

DIVERSITY OF MICROFUNGI ON BRANCHES OF *CASTANEA SATIVA* IN SLOVAKIA

KATARÍNA ADAMČIKOVÁ, GABRIELA JUHÁSOVÁ,
MAREK KOBZA & EMÍLIA ONDRUŠKOVÁ

Abstract. Localities in *Castanea sativa* Mill. plantations were visited in a study aimed at identifying the mycoflora of *C. sativa* in Slovakia [excluding *Cryphonectria parasitica* (Murr.) Barr for which much data is available]. Samples from chestnut tree branches and stems were examined visually and microscopically. Seven species of microfungi were recorded, three with their anamorphs. *Coryneum modonium* (Sacc.) Griffon & Maubl. and *Phomopsis castaneae* Woron. were the most common. *Libertella quercina* Tul. & C. Tul. was identified in both states (anamorph and teleomorph) at two new localities. The records of *Glioniopsis praelonga* (Schwein.) Underw. & Earle and *Dothidothia celtidis* (Ellis & Everh.) M. E. Barr are the first for Slovakia. These species were rare, found only in one locality. Two microscopic fungi were detected: *Valsa ambiens* (Pers.) Fr. [*Cytospora ambiens* (Nitschke) Sacc.] and *Diplodina castaneae* Prill. & Delacr.

Key words: European chestnut, microscopic fungi, diversity, *Glioniopsis praelonga*, *Dothidothia celtidis*

Katarína Adamčíková, Gabriela Juhásová, Marek Kobza & Emília Ondrušková, Institute of Forest Ecology, Slovak Academy of Sciences, Zvolen, Branch for Woody Plant Biology, Nitra, Akademická 2, 949 01 Nitra, Slovak Republic; e-mail: katarina.adamcikova@sav.savzv.sk

INTRODUCTION

European chestnut forms extensive forests, natural and naturalized, from the Caucasus through Turkey to the Balkans and westward to Italy, France, Spain and Portugal. It grows wherever rainfall and soil conditions allow. European chestnut (*Castanea sativa* Mill.) is an introduced woody plant in Slovakia. Its distribution is scattered and it does not form continuous forests. It is located in areas with warmer climate on downs in south and southeast Slovakia at 200–500 m a.s.l. (Bolvanský *et al.* 2008). *Castanea sativa* has been threatened by many parasitic microfungi for several decades, some causing whole chestnut plantations to decline (Juhásová 1999). One of them is *Cryphonectria parasitica*, which has been causing chestnut blight for more than 70 years throughout Europe (Robin & Heiniger 2001). There is a large amount of data on its distribution in Slovakia (Adamčíková *et al.* 2006, 2010; Juhásová & Bernadovičová 2001) but less about the occurrence of other microfungi on branches or stems of European chestnut.

In this study we surveyed the incidence of microfungi on *Castanea sativa* branches in different regions of Slovakia, excluding *C. parasitica* for the reason given above. Some are reported as new species for the Slovak mycobiota.

MATERIAL AND METHODS

Sites with *Castanea sativa* plantations in Slovakia were visited during our research on fungi inhabiting bark, periderm and wood in 2006–2011 (Table 1). Samples taken from chestnut tree branches were examined visually and microscopically in the laboratory.

We used damp chambers at room temperature to release spores. After dampening the samples with distilled water and incubating them for one day they were observed under a binocular microscope, and fruit bodies were removed for microscopic examination. The morphological structures of microfungi were evaluated and measured on mounts in distilled water. The fungi were identified by the appearance of the fruit bodies and spores under LM.

Spores were scanned with an Olympus Artcam camera and measured to 0.1 µm accuracy using Quick

Table 1. Characteristics of the studied *Castanea sativa* Mill. sites. A – estimated age of trees, N – number of *C. sativa* trees. g – private garden, f – forest, o – orchard, p – public park.

