

## Mycological Complex on the Leaves and Bark of *Salix* Species in Central Danube Basin

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**Abstract** – This work shows the results of the research on the microflora of willows (*Salix* spp.) in the area of Srednje Podunavlje. Dendro-material with disease and damage symptoms was collected in the field. The material was deposited in herbarium, the fungi were identified and isolated in laboratory on nutritive medium. During the research 36 fungal species were recorded on leaves and bark of willows. The most significant species causing economic damages and death of the plants were: *Colletotrichum gloeosporioides* Penz., *Glomerella miyabeana* (Fuk.) v. Arx, *Marssonina salicicola* (Bres.) Magn., *Sphaeropsis malorum* Peck., *Valsa salicina* Fr, *Phyllosticta salicicola* Thuem., *Fusarium oxysporum* Schlecht. and *Melampsora epitea* Thüm.

**willow / *Salix* spp./ bark / fungi / Srednje Podunavlje**

**Kivonat** – *Salix* fajok levél- és kéreggombái a Közép-Duna medencében. A munka a fűzek (*Salix* spp.) mikroflóra kutatásának eredményeit mutatja be Srednje Podunavlje térségében. Betegség- és károsodási tüneteket mutató fás anyagot gyűjtöttünk a terepen. Az anyagot herbáriumba helyeztük, a gombákat meghatároztuk és táptalajra kitenyésztettük. A kutatás során a fűzek leveléről és kérgéről 36 gombafajt azonosítottunk. A legjelentősebb, gazdasági károkat és a növények pusztulását okozó fajok a következők: *Colletotrichum gloeosporioides* Penz., *Glomerella miyabeana* (Fuk.) v. Arx, *Marssonina salicicola* (Bres.) Magn., *Sphaeropsis malorum* Peck., *Valsa salicina* Fr, *Phyllosticta salicicola* Thuem., *Fusarium oxysporum* Schlecht. és *Melampsora epitea* Thüm.

**fűz / *Salix* fajok / kéreg / gombák / Srednje Podunavlje**

### 1 INTRODUCTION

The restricted opportunities of wood production in natural forests and a rapid development of industrial processing of wood have brought about the decrease of timber supply and to the creation of the ever increasing deficit of wood as raw material. This problem has been somewhat mitigated by wood production in the plantations of fast growing tree species (primarily poplars and willows).

The fact that forests in Vojvodina are mainly concentrated along the rivers and that there are significant land areas that are not suitable for agricultural production increase the

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significance of establishment of willow plantations, which as a pioneer species occupies the lowest part of the microrelief.

Willow diseases, in contrast to poplar diseases, have not drawn any special attention. By the establishment of new willow clonal plantations, this problem has become outstanding and has made it necessary to undertake such investigations.

The aim of this paper was to identify the most significant parasitic and saprophytic fungi in willow natural stands and clonal plantations.

## 2 MATERIAL AND METHOD

The field research and collection of material was performed at several localities in willow plantations, natural populations and nurseries in the area of Central Danube Basin situated between 45°08'18" and 45°42'50" north latitude and 17°10'10" and 17°58'50" east longitude, at the altitude from 73 to 79 metres. The climate of this area is temperate continental with the characteristics of the Pannonian-steppe temperate-continental climate. The research was performed in the flooded area of the Danube Basin marshes, on the flat land intersected with micro-depressions and narrow land strips with the altitudinal difference between 1 and 6 metres. This is the case of the microrelief, which together with the level of underground water (which directly depends on the Danube water level), creates the special conditions for the development of some plant communities, and they are especially very favourable conditions for the development of willows.

The material collected in the field consisted of the diseased leaves, dead branches and branches with necroses and tumours, etc. All the material was kept in the herbaria and brought to the laboratory for the identification and isolation of fungi on the nutritive media. The determination of fungi was preceded by the preparation of the temporary microscopic preparations or by the cultivation of pure fungal cultures. The species determination was based on the appearance of the fruiting bodies, sporiferous organs, organs for reproduction, and the appearance of fungal mycelia.

## 3 RESULTS

During these researches, the following most frequent parasitic and saprophytic fungi which occur on willows were identified (*Table 1*).

