

Birch necrotic leaf spots caused by fungal pathogens

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Abstract

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Microscopic fungi associated with lesions on the leaves of *Betula pendula* Roth. species growing in different greenery types of Nitra town are causal factors weakening the health state and vitality of these trees. Many of them caused anthracnose and formed small, irregular, circular brown spots with dark brown margins or broad necrotic blotches. Disease symptoms begin as a large spots or blotches, which rapidly widen and join, resulting in large areas of dead tissue. The fungi overwinter on infected fallen leaves. Sporulation begins when spring conditions become warm and wet. During the study of the mycoflora of birch leaves ten fungal species were recorded: *Asteroma microspermum*, *Cryptocline betularum*, *Discula betulae*, *Marssonina betulae*, *Melanconium betulinum*, *Phoma* sp., *Phomopsis* sp., *Phyllosticta betulina*, *Pyrenopeziza betulicola*, *Stemphylium* sp. In this work were described disease symptoms and some distinctive morphological features.

Keywords

Betula pendula Roth., fungal pathogens, greenery types, necrosis of leaves, Nitra town

Introduction

The common birch (*Betula pendula* Roth) belongs among most frequent tree species in urban greenery. Birches grow in the squares, parks and streets of the cities. Urban birches lose resistance to several species of pathogenic fungi which are associated with lesions on leaves. Many fungal species which overwinter on leaf litter have been described as the causal agents of necrotic leaf spots of the genus *Betula* that can lead to premature defoliation. The development of symptoms depends on the weather conditions and may take a week before the symptoms of diseases become clearly visible.

The aim of this study was to identify the microscopic fungi associated with the leaves of *Betula pendula* species in different greenery types of Nitra town and to describe the distinctive morphological features for these ten fungi as causal factors involved in the decline of health state and vitality weakening of *B. pendula* species.

Material and methods

The samples of birch leaves exhibiting necrotic lesions were collected from 25 trees at 5 sites (SP, HE, NRP, PP, SPG) in Nitra town during the growing season in 2013. Altogether 125 birch trees grown in these five different greenery types were evaluated. The age of evaluated trees was between 10–40 years. The number of fungal taxa found in different categories of urban vegetation in individual localities of town is in Table 1. Pathogenic fungi have been isolated *in vitro* from infected ulcerous places of birch. Cultures of the fungi were obtained from tissues of leaf spots, and grown on potato-dextrose agar (PDA). The holotypes of the species were deposited in the Herbarium of the Branch for Woody Plants Biology in Nitra of the Institute of Forest Ecology of the Slovak Academy of Sciences in Zvolen.

Classical phytopathological approaches were used to isolate and obtain pure cultures. The leaf parts cut from the diseased plants were surface-sterilized by immersion in sodium hypochlorite solution (1% available

Table 1. The numbers of fungal taxa found in Nitra town

Location Categories of planting Taxon	Nitra/Chrenová SP, HE, NRP, PP, SPG	Nitra/Zobor SP, NRP	Nitra/Centrum SP, PP	Nitra/Čermán SP, NRP	Nitra/Kalvária SP, NRP, SPG	Nitra/Klokočina SP, HE, SPG, NRP
<i>Discula betulae</i>		+	+	+		
<i>Marssonina betulae</i>	+					+
<i>Asteroma microspermum</i>	+	+	+	+		
<i>Pyrenopeziza betulicola</i>				+		+
<i>Phoma sp.</i>	+	+	+	+	+	+
<i>Phomopsis sp.</i>	+	+	+	+	+	+
<i>Stemphylium sp.</i>		+	+	+	+	+
<i>Cryptocline betularum</i>	+	+		+	+	+
<i>Melanconium betulinum</i>	+		+			
<i>Phyllosticta betulina</i>		+			+	+

SP, plantings along streets; HE, housing estate; NRP, main roadside plantings; PP, park plantings; SPG, special purpose greenery.

