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Mycological complex of poplars in Serbia

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ABSTRACT: Poplars are fast-growing broadleaved tree species inhabiting river banks and sites with accessible water supplies. Vegetative propagation makes them suitable for establishing highly productive plantations along big rivers and in flooded plains. The production of large quantities of biomass provides a good substrate for various organisms. The aim of this study was to identify fungal species occurring in the poplar plantations in Serbia and to determine their frequency and role in decomposition of tree parts. Fifty species belonging to the divisions *Ascomycota*, *Basidimycota* and *Deuteromycota* as well as two species from *Oomycota* (the genus *Phytophthora*) were reported. Bark was the substrate for 27 species, 14 species were found on leaves and 9 species were wood-decaying fungi.

Keywords: *Populus* spp.; mycobiota diversity; taxonomy; trophic groups

In their natural range, poplars grow in alluvial plains along large rivers. These sites are characterised by specific climatic conditions with the dominance of higher air humidity and soil moisture (De Bell, Harrington 1997; Demchik et al. 2002). Alluvial plains along the rivers Danube, Sava, Tisa, Ibar and Morava are suitable for the growth of several broadleaved tree species (*Quercus robur* L., *Fraxinus angustifolia* Vahl., *Populus* spp.). The high production potential of hybrid poplars led to an increase of natural stands and establishment of plantations along river banks (Keča et al 2012). Production potential and mechanical characteristics of the wood of *P.* × *euramericana* cl. *I-214*, and also *Ostia, I-154*, and recently cv. *Pannónia*, favoured a wide use of these clones in plantations (Herpka 1986; Keča 2003a). According to the National Forest Inventory (NFI) poplar stands cover about 48,000 ha, or 2.1% of the total forest area in Serbia (Banković et al. 2009). Some of the habitats along major rivers represent Europe's best sites for poplar growth, with timber volumes up to 605 $m^3 \cdot ha^{-1}$ and an average of 350 $m^3 \cdot ha^{-1}$ in 15–20 years (Keča et al. 2012).

The high humidity, as well as the intensive growth of poplar trees, provides favourable conditions for the colonisation of poplars by a large number of fungal and bacterial organisms. A partial inventory of the mycoflora was reported by Krstić et al. (1958) and most of earlier research was focused on the most important diseases e.g. *Dothichiza populea* Sacc. et Br., *Marssonina brunnea* (Ell. et Ev.) P. Magn., *Melampsora* spp. (Keča 2003a,b).

The aim of this study was to (*i*) explore the diversity of fungi in poplar natural stands and plantations and (*ii*) to determine the significance of fungal species and their role in the decline of individual trees.

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MATERIAL AND METHODS

Our study was performed in the most productive poplar stands and plantations in the northern part of Serbia – the autonomous provenance of Vojvodina (P.E. "Vojvodinašume"), and also in the central part along the biggest rivers Morava and Ibar (P.E. "Srbijašume").

From 2005 to 2012, the material (leaves, bark, shoots, buds and wood) was collected from 44 localities in order to identify species, assess the density, distribution and significance of individual fungal organisms occurring in the plantations on different poplar clones and cultivars, and also in other stands of native poplars from the sections *Aigeiros* and *Leuce*. Some of the plots were visited three times a year (spring, summer and autumn), while others were controlled only once a year (autumn). Established plots were studied in order to follow the presence and dynamics of species appearance. Two tracks per plot, 12 m wide (two rows in a planting pattern of 6 × 6 m), evenly distributed through the stand were examined, both in natural stands and plantations, for the presence of symptoms. The track length ranged from 70 to 300 m, with an average length of 100 m.

Leaves, bark, shoots, buds, wood with symptoms were collected and checked for the presence of fungal fruiting structures. Material without fruiting structures was transferred to the moist chambers. For isolating *Phytophthora* spp., soil was collected in the form of monoliths ~ 25 × 25 × 25 cm in size. The isolation tests were performed using the baiting techniques (Jung et al. 1996, 2000), and young leaves of oak and beech were used as baits. Isolations from water, collected in sterilized 1-litre plastic bottles, were performed using the same techniques.

