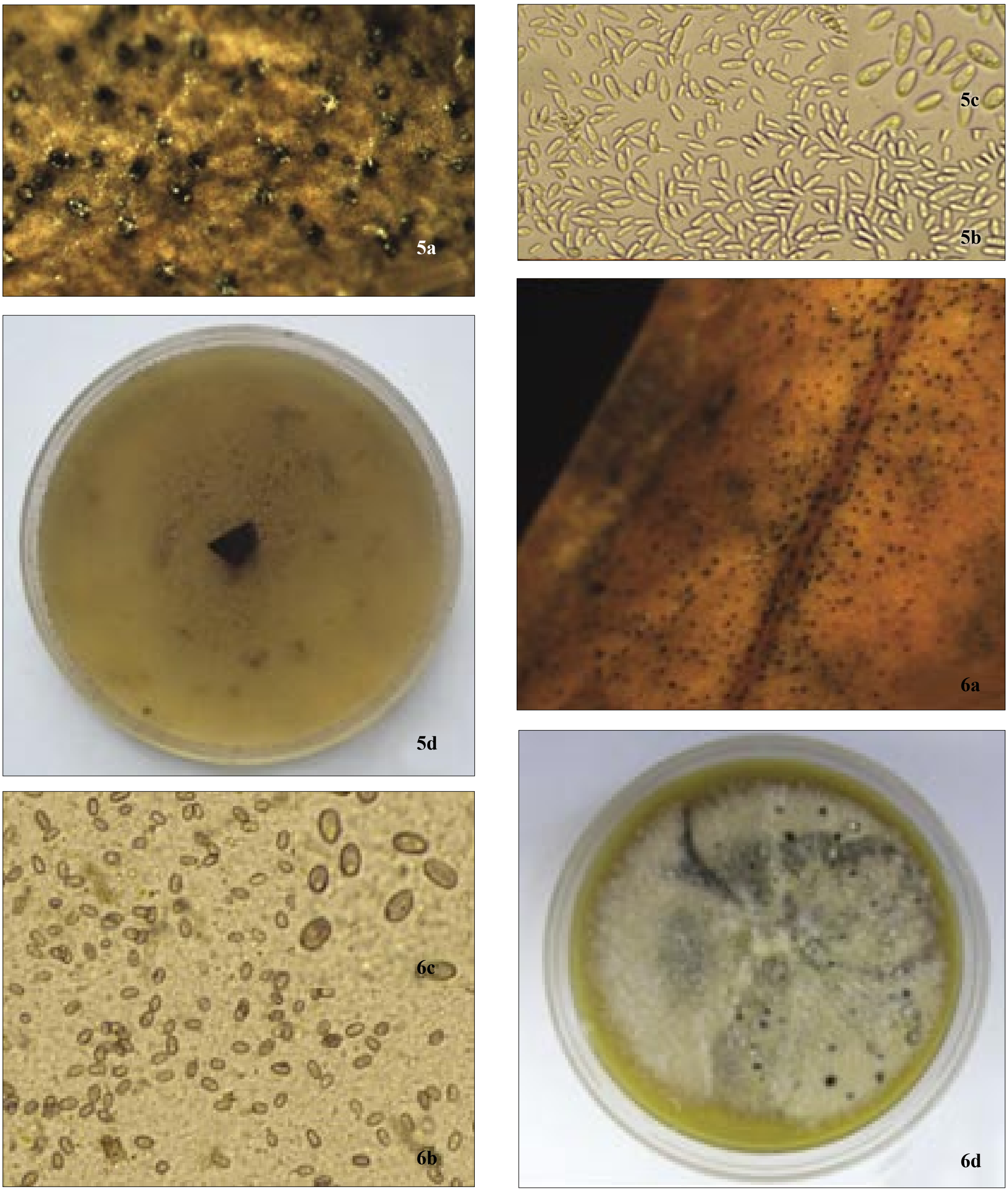
Acknowledgement

This study was carried out with financial support from the scientific project grant agency VEGA of Slovak Academy of Sciences No. 2/0149/10 and with grant of the Science and Technology Assistance Agency No. APVV-0421-07.

References

Abbas, S.Q., Niaz, M., Ghaffar, A. 2007. Thielaviopsis basicola: A potential threat to agriculture and forestry in Pakistan. *Pakist. J. Bot.*, 39: 985–990.

Andersen, B., Krøger, E., Roberts, R.G. 2002. Chemical and morphological segregation of Alternaria arborescens, A. infectoria and A. tenuissima species-groups. *Mycol. Res.*, 106: 170–182.