chlorine) for 20 minutes, rinsed twice or three times in sterile distilled water and then dried carefully with filter paper. The plant samples were subsequently cut to 3–5 mm large fragments which were placed on potato-dextrose agar (PDA) in plastic Petri dishes. This was followed by cultivation at 24 ± 1 °C and 45% humidity in dark conditions in a versatile environmental test chamber MLR-351H (Sanyo) and subsequent isolation on the culture medium. Pure fungal cultures were obtained by using multiple purifications. The obtained isolates were transferred on PDA medium to induce sporulation. Study of fungal structures was performed with a clinical microscope BX41 (Olympus) in water-based mounting medium under 400 \times and 1,000 \times magnification. Software Quick Photo Micro Version 2.3 was used for measurements of the size of microscopic objects. Fungal species were identified and the morphometric values were ascertained by routine mycological methods using morphological keys (PEI et al., 2010) and data of morphological studies (ELLIS and ELLIS, 1997; PAAVOLAINEN et al., 2000; WATANABE, 2002; ERIKSSON, 2006a, 2006b; FARR et al., 2007 and GOMES et al., 2013).

Results and discussion

As a result of the extremely wet spring this year, we found a lot of common diseases, as well as uncommon diseases. Occurrence of these fungi is common in years with wet rainy spring weather. This disease appears as a spotting on the leaf tissue and may cause some defoliation. Under optimum disease conditions run together to form larger blotches. The first lesions which produce conidia usually appear in the beginning of July at the earliest.

During the study of the mycoflora of birch leaves fungal species: *Asteroma microspermum*, *Cryptocline betularum*, *Discula betulae*, *Marssonina betulae*, *Melanconium betulinum*, *Phoma sp.*, *Phomopsis sp.*,

Phyllosticta betulina, *Pyrenopeziza betulicola* and *Stemphylium sp.* were recorded as a causal factors involved in the decline of health state and vitality weakening of *B. pendula* species.

Phoma Sacc. represents a complicated asexual genus of fungi generally considered to be a taxonomically difficult group of mitosporic fungi with a wide geographical range, more than 2,000 described infrageneric taxa (MONTEL et al., 1991) and a limited range of useful morphological characters for distinguishing species. Species are found in numerous ecological niches, include as opportunistic or primary plant pathogens, saprobic soil or water organisms, or parasites of other fungi, lichens, insects and vertebrates (CROUS et al., 2009; AVESKAMP et al., 2010) occurring on leaves. Pycnidia were scattered, erumpent, globose, black, smooth with a subrimose ostiole. In our experiments the conidia were $7-9 \times 2.5-3$ μm (Fig. 1a, b). Chlamydo spores were absent. This data, which are obtained by cultivation on PDA medium are in scope of variability on this species. According to literature data the dimensions of conidiogenous cells were $4-6 \times 3.5-5$ μm , globose to bottle shape. Conidia are ellipsoidal, occasionally curved, with dimensions of $3.5-4.5 \times 1.5-1.75$ μm , on average 3.9×1.48 μm , with 2 or 3 guttules, often fusoid, biguttulate, 7–10 μm long or hyaline, ellipsoidal, one-celled, with size $6(7)-8(9) \times 3(2.5)$ μm , (on avg. 7.5×2.58 μm). According to HEČKOVÁ et al. (2013) ellipsoid, hyaline and single-celled conidia occurring on fresh leaves of *Betula pendula* were $11-6 \times 6-3$ μm , (mean 9×4 μm) large.

Phomopsis sp., asexual states of *Diaporthe* Nitschke, includes important taxa occurring as plant pathogens, endophytes and saprobes on a wide range of hosts (ROSSMAN and PALM-HERNÁNDEZ 2008; UDAYANGA et al., 2011, 2012). This fungus is widely distributed and some species can incite cankers, rots, wilts, and die-backs in some economically important plants, includ-