Fungi were identified conventionally according to their macroscopic and microscopic features. Isolations were performed using different artificial media (MEA, PDA, V8A-PARPNH, CA). Sample fragments were surface sterilized in 1% NaOCl (diluted from a commercial 5% stock solution) for 2 min and 1 min in 20% ethanol before plating. Petri dishes were sealed with Parafilm® and kept at room temperature ~22 to 24°C in the dark. The cultures are archived in the Laboratory for Forest Pathology at the University of Belgrade, Faculty of Forestry.

Identification of morphological features was based on the use of identification keys: Grove (1935, 1937); Lanier et al. (1976); Dennis (1978), Sutton (1980); Breitenbach and Kräntzlin (1981); Ellis and Ellis (1985), Barnett and Hunter (1987), Stamps et al. (1990), Erwin and Ribeiro (1996), Jung and Burgess (2009).

Frequency of occurrence and the intensity of host affection by trophical groups were estimated on an altered 5-point scale, proposed by Karadžić (1987) as follows: – typical saprophytes; (+) decaying fungi; + weak pathogen; ++ facultative pathogen causing problems only exceptionally; +++ strong pathogen which is the practical problem in raising and maintaining poplar plantations.

Species for which we could not assess parasitic behaviour were marked with ??.

Frequency of occurrence, for each year, was calculated as the number of positive samples, for identified species, in relation to the total number of taken samples on 44 studied localities. The average number of observations (in %) was calculated for the period 2005–2012. The significance of differences was tested by simple one-way ANOVA. The data obtained were processed statistically in SPSS 10 (IBM, USA).

RESULTS

In total 612 samples from different localities were processed. In the process of identification of isolates and reproductive organs 50 species of fungi were identified and two *Phytophthora* species. Forty-seven percent of identified species belong to the phylum *Ascomycota* (47%), while 24% and 27% of observed species were from the phyla *Basidiomycota* and *Deuteromycota*, respectively (Fig. 1). Observed differences in the occurrence of fungal phyla on poplar trees were not statistically significant ($P = 0.54$). Fourteen species were found in leaf tissues, 27 in cortical tissues, and 9 species are decaying fungi. *Phytophthora* species were isolated from the soil and water collected under poplar trees (Table 1).

Sixteen percent of the observed species acted as saprotrophs, while 10% of species demonstrated high pathogenicity in poplar plantations, and also in natu-