Figs 5–6. Coelomycetous fungi isolated from affected leaves and twigs of *Prunus laurocerasus*:

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

Arsenijević, M. 2003. Neke karakteristike sojeva Phomopsis spp. izolovanih sa raznih voćaka, vinove loze i ukrasnih biljaka u Srbiji [Some characteristics of the Phomopsis spp. strains isolated from various fruit-trees, grapevine and ornamental plants in Serbia]. *Zašt. Bilja*, 54: 5–18.

Arx, J.A. von. 1970. *A revision of the fungi classified as Gloeosporium*. Lehre: J. Cramer. 203 p.

Arx, J.A. von. 1981. *The genera of fungi sporulating in pure culture*. Vaduz: J. Cramer. 424 p.

Avanzato, M.V., Rothrock, C.S. 2010. Use of selective media and baiting to detect and quantify the soilborne plant pathogen Thielaviopsis basicola on pansy. *The plant health instructor.* DOI: 10.1094/PHI-I-2010-0610-01.

Barnett, H.L., Hunter, B.B. 1972. *Illustrated genera of imperfect fungi*. Minneapolis: Burgess Publishing Company. 241 p.

Burgess, L.W., Liddel, C.M., Summerell, B.A. 1988. *Laboratory manual for Fusarium research*. Sydney: Univ. of Sydney. 156 p.

Burgess, L.W., Knight, T.E., Tesoriero, L., Phan, H.T. 2008. *Diagnostic manual for plant diseases in Vietnam*. ACIAR Monograph No. 129. Canberra: ACIAR. 210 p.

Cano, J., Guarro, J., Gené, J. 2004. Molecular and morphological identification of Colletotrichum species of clinical interest. *J. Clin. Microbiol.*, 42: 2450–2454.

Dickman, M.B., Alvarez, A.M. 1983. Latent infection of papaya caused by Colletotrichum gloeosporioides. *Pl. Dis*., 67: 748–750.

Ellis, M.B. 1976. *More dematiaceous Hyphomycetes*. Kew: CMI. 507 p.

Ellis, M.B., Ellis, J.P. 1985. *Microfungi on land plants*: *An identification handbook*. London, Sydney: Croom Helm. 868 p.

Ellis, M.B., Ellis, J.P. 1987. *Microfungi on land plants: An identification handbook*. London, Sydney: Croom Helm. 818 p.

Ellis, M.B., Ellis, J.P. 1997. *Microfungi on land plants*: *An identification handbook*. Slough: The Richmond Publishing Co. 868 p.

Farr, D.F., Bills, G.F., Chamuris, G.P., Rossman, A.Y. 1989. *Fungi on plants and plant products in the Unites States*. St. Paul, Minnesota: APS Press. 1252 p.

Gangadevi, V., Muthumary, J. 2008. Isolation of Colletotrichum gloeosporioides, a novel endophytic taxol-producing fungus from the leaves of a medicinal plant, Justicia gendarussa. *Mycol. balc.,* 5: 1–4.

Garić, R., Arsenijević, M. 1990. Phomopsis – uzročnik nove bolesti kajsije [Phomopsis – the causal agent of a new disease of apricots]. *Zast. Bilja*, 193: 269–274.

Guarro, J., Gene, J. 1992. Fusarium infections. Criteria for the identification of the responsible species. *Mycoses*, 35: 109–114.

Hanlin, R.T. 1973. The distribution of peanut fungi in the southeastern United States. *Mycopath. et Mycol. appl.*, 49: 227–241.

Jones, R.K. 2000. Assessments of Fusarium head blight of wheat and barley in response to fungicide treatment. *Pl. Dis*., 84: 1021–1030.

Kim, W.G., Lee, B.D., Cho, W.D, Shin, D.B. 2001. Anthracnose of perilla caused by Colletotrichum spp. and Glomerella cingulata. *Pl. Pathol. J.*, 17: 236–241.

Kokubun, T., Harborne, J.B., Eagles, J. 1994. 2′,6′-Dihydroxy-4′-methoxyacetophenone, a phytoalexin from the roots of Sanguisorba minor. *Phytochemistry*, 35: 331–333.

Leslie J.F., Summerell, B.A. 2006. *The Fusarium laboratory manual*. Ames, Iowa: Blackwell Professional Publishing. 385 p.

Maiti, C.K., Sen, S., Paul, A.K., Acharya, K. 2007. First report of leaf blight disease of Gloriosa superba L. caused by Alternaria alternata (Fr.) Keissler in India. *J. Gen. Pl. Path.*, 73: 377–378.

Martínez, E.P., Hío, J.C., Osorio, J.A., Torres, M.F. 2009. Identification of Colletotrichum species causing anthracnose on Tahiti lime, tree tomato and mango. *Agron. colomb.*, 27: 211–218.

Mordue, J.E.M. 1971. *Glomerella cingulata*. *CMI descriptions of pathogenic fungi and bacteria.* No. 315. Kew: CMI. 2 p.