Locality	Coordinates	A	N	Type of stand
Arborétum Mlyňany, Vieska nad Žitavou	48°19'30"N, 18°22'15"E	30	250	o
Bratislava Jeséniova	48°10'09"N, 17°06'15"E	60–120	61	p
Bratislava Rača	48°12'35"N, 17°09'24"E	50–60	1	g
Suchá nad Parnou	48°24'33"N, 17°29'25"E	5–10	100	o
Sološnica	48°27'54"N, 17°13'52"E	90	50	f
Horné Otrokovce	48°29'35"N, 17°52'59"E	25	1	g
Modrý Kameň	48°14'30"N, 19°20'20"E	30–35	216	o
Svätý Jur	48°15'02"N, 17°11'42"E	10–100	35	g

Micro Photo ver. 2.1. Enlarged images of spores were used for measurement. Species determination followed the keys of Bánhegyi *et al.* (1985), Ellis and Ellis (1997) and Grove (1935, 1937), and fungal nomenclature follows *Index Fungorum*.

RESULTS

We recorded seven microscopic fungi during our research on *Castanea sativa* in Slovakia. The species are given below.

Melanconis modonia Tul. & C. Tul.

Coryneum modonium (Sacc.) Griffon & Maubl.

This fungus was determined in both its states. The anamorph, *C. modonium*, was most common. It was found on dead branches. Acervuli are pulvinate, dark brown to black, up to 0.5 mm in diameter, embedded in bark tissues. Conidia are slightly curved, fusiform, pale brown, with 3 to 7 pseudosepta, 15–21 × 43–72 µm. Perithecia are black, deeply embedded in stromata. Ascospores are hyaline, with one septum, 26–38 × 8–12 µm. This was the fungus most commonly observed (Table 2).

Phomopsis castaneae Woron.

Pycnidia, found in dead tissues, are black to grey. Two types of spores, both of which hyaline and one-celled, are produced in each pycnidium. One type, called α conidia, are ellipsoid to fusi-

form, germinate easily, and contain two oil guttules at each end of the spore. The second, called β conidia, are curvilinear and did not germinate. α conidia measure 10–12 × 2.0–2.5 µm, and β conidia 30–38 × 1–2 µm.

Symptoms of *Ph. castaneae* are commonly seen on European chestnut in Slovakia, but during this research the fruit bodies were identified at three localities (Table 2).

Diatrypella quercina (Pers.) Cooke

Libertella quercina Tul. & C. Tul.

This fungus was found as teleomorph and anamorph. Present in the bark were conidial stromata of the fungus, in the form of glossy red-orange subepidermal acervuli. In moist conditions the conidia are released from the acervuli in a sticky orange substance. This substance contains long, curved, hyaline conidia, 23.9–41.2 × 0.7–0.8 µm.

This fungus was detected in both states at two localities (Suchá nad Parnou, Bratislava, Table 2). The perithecia of *D. quercina* are dark brown to black, up to 0.6–0.7 mm in diameter. The asci are polysporous. The ascospores are curved, hyaline, and pale gold to brown in mass, 8–12 × 2–3 µm.

Valsa ambiens (Pers.) Fr.

Cytospora ambiens (Nitschke) Sacc.

Both states of this fungus were found on young living twigs. Stromata of the anamorph state are

visible to the naked eye as slightly conical formations 0.3–0.6 mm in diameter. The stromata are yellow at first, later brown to black, and pale or white on the margin. Conidia are tiny, slightly curved, hyaline, single-celled, $6\text{--}7 \times 1 \mu\text{m}$. The conidia are released from conidiomata in moist conditions as curling orange-yellow tendrils. The perithecia of *V. ambiens* are formed in the same stromata as pycnidia or separately. Erumpent ectostromatal discs are dark grey, grey or pale grey. Asci are 8-spored. Ascospores are allantoid, hyaline, one-celled, $22\text{--}27 \times 5\text{--}8 \mu\text{m}$. This species was found at two localities during the study (Table 2).

Glioniopsis praelonga (Schwein.) Underw. & Earle

It was detected at only one locality (Bratislava, Table 2). Black fruit bodies were formed on the bark surface. They are brittle, ellipsoid to elongated, 1.5 mm long and 0.3–0.4 mm wide. One ascus contains eight ascospores. Ascospores are $24\text{--}31 \times 12\text{--}14 \mu\text{m}$, with 5 to 7 (sometimes

more) transverse septa and 1 to 4 longitudinal septa.