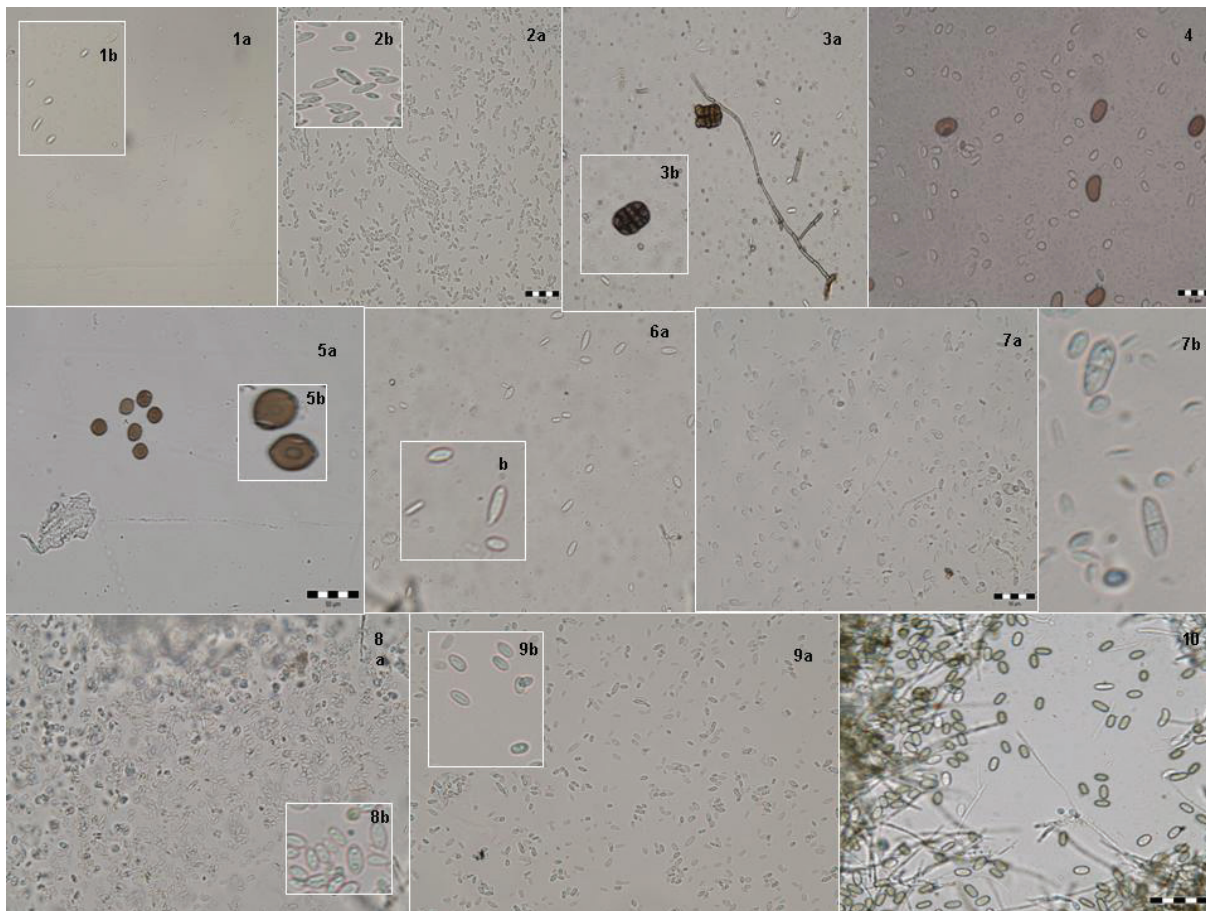


Fig. 1–10. Fungal pathogens caused birch necrotic leaf spots: 1a, b, *Phoma* sp.; 2a, b, *Phomopsis* sp.; 3a, b, *Stemphylium* sp.; 4, *Melanconium betulinum*; 5a, b, *Cryptocline betularum*; 6a, b, *Discula betulae*; 7a, b, *Marssonina betulae*; 8a, b, *Asteroma microspermum*; 9a, b, *Pyrenopeziza betulicola*; 10, *Phyllosticta betulina*.

ing birch, causing severe damage, dieback, leaf spots, blights, decay, wilt and significant losses (SANTOS et al., 2011; THOMPSON et al., 2011; GOMES et al., 2013).