Nelson, P.E., Horst, R.K., Woltz, S.S. 1981. Fusarium diseases of ornamental plants. In Nelson, P.E., Toussoun, T.A., Cook, R.J. (eds). *Fusarium*: *Diseases, biology and taxonomy.* Pennsylvania: Pennsylvania State University Press, p. 121–128.

Nelson, P.E., Dignani, M.C., Anaissie, E.J. 1994. Taxonomy, biology, and clinical aspects of Fusarium species. *Clin. Microbiol. Rev.,* 7: 479–504.

Peres, N.A., Timmer, L.W. 2006. Evaluation of the Alter-Rater model for spray timing for control of Alternaria brown spot on Murcott tangor in Brazil. *Crop Protec*., 25: 454–460.

Pring, R.J, Nash, C., Zakaria, M., Bailey, J.A. 1995. Infection process and host range of Colletotrichum capsici. *Physiol. Mol. Pl. Path.*, 46: 137–152.

Prusky, D., Freeman, S., Dickman, B. 2000. *Colletotrichum. Host specificity, pathology, and host-pathogen interaction.* St. Paul, Minnesota: APS Press.

Punja, Z.K., Sun, L.J. 1999. Morphological and molecular characterization of Chalara elegans (Thielaviopsis basicola), cause of black root rot on diverse plant species. *Can. J. Bot.*, 77: 1801–1812.

Rensburg, J.C.J., Lamprecht, S.C., Groenewald, J.Z., Castlebury, L.A., Rous, P.W. 2006. Characterisation of Phomopsis spp. associated with die-back of rooibos (Aspalathus linearis) in South Africa. *Stud. Mycol*., 55: 65–74.

Roberts, P.D., Pernezny, K.L., Kucharek, T.A. 2009. *Anthracnose caused by Colletotrichum sp. on pepper*. University of Florida*,* Institute of Food and Agricultural Sciences. [cit. 2010-09-09]. http://edis.ifas.ufl.edu/pp104

Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O. 2002. *Introduction to food- and airborne fungi.* Utrecht: Centraalbureau voor Schimmelcultures. 389 p.

SHAMSI, S., SULTANA R. 2008. Trichothecium roseum – A new record of hyphomycetous fungus for Bangladesh. *Bangladesh J. Pl. Taxon*, 15: 77–80.
SHEW, H.D., MEYER, J.R. 1992. Thielaviopsis. In SINGLETON, L.L., MIHAIL, J.D., RUSH, C.M. (eds). *Methods for research on soilborne phytopathogenic fungi*. St. Paul, MN: The American Phytopathological Society, p. 171–174.
SILVA, C.M., MELO, I.S. 1999. Requisitos nutricionais para o fungo Alternaria alternata [Nutritional requirements for the fungus Alternaria alternata]. *Pesq. agropec. bras*., 34: 499–503.
SIMMONS, E.G., ROBERTS, R.G. 1993. Alternaria themes and variations (73). *Mycotaxon*, 40: 109–140.
SINCLAIR, W.A., LYON, H.H. 2005. *Diseases of trees and shrubs.* Ithaca, London: Cornell University Press. 660 p.
SUTTON, B.C. 1980. *The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata.* Kew: Commonwealth Mycological Institute. 696 p.
SUTTON, B.C. 1992. The genus Glomerella and its anamorph Colletotrichum. In BAILEY, J.A., JEGER, M.J. (eds). *Colletotrichum: biology, pathology and control*. Wallingford: CAB International, p. 1–26.
TIMMER, L.W., PEEVER, T.L., SOLEL, Z., AKIMITSU, K. 2003. Alternaria diseases of citrus – novel Pathosystems. *Phytopath. mediterr*., 42: 99–112.
TRUJILLO, E.E., OBRERO, F.P. 1969. Anthracnose of papaya leaves caused by Colletotrichum gloeosporioides. *Pl. Dis. Rep*., 53: 323–325.
UECKER, F.A. 1988. A world list of Phomopsis names with notes on nomenclature, morphology, and biology. *Mycol. Mem*. 13: 1–323.
WONG, M.Y. 2003. *Fusarium oxysporum f. sp. lycopersici (Sacc.) W.C. Snyder and H.N. Hans*. PP728 Soilborne Plant Pathogen Class Project. [cit. 2010-09-10].
http://www.cals.ncsu.edu/course/pp728/Fusarium/Fusarium_oxysporum.htm
WRIGHT, E.R., PIZZINGRILLI, P., CALIGARIS, M.V. 2007. Rose dieback caused by Trichothecium roseum in Argentina. *Pl. Dis.*, 91: 631.

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

Súhrn

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

Received December 13, 2010
Accepted March 28, 2011