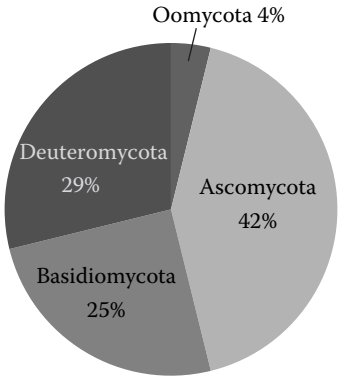

Fig. 1. The taxonomic structure of *Populus* spp. mycobiota in Serbia

Table 1. The diversity of mycobiota on poplar species (*Populus* spp.) in Serbia

No.	Species	Plant parts colonized	Frequency of occurrence (%)	Trophic behaviour[2]	Taxonomic affiliation[1]
Fungi colonizing leaves (Σ14)					
1	*Alternaria* sp.	leaves	1.5	+	D
2	*Botrytis cinerea* Pers.	leaves and sprouts	0.5	+	D
3	*Cladosporium epiphyllum* (Pers.) Nees	leaves and shoots	2.1	+	D
4	*Drepanopeziza punctiformis* Gremmen	leaves and shoots	13.2	+++	A
5	*Melampsora allii-populina* Kleb.	leaves	11.0	+++	B
6	*Melampsora larici-populina* Kleb.	leaves	9.8	+++	B
7	*Astreromella osteospora* Sacc.	leaves	2.2	+	D
8	*Stictochorella populi-nigrae* (Allesch.) Petr.	leaves	3.6	+	D
9	*Ascochyta populorum* (Sacc. & Roum.) Voglino	leaves	1	+	D
10	*Phyllosticta populina* Sacc. (Mich.)	leaves	2.8	+	D
11	*Venturia populina* (Vuill.) Fabric.	leaves and shoots	5.5	++	A
12	*Taphrina populina* Fr.	leaves	2	+	A
13	*Torula* sp.	dead leaves	3.3	-	D
14	*Erysiphe adunca* (Wallr.) Fr.	leaves	3.2	+	A
Fungi colonizing bark (Σ26)					
15	*Botryosphaeria dothidea* (Moug.) Ces. & De Not.	branches and trunk	4.0	++	A
16	*Cryptosporiopsis fasciculata* (Tode ex Tul.) Petrak	young shoots	0.1	-	A
17	*Dothichiza populea* Sacc et Briard	branches and trunk	12.3	+++	A
18	*Dothiorella populina* P. Karst.	branches and trunk	0.5	++	D
19	*Sirodothis populnea* (Thüm.) B. Sutton & A. Funk	branches and trunk	0.3	++	D
20	*Epicoccum nigrum* Link	bark	6.6	-	D
21	*Gibberella avenacea* R.J. Cook	branches and trunk	1.7	++	A
22	*Hendersonula* sp.	branches and shoots	> 0.1	+	D
23	*Hypoxylon rubiginosum* (Pers.) Fr.	branches and trunk	> 0.1	+	A
24	*Leptospora rubella* (Person) Rabenh.	dead shoots	> 0.1	-	A
25	*Valsa nivea* (Hoffm.) Fr.	branches and trunk	3.2	+	A
26	*Macrophoma* sp.	shoots	> 0,1	+	D
27	*Neonectria galligena* (Bres.) Rossman & Samuels	branches and trunk	1	++	A
28	*Patellaria atrata* (Hedw.) Fr.	young shoots	> 0.1	-	A
29	*Periconia cookei* E.W. Mason & M.B. Ellis	dead shoots	> 0.1	-	D
30	*Pezicula ocellata* (Pers.) Seaver	shoots (current vegetation)	> 0.1	+	A
31	*Boeremia exigua* (Desm.) Aveskamp, Gruyter & Verkley	branches and shoots	0.1	+	A
32	*Phoma urens* Ell. et Ev.	shoots	0.5	+	A
33	*Phomopsis putator* (Nitschke) Traverso	branches and trunk	0.5	++	D
34	*Roselinia necatrix* Berl. ex Prill.	butt	1.0	++	A
35	*Apiosporopsis carpinea* (Fr.) Mariani	shoots	> 0.1	-	A
36	*Botryosphaeria stevensii* Shoemaker	branches and trunk	> 0.1	+	A
37	*Tremella mesenterica* Retz.	bark surface	> 0.1	-	B
38	*Nectria cinnabarina* (Tode) Fr.	branches and trunk	1.8	++	A
39	*Cryptosphaeria ligniota* (Fr.) Auersw.	branches and trunk	0.1	++	A
40	*Valsa sordida* Nitschke	branches and trunk	4.1	+++	A
Soil and water (Σ2)					
41	*Phytophthora plurivora* T. Jung and T.I. Burgess	root and butt	> 0.1	??	O
42	*Phytophthora cactorum* (Lebert & Cohn) J. Schröt.	root and butt	> 0.1	??	O
Total			**100**		

Table 1. to be continued

Fungi decaying wood (Σ10)					
43	*Armillaria mellea* (Vahl.:Fr.) Kumm. s.s.	root and butt rot	n.a.	(+)	B
44	*Chondrostereum purpureum* (Pers.) Pouz.	logs	n.a.	(+)	B
45	*Flamulina velutipes* (Curt.) Sing.	logs & stumps	n.a.	(+)	B
46	*Fomes fomentarius* (L.) Fr.	trees	n.a.	(+)	B
47	*Laetiporus sulphureus* (Bull.) Murrill	trees	n.a.	(+)	B
48	*Hemipholiota populnea* (Pers.) Bon	logs & stumps	n.a.	(+)	B
49	*Pleurotus ostreatus* (Jacq.) Kummer	trees	n.a.	(+)	B
50	*Schizophyllum commune* Fr.	dead parts of trunk	n.a.	(+)	B
51	*Trametes suaveolens* (L.) Fr.	branches and trunk	n.a.	(+)	B
52	*Trametes versicolor* (L.) Lloyd	branches and trunk	n.a.	(+)	B

[1]A – *Ascomycota*, B – *Basidiomycota,* D – *Deuteromycota,* O – *Oomycota*; [2]typical saprophytes; (+) decaying fungi; + weak pathogen; ++ facultative parasites, they cause problems only exceptionally; +++ strong pathogens which are the practical problem in raising and maintaining poplar plantations; ?? unknown trophic behaviour; n.a. – frequency not assessable