Altogether 36 species of fungi were identified on willow foliage, on catkins and bark, of which on the foliage 7, on catkins 1, on bark 26, and on bark and foliage 2. One species was determined (*Darluca filum* (Biv.) Cast.), which behaves as a hiper-parasite on uredosori of the fungus *Melampsora epitea* Thüm. The above fungi were identified both on the willows in natural stands and on the willows in clonal plantations. Of all the identified species, the most significant are the following: *Phyllosticta salicicola*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Sphaeropsis malorum*, *Valsa salicina*, *Marssonina salicicola* and *Melampsora epitea*.

During the above research on willows, 22 parasitic and saprophytic species of fungi were identified for the first time, i.e. they have not been identified so far on willows on the territories of Serbia and Montenegro. Of the 22 new identified species of fungi, 19 species have never been identified in the study area, and three species were identified, but on other hosts (e.g. on poplars).

Table 1. The most frequent parasitic and saprophytic fungi on willow leaves, catkins and bark

| Name of fungus  | Sistematical place  | The infect. part | The first time found in Serbia and Montenegro | The first time found on willow |
|---|---|------------------|---|--------------------------------|
| <i>Ascochyta salicicola</i> Pass.                               | Subphylum<br><i>Deuteromycotina</i><br>Ord. <i>Sphaeropsidales</i><br>Fam. <i>Sphaerioidaceae</i> | Leaf             | +   | +                              |
| <i>Botryosphaeria dothidea</i><br>(Moug. ex Fr.) Ces. & de Not. | Phylum <i>Ascomycota</i><br>Ord. <i>Dothideales</i><br>Fam. <i>Botryosphaeriaceae</i>             | Bark             | -   | +                              |
| <i>Capnodium salicinum</i> Mont                                 | Phylum <i>Ascomycota</i><br>Ord. <i>Dothideales</i><br>Fam. <i>Capnodiaceae</i>                   | Leaf             | -   | -                              |
| <i>Cladosporium herbarum</i><br>(Pers.) Link ex SF. Gray        | Subphylum<br><i>Deuteromycotina</i><br>Ord. <i>Hyphomycetales</i><br>Fam. <i>Dematiaceae</i>      | Bark             | -   | -                              |
| <i>Colletotrichum gloeosporioides</i><br>Penz.                  | Subphylum<br><i>Deuteromycotina</i><br>Ord. <i>Melanconiales</i><br>Fam. <i>Melanconiaceae</i>    | Bark / Leaf      | +   | +                              |
| <i>Cryptodiaporthe salicina</i> (Pers.)<br>Wehmeyer             | Phylum <i>Ascomycota</i><br>Ord. <i>Diaporthales</i><br>Fam. <i>Valsaceae</i>                     | Bark             | +   | +                              |
| <i>Crypthodiaporthe salicella</i> (Fries)<br>Petraik            | Phylum <i>Ascomycota</i><br>Ord. <i>Diaporthales</i><br>Fam. <i>Valsaceae</i>                     | Bark             | +   | +                              |
| <i>Cytospora ambiens</i> Sacc.                                  | Subphylum<br><i>Deuteromycotina</i><br>Ord. <i>Sphaeropsidales</i><br>Fam. <i>Sphaerioidaceae</i> | Bark             | -   | -                              |
| <i>Cytospora chrysosperma</i> (Pers.) Fr.                       |   |                  | -   | -                              |
| <i>Cytospora fertilis</i> Sacc.                                 |   |                  | +   | +                              |
| <i>Cytospora nivea</i> Sacc.                                    |   |                  | -   | -                              |
| <i>Cytospora salicis</i> Rab.                                   |   |                  | +   | +                              |
| <i>Cytospora translucens</i> Sacc.                              |   |                  | +   | +                              |
| <i>Darluca filum</i> (Biv.) Cast.                               | Subphylum<br><i>Deuteromycotina</i><br>Ord. <i>Sphaeropsidales</i><br>Fam. <i>Sphaerioidaceae</i> | Hyper-parasite   | -   | -                              |
| <i>Dasyscyphus pudibundus</i> (Quelet)<br>Sacc.                 | Phylum <i>Ascomycota</i><br>Ord. <i>Leotiales</i><br>Fam. <i>Hyaloscyphaceae</i>                  | Bark             | +   | +                              |
| <i>Diatrype bullata</i> (Hoffm.) Fr.                            | Phylum <i>Ascomycota</i><br>Ord. <i>Diatrypales</i><br>Fam. <i>Diatrypaceae</i>                   | Bark             | +   | +                              |
| <i>Diplodina salicis</i> Westd.                                 | Subphylum<br><i>Deuteromycotina</i><br>Ord. <i>Sphaeropsidales</i><br>Fam. <i>Sphaerioidaceae</i> | Bark             | +   | +                              |
| <i>Epicoccum purpurescens</i> Ehrenb                            | Subphylum<br><i>Deuteromycotina</i><br>Ord. <i>Hyphomycetales</i><br>Fam. <i>Tuberculariaceae</i> | Bark             | -   | -                              |
| <i>Fusarium oxysporum</i> Schlecht.                             | Subphylum<br><i>Deuteromycotina</i><br>Ord. <i>Hyphomycetales</i><br>Fam. <i>Tuberculariaceae</i> | Bark             | -   | +                              |