The major morphological characters of *Phomopsis* are conidiomata ostiolate, conidiophores phialidic, conidia dimorphic, with usually fusiform and biguttulate alpha conidia and usually filiform, hamate, and non-guttulate beta conidia (REHNER and UECKER 1994; UDAYANGA et al., 2011). According to GAO et al. (2013) alpha conidia are unicellular, hyaline, aseptate, oval or fusiform, usually with one guttule at each end, rounded at both ends, present more frequently than beta conidia. This fact was confirmed also in our experiments: colonies were woolly to cottony, white, later light brown. Conidiomata were pycnidial, stromatic, dark brown to black, single or aggregated in culture. Alpha conidia were unicellular, hyaline, straight, biguttulate and rounded at both ends, with dimensions of 4–8 (10) × 2–3 (4) μm, beta conidia were not seen (Figs. 2a, b). This data are in scope of variability on this species. Dimensions of spores of *Phomopsis* sp. on *Betula* reported from examined material are 11 (12)–15 × 5 (6) 8 μm (on avg. 13 × 5.87 μm). According to ARNOLD (1967)

on *Betula papyrifera* and *Betula alleghaniensis* (*Betula lenta*) were dimensions of conidiomata 200–250 × 100–170 μm, conidiophore 3–26 × (1–) 1.5–2 (–2.5) μm, alpha conidia (4–) 5–8 × (1–) 1.5–2 (–2.5) μm and beta conidia 14–21 (–25) × 0.7 (–1) μm. Author expressed the view that the fungus could be a potentially serious pathogen for seedlings subjected to low light intensity and high humidity in their natural habitat.

Most species of *Stemphylium* are plant pathogens, and are less commonly isolated from soil and decaying plant material. *Stemphylium* sp. in our experiments was isolated from necrotic leaf spots on *Betula pendula*. Colonies on PDA were pale brown, cottony. Mycelium was superficial, hyphae branched, septate, pale brown, smooth, 4.5–5 μm wide. The spores of this fungus are fairly large, 25 (30)–35 × 15 (18)–20 μm, (on avg. 29.8 × 18.6 μm), dark, and have internal cross walls running crosswise and lengthwise, dividing the spore into a number of segments and are typically constricted at the central septum (Figs. 3a, b). Conidia developing singly at the apex of each conidiophore are oblong, ovoid or broadly ellipsoidal, conical at the apex and rounded or subtruncate at the base, with 2–3 transverse septa and 1

(–3) longitudinal or oblique septa. Data are in scope of variability on this species. According to SZABÓ (2001) colonies of *Stemphylium* are fast growing, suede-like to cottony in texture, and gray, brown, or brownish-black in colour. It grows well on general cellulose surfaces but spores may take longer than normal to develop or may be completely absent. SIMMONS (1967) delineated this genus, which shares several characters with *Alternaria* and *Ulocladium*, including muriform, usually pigmented conidia. *Stemphylium* is separated from *Alternaria* and *Ulocladium* by the principal morphological characteristic of currently proliferating conidiophores. Taxonomy of *Stemphylium* species was based primarily on conidial morphology, including variations in conidial shape, size, length/width ratio, colour, septation and ornamentation (SIMMONS, 2004). According to LEACH and ARAGAKI (1970) and HOSEN et al. (2009) morphological characteristics in *Stemphylium* sp. may vary depending on environmental conditions such as temperature and substrate. PEI et al. (2010) isolated from diseased leaves of *Lycium chinense* the *Stemphylium lycii* and this species was described by morphological and molecular phylogenetic analyses.

Melanconium betulinum (Kunze et Schm) is considered a weak parasite. This fungus invades weakened or dead tissue and causes what is referred to as *Melanconis* dieback. *Melanconium bicolor* is known as a frequent colonizer of damaged or declining birch shoots (PEACE, 1962; BENNELL and MILLAR, 1984) and attacks the tree when it has been weakened by drought conditions, winter kill or phenoxy-acetic acid herbicide exposure causing progressive dieback. First the foliage becomes thin, with chlorotic or curled leaves at the shoot tips. Twigs then become bare because of lack of sufficient vigour to re-foliate. Then the branches and parts of the crown die, below which a bunching of the foliage develops that tends to be confined to the lower part of crown. Death usually takes place within three to five years after the onset of symptoms. *Melanconium* produces black acervuli and ovoid to ellipsoid, 1-celled, dark brown conidiospores that average 13–18 (19) × 5–7 (9) µm. In our experiments conidia measured 10 (13)–16 × 6–9 µm were one-celled, ovoid, dark brown (Fig. 4). All data are in scope of variability on this species.