Dothidothia celtidis (Ellis & Everh.) M. E. Barr

Pseudothecia are solitary, immersed, dark, up to 0.4 mm in diameter. Asci are cylindrical to clavate, with 8 spores, $115\text{--}125 \times 16\text{--}18 \mu\text{m}$. Ascospores are dark brown, ellipsoid, with a thick smooth wall, one-septate, not or sometimes slightly constricted at the septum, $19\text{--}22 \times 9\text{--}11 \mu\text{m}$. It was found at only one locality on dead branches (Table 2).

Diplodina castaneae Prill. & Delacr.

This fungus was detected only in its anamorph state (*D. castaneae*); the teleomorph state was not recorded (Table 2). It was identified on angular cracks in bark of dead branches. The conidiomata of the fungus are dark subepidermal pycnidia. Conidia are released under wet conditions as flesh-colored tendrils which are hyaline, fusiform, $10\text{--}13 \times 1\text{--}3 \mu\text{m}$ (mean 10.9×1.96), with one septum.

Table 2. List of microfungi recorded on European chestnut during the study period at sites in Slovakia. t – teleomorph, a – anamorph.

Fungus	Date of sampling	t/a	Locality
<i>Melanconis modonia</i> Tul. & C. Tul., <i>Coryneum modonium</i> (Sacc.) Griffon & Maubl.	8 April 2008	a	Arborétum Mlyňany, Vieska nad Žitavou
	22 May 2011	a	Suchá nad Parnou
	29 June 2009	a	Sološnica
	23 May 2006	a	Horné Otrokovce
	20 April 2010	a/t	Modrý Kameň
<i>Valsa ambiens</i> (Pers.) Fr., <i>Cytospora leucosperma</i> (Pers.) Fr.	26 September 2011	a/t	Suchá nad Parnou
	22 October 2010	a/t	Modrý Kameň
<i>Diaporthe eres</i> Nitschke, <i>Phomopsis castaneae</i> Woron.	19 April 2010	a	Modrý Kameň
	5 June 2007	a	Arboretum Mlyňany, Vieska nad Žitavou
	26 September 2011	a	Suchá nad Parnou
<i>Diatrypella quercina</i> (Pers.) Cooke <i>Libertella quercina</i> Tul. & C. Tul.	22 September 2010	a/t	Suchá nad Parnou
	28 June 2006	a	Horné Otrokovce
	8 April 2011	a/t	Bratislava, Jeséniova
<i>Glioniopsis praelonga</i> (Schwein.) Underw. & Earle	21 April 2011	t	Bratislava Rača
<i>Dothidothia celtidis</i> (Ellis & Everh.) M. E. Barr	19 September 2011	t	Svätý Jur
<i>Diplodina castaneae</i> Prill. & Delacr., <i>Cryptodiaporthe castanea</i> (Tul. & C. Tul.) Wehm.	19 April 2010	a	Modrý Kameň
	26 September 2011	a	Suchá nad Parnou

DISCUSSION

Melanconis modonia (*Coryneum modonium*) was the most common microscopic fungus determined on branches of European chestnut in Slovakia (except for *Cryphonectria parasitica*; Juhásová *et al.* 2005). It was first recorded in Slovakia in 1974 in Zemberovce. Both states (teleomorph and anamorph) were recorded on dead and weakened branches and rarely on stems (Juhásová 1978). In the following years it was noted from all studied localities on weakened trees cultivated in unsuitable conditions (Juhásová 1999). It is an endophytic, nonpathogenic fungus (Bissegger & Sieber 1994). *M. modonia* has been reported on European chestnut in other European countries: Switzerland, Bulgaria, Romania, France and Belgium (Farr & Rossman 2013). Diffuse cankers caused by *M. modonia* were recorded previously in Portugal, but only on senescent bark or on chestnuts weakened by biotic and abiotic factors. During 1995, however, there was a widespread and unexpected outbreak of diffuse canker on young chestnuts; high infection by *M. modonia* caused significant economic loss in chestnut orchards in Portugal (Abreu 1996). The fungus has been recorded on *Castanea* species other than *C. sativa*, and on other woody plant species (Farr & Rossman 2013).