Table 1 cont. The most frequent parasitic and saprophytic fungi on willow leaves, catkins and bark

| Name of fungus  | Sistemical place   | The infect. part | The first time found in Serbia end Montenegro | The first time found on willow |
|---|--|------------------|---|--------------------------------|
| <i>Glomerella miyabeana</i> (Fuk.) v. Arx             | Phylum <i>Ascomycota</i><br>Ord. <i>Phyllachoraceales</i><br>Fam. <i>Phyllachoraceae</i>       | Bark             | -   | -                              |
| <i>Leucostoma nivea</i> (Persoon ex Fries) van Höhner | Phylum <i>Ascomycota</i><br>Ord. <i>Diaporthales</i><br>Fam. <i>Valsaceae</i>                  | Bark             | -   | -                              |
| <i>Marssonina salicicola</i> (Bres.) Magn.            | Subphylum <i>Deuteromycotina</i><br>Ord. <i>Melanconiales</i><br>Fam. <i>Melanconiaceae</i>    | Bark / Leaf      | +   | +                              |
| <i>Melampsora epitea</i> Thüm                         | Phylum <i>Basidiomycota</i><br>Ord. <i>Uredinales</i><br>Fam. <i>Melampsoraceae</i>            | Leaf             | -   | -                              |
| <i>Mollisia</i> sp.                                   | Phylum <i>Ascomycota</i><br>Ord. <i>Leotiales</i><br>Fam. <i>Dermataceae</i>                   | Bark             | +   | +                              |
| <i>Monostichella salicis</i> (Westd.) v. Arx.         | Subphylum <i>Deuteromycotina</i><br>Ord. <i>Melanconiales</i><br>Fam. <i>Melanconiaceae</i>    | Leaf             | +   | +                              |
| <i>Mycosphaerella tassiana</i> v. Arx.                | Phylum <i>Ascomycota</i><br>Ord. <i>Mycosphaerellales</i><br>Fam. <i>Mycosphaerellaceae</i>    | Bark             | -   | -                              |
| <i>Nectria flavo-viridis</i> (Fuckel) Wollenweder     | Phylum <i>Ascomycota</i><br>Ord. <i>Hypocreales</i><br>Fam. <i>Hypocreaceae</i>                | Bark             | +   | +                              |
| <i>Phoma glyptica</i> Cooke end Massel                | Subphylum <i>Deuteromycotina</i>   | Bark             | +   | +                              |
| <i>Phoma salicina</i> Westol                          | Ord. <i>Sphaeropsidales</i>  |                  | +   | +                              |
| <i>Phomopsis salicina</i> Died.                       | Fam. <i>Sphaerioidaceae</i>  |                  | +   | +                              |
| <i>Phyllosticta salicicola</i> Thuem                  | Subphylum <i>Deuteromycotina</i><br>Ord. <i>Sphaeropsidales</i><br>Fam. <i>Sphaerioidaceae</i> | Leaf             | +   | +                              |
| <i>Sphaeropsis malorum</i> Peck.                      | Subphylum <i>Deuteromycotina</i><br>Ord. <i>Sphaeropsidales</i><br>Fam. <i>Sphaerioidaceae</i> | Bark             | +   | +                              |
| <i>Trichothecium rosae</i> (Link)                     | Subphylum <i>Deuteromycotina</i><br>Ord. <i>Hyphomycetales</i><br>Fam. <i>Mucedinaceae</i>     | Catkins          | -   | -                              |
| <i>Uncinula salicis</i> Wint.                         | Phylum <i>Ascomycota</i><br>Ord. <i>Erysiphales</i><br>Fam. <i>Erysiphaceae</i>                | Leaf             | -   | -                              |
| <i>Valsa salicina</i> Fr.                             | Phylum <i>Ascomycota</i><br>Ord. <i>Diaporthales</i><br>Fam. <i>Valsaceae</i>                  | Bark             | -   | -                              |
| <i>Venturia saliciperda</i> Nüesch                    | Phylum <i>Ascomycota</i><br>Ord. <i>Dothideales</i><br>Fam. <i>Venturiaceae</i>                | Bark             | -   | +                              |