Anthraco-nose of birch leaves exhibits brown spots with dark brown to black margins and is caused by fungus ***Cryptocline betularum*** (Ellis & G. Martin) Arx. JOHNSON et al. (2001) evaluated eight cultivars of birch (*Betula* spp.) for resistance to that birch leaf-spot disease. *Betula pendula* Roth. together with *B. nigra* L. and *B. nigra* L. cv. Heritage were most susceptible to defoliation caused by birch leaf spot disease. Infected leaves fall from tree even if still partly green. Leaves on lower branches are most affected. Leaf spots may be 3–10 mm wide brown spots to large brown blotches surrounded by yellow tissue. This fungus produces

acervuli which are intra- or subepidermal (VON ARX, 1970). The conidia are aseptate and measure 10 (13)–15 (17) × 7 (8)–9 µm (on avg. 13.5 × 8 µm). In our experiments the conidia measured 12 (13)–16 (17) × 10 (11) µm and were aseptate, dark brown, oblong and tapered on one end (Figs. 5a, b). This data, which are obtained by cultivation on PDA medium are in scope of variability on this species.

Discula betulae (Westend.), (syn. *Discula betulina* (J. Kickx f.) Arx., syn. *Gloeosporium betulinum* (J. Kickx f.) is a common foliar pathogen on birch in whole Europe causing characteristic leaf spots that can lead to premature defoliation (ADAMSKA, 2005; GREEN and CASTLEBURY, 2007; GREEN and MACASKILL, 2007). Dieback of shoots is caused only when it is combined with other stress factors initiated by unsuitable climatic or site conditions. This fungus, which is generally regarded as a leaf disease, forms brown lesions with dark margins on both sides of the leaves. Leaf spots may be 3–4 mm wide brown rings with a light centre surrounded by yellow tissue. Fungus is producing fast-growing colonies on PDA agar. The hyaline conidia are ovoid, aseptate, large 4–10 × 2.5–3 µm (mean 7 × 2.5 µm). Spores in our experiments were large 4 (5)–8 × 2.5 (3)–4 µm (Figs. 6a, b). This data are in scope of variability on this species. According to GREEN and CASTLEBURY (2007) diseased shoots and leaves of birch after 4–5 weeks incubation on 2% malt agar producing fast-growing colonies of white aerial mycelium and aseptate hyaline conidia large 4–8 × 1.5–2.5 µm (mean 6 × 2 µm). HEČKOVÁ et al. (2013) reported, that dimensions of *D. betulae* spores isolated from fresh silver birch leaves were 5–9 × 3–2 µm (mean 7 × 2 µm) and according to SZABÓ (2001) the conidia were large 6–16 × 2.5–4 µm.

Marssonina betulae (Lib.) Sacc. (syn. *Gloeosporium betulae* (Lib.) Mont) is a primary pathogen on shoots and stems of birch, causing cankers and dieback. This fungus belong to foliar pathogen on birch, where causes characteristic leaf spots (PEACE, 1962; BENNELL and MILLAR, 1984). Together with other factors, such as unsuitable provenance of birch, incorrect site selection, poor silvicultural management and climatic damage, may also cause birch dieback or predispose trees to disease. *M. betulae* caused dieback of shoots without requiring prior wounding, which indicated that this fungus is a more aggressive pathogen than suggested in the current literature (GREEN, 2004; GREEN and MACASKILL, 2007). The sporulation of this fungus made leaf lesions on birch. Primary infections caused by fungus *Marssonina* spp. tend to occur in spring shortly after the leaves emerge on the host, and are initiated by conidia from acervuli overwintering in lesions on shoots and fallen leaves. *Marssonina* was able to form acervuli on inoculation lesions, with the potential to release conidia

which are probably spread via rain splash (SINCLAIR and LYON, 2005). Conidia, which occurred in our experiment were two celled, hyaline. Dimensions of conidia were $19 (22) \times 5 (7) \mu\text{m}$ (Figs. 7a, b) and are in scope of variability on this species. According to SZABÓ (2001) conidia were large $17\text{--}22 \times 8\text{--}10 \mu\text{m}$ and in experiments of GREEN (2004), GREEN and MACASKILL (2007) conidia measured $10\text{--}12 (14)\text{--}18 (20) \times 5 (6)\text{--}7 (8) \mu\text{m}$, (on avg. $14.1 \times 7 \mu\text{m}$).