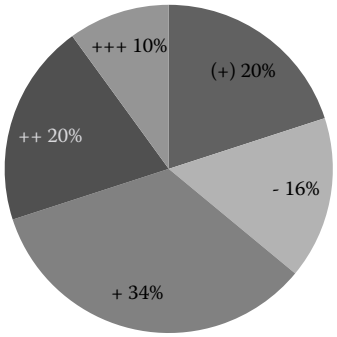

Fig. 2. Frequency of occurrence and intensity of mycobiota observed on *Populus* spp.

ral stands (Fig. 2). Fifty-four percent of identified species act as weak pathogens of facultative parasites.

Observed species are less specialised to poplars and 71% of them develop on a wide variety of woody hosts. The greatest damage to poplar cultivation was caused by the species highly specialized to poplars e.g. *Dothichiza populea*, *Marssonina brunnea*, *Melampsora allii-populina*, *M. larici-populina* and *Valsa sordida.*

Phytophthora species were found in the soils of young hybrid poplar *I–214* plantations. They were identified as *P. cactorum* and *P. plurivora*, which are also present in neighbouring pedunculate oak (*Quercus robur*) and narrow-leaved ash (*Fraxinus angustifolia*) stands. No damage to poplar plantations where *Phytophthora* species were present has been observed so far.

DISCUSSION

There are numerous studies on pathogenic and saprophytic fungi on poplars. Most of the studies are from the mid-20th century, when establishment of plantations was seen as solutions to the shortage of raw wood of broadleaved species (Cagelli, Lefevre 1995). Butin (1957) listed 142 fungal species on poplars from the sections *Aigeiros*, *Populus* and *Tacamahaca* and reported 63 species from Germany. In former Yugoslavia the first study was done by Krstić et al. (1958) and 33 species were reported. During the 60's and 70's several researchers in Yugoslavia reported mycobiota and epidemics of the most important poplar diseases (Vujić et al. 1967; Gojković 1971, 1974). Description and impact on poplar plantations were overviewed for about fifteen years by Italian researchers Cellerino and Gennaro (1999).

This study provided an insight into the diversity of fungi, pseudofungi and bacteria present in poplar plantations and stands along main rivers (Danube, Sava, Tisa, Ibar and Morava) in Serbia. Although over the last 60 years several studies were performed on the poplar diseases (Krstić 1958; Kišpatić 1959; Gojković 1974), there are few data relating to the presence of facultative biotrophs. During this study eighteen species have been found for the first time on *Populus* spp. in Serbia. According to the previous studies *Glomerella miyabeana* (Fuck.) v. Arx. (Gojković 1974) and *Cytospora foetida* Vl. et Kr. (Naidenov 1984) can cause significant damage to poplars, but they were not found during this study.

During the studied period of local epidemics, caused by rainy and mild summers/autumns, *Melampsora* spp., *Marssonina burnnea* and *Dothichiza populea* were observed. Forestry practitioners are aware of them and usually apply silvicultural measures, even fungicides (Keča 2003b), to prevent increment loss and decline of trees (Keča, Karadžić 2004). In addi-

tion, the study reports about 25% (Table 1 – with ++ for significance) of the species that can change their trophic behaviour. Environmental conditions can stimulate fungal or destimulate host development and affect the host-pathogen interaction. Further monitoring in natural stands and plantations is necessary because behavioural changes were already observed for some species e.g. *Botryosphaeria* complex (Karadžić et al. 2000; Slippers, Wingfield 2007; Zlatković et al. 2013).

CONCLUSIONS

During these studies, 50 species of fungi and two *Phytophthora* species were identified on poplars. Bark was a substrate for 27 species, 14 biotrophic and saprotrophic species were found on leaves and 9 non-specialized species were associated with wood decomposition.

The dominant taxonomic group of poplar associated fungi is *Ascomycota*. Those are cosmopolitan species growing on above- and belowground parts of *Populus* spp. Most of the identified species belong to saprobic fungi (91%), but some species are capable to change their trophic behaviour depending on the host condition.

The most important species, as expected from earlier studies, were *Dothichiza populea*, *Marssonina brunnea*, *Melampsora allii-populina*, *M. larici-populina* and *Valsa sordida*, while *Fomes fomentarius* caused the decay of solitary trees.

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