Phomopsis castaneae is another common microfungus on European chestnut in Slovakia. It was found frequently during earlier research in 1965–1991 at 45 localities throughout Slovakia (Juhásová 1992), but was not included in the *Checklist of non-vascular and vascular plants of Slovakia* (Lizoň & Bacigálová 1998). Two of the three localities recorded in this paper (Modrý Kameň, Arboretum Mlyňany) were also reported by Juhásová (1992). The new one is Suchá nad Parnou, where a new chestnut orchard was planted in 2007. The fungus was recorded mainly on seedlings and young trees with smooth bark (Juhásová 1999). *Phomopsis* is an endophyte not known to be a chestnut pathogen (Bissegger & Sieber 1994). According to Juhásová (1999) it causes cankers and withering of branches and young stems. The teleomorph state (*Diaporthe*) was not detected during our research. It has been described from

Ukraine, France, Germany and Italy on *C. sativa*, and on dead branches of *Castanea dentata* from the United States (Farr & Rossman 2013). Besides occurring on branches, *P. castanea* has also been identified in nuts. It occurred as an endophyte in stems (except xylem), leaves, flowers and immature burrs of chestnut trees (Wadia *et al.* 2000).

Libertella quercina was first determined on European chestnut in Slovakia in 2006, on dead branches; it has not been successfully isolated and its pathogenicity to chestnut is unknown (Adamčíková *et al.* 2011). During this study it was detected in both states at other two localities. Saccardo (1906) reported *Cytosporina quercina* (Tul. & Tul.) Traverso (= *Libertella quercina* Tul. & Tul.) on branches of *Quercus* and *Castanea* in Italy, France and Germany, with conidia described as cylindrical-filiform, arched curved, hyaline, 30–35 × 1.0–1.5 µm. *Libertella quercina* has been reported from England (Grove 1937), Lithuania (Irsenaite & Treigienė 2001) and Hungary (Magyar & Tóth 2003). Acervuli of *L. quercina* occurred abundantly on *Quercus* bark in England and France (Grove 1937). *Diatrypella quercina*, the teleomorph state of the anamorph *L. quercina* (Grove 1937), was recorded on more woody plant species in eastern North America and Europe.

Valsa ambiens and *Cytospora ambiens* are cosmopolitan microfungi causing canker disease of hardwood trees, especially those stressed or damaged (Sivanesan & Holliday 1970; Hayova & Minter 1998a, b). Two species of *Cytospora* have been recorded on *C. sativa*: *C. ambiens* (Spaulding 1961) and *C. ceratophora* (Truszkowska & Chlebicki 1983). Saccardo (1879) described *C. ambiens* conidia (hyaline, 6 × 1 µm, sausage-shaped) on branches of *Juglans* and *Tilia*, and *C. ceratophora* conidia (4 × 1 µm, sausage-shaped) on branches of *Fraxinus*. *C. leucosperma* is common on bark of *Castanea* twigs and branches; the spores are sausage-shaped, 5–7 × 1 µm, issuing white, then yellowish tendrils or mass (Grove 1935). In Europe *C. ambiens* has been determined on *C. sativa* in Belgium and on *C. crenata* in France (Farr & Rossman 2013). Juhásová (1992) recorded *C. intermedia* as a cause of branch withering in European chestnut at 28 localities in Slovakia; the conidia were described

as crescent-shaped, colorless, $4\text{--}5 \times 1\text{--}2 \mu\text{m}$. *Valsa ambiens* was recorded on *Castanea* species in Japan (Farr & Rossman 2013).