Asteroma microspermum (Peck) Sutton (syn. *Gloeosporium betulicola* Sacc. & Dearn.) occurred on affected leaves and formed roundish, irregular spots, several mm in size. Spore bearing structures in the spots are formed in summer and in early autumn. In our experiments spores, which were observed on the surface of agar medium (PDA) were hyaline, 1-celled with thick cell wall and measured $10 (15)\text{--}20 \times 5\text{--}7 \mu\text{m}$ (Figs. 8a, b). *Asteroma* conidia with dimensions $10 (14)\text{--}18 (24) \times 4 (5)\text{--}7 \mu\text{m}$ (on avg. $16.7 \times 5.28 \mu\text{m}$) are produced on the same kind of lesions as of *Gloeosporium* (*Discula* sp.) conidia, but they are smaller than *Gloeosporium* conidia (KURKELA, 1995) and have not been induced to germinate on agar. It is possible that *Asteroma* is the microconidial form of *Gloeosporium* (LILJA et al., 1997). Its presence on *Betula pendula* host was confirmed by CHLEBICKI and MULENKO (1992) and MULENKO (1996).

Pyrenopeziza betulicola (Fuckel), (anamorpha: *Cylindrosporium concentricum* Grev.) has recently been identified to be the major causative agent of leaf spot disease on *Betula pendula* and *Betula pubescens* (PAPPINUM et al., 2002). Fungus *Pyrenopeziza betulicola* forms the small circular brown spots without definite borders in the leaves. The fungus is the causative agent of the symptoms occurring in form of spots on the birch leaves (PAAVOLAINEN et al., 2000). This pathogen causes premature yellowing and falling of leaves, but not complete defoliation has been observed so far. The economic loss resulting from this disease involves premature leaf-fall, reduction of photosynthesis and may cause problems in frost hardening. Conidia $10\text{--}15 \times 3 \mu\text{m}$ long (Figs. 9a, b) are in scope of variability on this species.

Phyllosticta betulina Sacc. fungus spreads easily and causes unsightly blemishes on the leaves. The leaves may eventually die and drop. The first signs of a *Phyllosticta* infection appear as elongated tiny purplish or black spots along the veins of the leaves. These lesions gradually grow in size and eventually take on the characteristic eyespot appearance. It is most common in warmer climates, but can be found throughout the world on a number of different types of trees. ADAMSKA (2005) confirms the occurrence of 9 species of parasitic fungi of plants of the genera *Betula* in Poland. Between them *Phyllosticta betulina* is rare. This fact was confirmed by CHLEBICKI and MULENKO (1992) and MULENKO (1996) on *Betula pendula* host. Two types of spores of this fun-

gus germinate when moisture is present: ascospores and conidia. The ascospores are discharged into the air and can travel between plants on a breeze or current. If they land on a moist leaf, ascospores germinate. Conidia can quickly be carried from diseased plants to healthy ones by splashing rainwater, sprinklers or watering. Conidia which formed in great amount on PDA medium are in scope of variability on this species. In our experiments they were elliptical, 1-celled, pale brown, with thick wall, $13\text{--}14 (16) \times 6 (7) 8 \mu\text{m}$ long (Fig. 10).

Conclusions

Parasitic mycoflora of *Betula pendula* growing in different greenery types of Nitra town had considerable diversity. The microscopic parasitical fungi caused premature drying of assimilative organs, branches and also individual trees and these causal factors were weakening the health state and vitality of these trees. The destruction effects of these fungi resulted in various large spots or broad necrotic blotches forming large areas of dead tissue. We have confirmed necessity of the causal agent diagnosis of these trees.

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