## 4 DISCUSSION

By the establishment of new clonal willow plantations in Serbia, the problem of willow diseases, which has so far not been paid much significance to (and therefore our literature on this subject is rather scarce), has become increasingly prominent. For this reason, it was necessary to research all harmful abiotic and biotic factors which occur both in willow natural stands and in clonal plantations, leading to tree decline and tree dying. Among the harmful biotic factors, special place is occupied by parasitic fungi.

In his capital work "Sylloge Fungorum", Sacardo (1898), was the first to describe more than 500 species of fungi on the material collected all over the world. Somewhat later, Grove (1935, 1937) described 42 species of fungi on willows, belonging to the genera *Sphaeropsidales Melanconiales*. Wilson and Henderson (1966) described 7 species of fungi of the genus *Melampsora* on willow leaves. Lanier et al. (1976) reported only the fungi which caused serious diseases on willows. Of altogether 33 species of fungi, 23 infest the leaves, and 10 species infest the bark. They point out especially the significance of the fungi *Marssonina salicicola* (teleomorph *Drepanopeziza sphaeroides*) and *Colletotrichum gloeosporioides*, which in addition to leaf spot on the leaves, also cause necrosis and canker on the shoots and branches. Karadžić (1979) emphasised the significance of the fungus *Diplodina salicina* which caused severe dying of branches on weeping willows (*Salix alba* var. *Vitalina* f. *Pendula*). During this research in the area of the Central Danube Basin, this fungus was not observed, but the fungus *Diplodina salicis* was identified on the diseased branches with similar symptoms. Ellis and Ellis (1985) described 69 species of parasitic fungi (on the leaves of 21 species, on catkins 1, and on bark and wood 47 species) on willows in Great Britain. In the Pacific north-west region of USA, Shaw (1980) reported 223 species fungi on willows. Butin (1989) in Germany reported that serious diseases on willows were caused by 11 species of fungi and 2 bacteria, with special reference to the significance of the fungi *Drepanopeziza sphaeroides* (anamorph *Marssonina salicicola*). In their study of leaf spot on forest tree species, Karadžić and Milijašević (2005) described three species on willows, of which the greatest significance was assigned to the species *Uncinula salicis*. If we compare the results of our research with those in the references, it can be concluded that it is generally agreed that the most important parasitic fungi are *Marssonina salicicola*, *Colletotrichum gloeosporioides* and "the rusts".

## 5 CONCLUSIONS

Based on the above research, we can conclude as follows:

- 36 species of fungi were identified on willow foliage, catkins and bark;
- of the identified species of fungi, 7 species were found on the foliage, 26 on bark, and 2 species synchronically on bark and foliage, 1 species of fungi was found on catkins;
- in contrast to the trees in natural stands, there were no fungal agents of wood decay on the trees in clonal plantations;
- 22 parasitic and saprophytic species of fungi were identified for the first time on willow leaves, catkins and bark, i.e. they have not been identified so far on willows on the territories of Serbia and Montenegro. Of 22 new identified species of fungi, 19 species have never been identified in the study area, and three species were identified, but on other hosts (e.g. on poplars).
- the fungus *Darluca filum* which behaves as a hyper-parasite on uredosori of the fungus *Melampsora epitea*, was also identified for the first time;

- of all the identified species, the most significant are the following *Phyllosticta salicicola*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Sphaeropsis malorum*, *Valsa salicina*, *Marssonina salicicola* and *Melampsora epitea*.

Based on the comparison of the mycoflora occurring on willow trees in natural stands and that occurring in plantations on clonal material, it was concluded that, on the clonal material, the dominant parasitic fungi are those which infest the leaves and bark, while on the old trees in natural stands, the dominant fungi are the agents of wood decay. To protect the juvenile willows in plantations (primarily against the parasites on the foliage) it is necessary to apply the chemical measures of protection, i.e. to apply the fungicides during the critical period for parasite infection.

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