Our present reports of *Gloniopsis praelonga* and *Dothidotthia celtidis* on *C. sativa* in Slovakia are the first for Slovakia. There are no published data on the occurrence of *G. praelonga* and *D. celtidis* in Slovakia; nor are they noted in the *Checklist of non-vascular and vascular plants of Slovakia* (Lizoň & Bacigálová 1998). Each was found at a single locality. *G. praelonga* is a cosmopolitan fungus recorded on many woody plant species. On *C. sativa* it has been reported from Portugal, Spain (Farr & Rossman 2013) and Hungary (Bánhegyi et al. 1985).

Didymosphaeria superapplanata Sivan. (= *Dothidotthia celtidis*) was described in 1977 on branches of *C. sativa* (Sivanesan 1977). Pseudothecia are scattered, immersed, erumpent, black, $360\text{--}400 \times 350\text{--}380 \mu\text{m}$, globose. Asci are cylindrical to cylindrical-clavate, thick-walled, 8-spored, $115\text{--}125 \times 16\text{--}18 \mu\text{m}$. Ascospores are brown, ellipsoidal, septate, not constricted or sometimes slightly constricted at septum, smooth, $20\text{--}25 \times 5\text{--}11 \mu\text{m}$, tapering to connate or rounded ends (Sivanesan 1977). It was also reported on *Castanea* sp. in the United Kingdom by Cannon et al. (1985) and by Ellis and Ellis (1997). Barr (1989) described this fungus as *D. celtidis* with ascomata immersed, scattered to gregarious, sphaeroid, $220\text{--}440 \mu\text{m}$ in diameter, asci $60\text{--}70 \times 25\text{--}28 \mu\text{m}$, ascospores $19\text{--}27 \times 9.0\text{--}12.5 \mu\text{m}$, dark brown, broadly ellipsoid, (0–)1-septate, not constricted, wall thick, smooth.

Diplodina castaneae was observed on young branches of *C. sativa* as a very harmful species: spores $6\text{--}7 \times 1\text{--}1.5 \mu\text{m}$, 1-septate (Saccardo 1895). Conidial size is $9\text{--}12 \times 2.5\text{--}3.0 \mu\text{m}$ according to Day (1930), but $6\text{--}7 \times 1\text{--}1.5 \mu\text{m}$ in the original description (Prillieux & Delacroix 1893). Grove (1935) adopted the latter measurements but noted the possibility of error. Ellis and Ellis (1997) described both states of this fungus. The *Diplodina* state was found on dead branches and twigs between May and October. It causes Javart disease of European chestnut (Prillieux & Delacroix 1893). According to Day (1930) this disease is character-

ized by small cankers on the stem of young coppice shoots, which are sometimes killed. The conidia are issued in flesh-colored tendrils, mostly $8\text{--}12 \times 2\text{--}3 \mu\text{m}$, and remain without a septum except in longer conidia just before germination (Ellis & Ellis 1997). We did not observe this dimorphism of conidia; nor did Bissegger and Sieber (1994), who gave the conidia dimensions as $8\text{--}13 \times 2.0\text{--}2.5 \mu\text{m}$. The fungus was recorded in the area of present-day France, Belgium, northern Italy and Germany (Saccardo 1895), and more recent records come from Bulgaria and Switzerland (Vanev et al. 1997; Bissegger & Sieber 1994). The fungus was also reported on *C. crenata* in Japan (Farr & Rossman 2013). It was first found in Slovakia in 1998 at Horné Lefantovce on seedlings and young trees, causing necrosis well visible on smooth bark (Juhásová 1999); it was not included in the *Checklist of non-vascular and vascular plants of Slovakia* (Lizoň & Bacigálová 1998). The teleomorph state (as *Cryptodiaporthe castaneae*) forms black clustered perithecia up to 0.8 mm in diameter. Ascospores are hyaline, appendaged, one-septate, $13\text{--}18 \times 2\text{--}4 \mu\text{m}$ (Ellis & Ellis 1997). We did not record the teleomorph during this study. It is considered to be a weak wound parasite sometimes causing dieback and cankers (Bissegger & Sieber 1994).

In this study we recorded seven different fungal species. Some of them co-occurred with the chestnut blight fungus *C. parasitica*. Their joint effect could be the reason for the poor health, drying and death of *Castanea sativa*.

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