

## Original papers

# The phyllosphere as a little-known reservoir of the *Fusarium* genus, a fungi of importance to medical mycology

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**ABSTRACT.** The phyllosphere is an ecological niche that includes the area of the leaf blade, which is a living environment for microorganisms of various taxonomic and trophic groups, including saprotrophic and phytopathogenic fungi. This study analysed the degree of colonisation of the phyllosphere of rush plants of selected bathing lakes by fungi of the *Fusarium* genus that are of significance to the etiology and epidemiology of fungal infections, and to toxicology and allergology. The research materials was plants of the littoral zone of Lakes Skanda and Kortowskie, which were subjected to a standard phytopathological assessment. The fungi obtained in macrocultures were used to prepare microscopic specimens required for the identification of morphological characteristics of the asexual and sexual stages. A total of 560 fungal isolates were obtained from the phyllosphere of nine plant species, of which 40 (7.14%) were fungi of the *Fusarium* genus. Most isolates were noted on *Phragmites australis*. Both macro- and microconidia of *Fusarium* were observed on this plant, while only macroconidia were observed on other plants. The results confirmed that the phyllosphere was an important reservoir of fungi that are potentially pathogenic to humans.

**Key words:** *Fusarium*, phyllosphere, bathing lakes, rush plants

## Introduction

As regards hydromycological issues associated with medical ecology, the sources of environmental fungal infections of various etiologies are increasingly analysed [1,2]. This primarily concerns bathing waters, the broadly understood cleanliness of which is of particular importance. To date, the most attention has been paid to yeasts and yeast-like fungi [3,4], and significantly less to mould fungi for which water is a transient environment. Mould fungi diaspores penetrate into these waters from the air bioaerosol, from the soil with allochthonous matter, and from the surfaces of leaves of plants surrounding water bodies [4].

The surface of leaves, referred to as the phyllosphere, is a specific ecological niche on which numerous bacteria and fungi of various taxonomic groups gather. They are directly and indirectly linked with morphological relationships

typical of biofilms, and trophic relationships resulting from common nutritional needs. Secretions of plants accumulated on the surfaces of leaves are most frequently polysaccharides most willingly used by saprotrophic and phytopathogenic fungi of various degrees of parasitism. Of the phytopathogens that are of significance to the etiology and epidemiology of fungal infections, and to toxicology and allergology, fungi of the *Fusarium* genus are becoming increasingly important.

Due to morphological and physiological variability of these fungi, they are extremely flexible and expansive. Even though they belong to polyphagous, facultative parasites of plants, they are capable of attacking the human body and exhibit affinity for the blood vessels. By growing into the vessel walls, they are a cause of embolisms, intravascular clots and tissue necroses [5,6]. Most frequently, they attack the skin, subcutaneous tissue and nails [7,8]. They are also capable of causing

Table 1. The number of fungal isolates of the *Fusarium* genus, obtained from the phyllosphere of plants of the littoral zone of Lakes Kortowskie and Skanda

Species of plant	Number/symbols of fungal isolates		Comments
	Lake Kortowskie	Lake Skanda	
<i>Typha latifolia</i> L.	<b>2:</b> K1a <sup>1</sup> , K1b <sup>2</sup>	<b>2:</b> S1a <sup>1</sup> , S2b <sup>1</sup>	macroconidia
<i>Phragmites australis</i> (Cav)Trin. Ex Steud	<b>11:</b> K1 <sup>1</sup> , K3a <sup>1</sup> , K1 <sup>2</sup> , K3 <sup>2</sup> , K6 <sup>2</sup> , K9 <sup>2</sup> , K1 <sup>3</sup> , K4b <sup>3</sup> , K5 <sup>3</sup> , K6 <sup>3</sup> , K7 <sup>3</sup>	<b>5:</b> S1b <sup>1</sup> , S7 <sup>2</sup> , S8a <sup>2</sup> , S10b <sup>2</sup> , S1b <sup>3</sup>	macroconidia and microconidia
<i>Nuphar lutea</i> (L.) Sibth. & Sm.	<b>1:</b> K8a	–	macroconidia
<i>Typha angustifolia</i> L.	–	<b>1:</b> S3b <sup>2</sup>	macroconidia
<i>Schoenoplectus lacustris</i> (L.) Palla	–	<b>1:</b> S2b	macroconidia
<i>Carex</i> sp. L.	<b>3:</b> K4 <sup>1</sup> , K1c <sup>2</sup> , K1b <sup>3</sup>	<b>2:</b> S3 <sup>3</sup> , S5b <sup>2</sup>	macroconidia
<i>Acorus calamus</i> L.	–	<b>4:</b> S5a <sup>2</sup> , S8c <sup>2</sup> , S4, S6 <sup>3</sup>	macroconidia
<i>Glyceria aquatica</i> (L.) Wahlenb.	<b>2:</b> K1a <sup>2</sup> , K3b <sup>2</sup>	<b>5:</b> S1 <sup>1</sup> , S2 <sup>1</sup> , S1 <sup>2</sup> , S9g <sup>2</sup> , S10a <sup>2</sup>	macroconidia
<i>Mentha aquatica</i> L.	–	<b>1:</b> S4b	macroconidia

Symbols of fungal isolates, example: K1a<sup>1</sup>- Lake Kortowskie, stations 1-10, plants a-g, ingathering 1-3, S1a<sup>1</sup>- Lake Skan

inflammatory conditions of the internal structures of the eye and joints. When they infect by the inhalation route, they locate in the lungs [9], but most frequently, the entry of infection is damaged skin [5,10]. In this context, the presence of fungi of the *Fusarium* genus in bathing waters takes on special significance.

The aim of the study was to determine the degree of colonisation of the phyllosphere of rush plants of selected bathing lakes by fungi of the *Fusarium* genus.

## Materials and Methods

The research materials were plants of the littoral zone of two bathing lakes, Lakes Skanda and Kortowskie, situated in the city of Olsztyn. Samples were taken three times during the year from 10 stations in late June and early July, in late July and early August, and in late August and early September, in accordance with the dates recommended in phytopathology. Fragments of one plant with visible lesions were assumed to be one sample.

In order to isolate fungi, moist chambers were prepared (Petri dishes with moist tissue paper). Sites with lesions were disinfected with 70% ethyl alcohol and fragments including these lesions were then cut out using sterilised scissors. They were placed in a moist chamber using sterile tweezers.

Macrocultures were incubated at room temperature for 48 hours. When fungal colonies emerged on the collected material, specimens were

prepared using Gerlach's method (aniline blue with lactophenol, sterile adhesive tape, glass slides and cover slips). Under sterile conditions, imprints of grown fungal colonies were made using an adhesive tape. Such material was transferred onto a glass slide, with the adhesive side up, a drop of a blue with lactophenol was applied and covered with a cover slip. The specimens were viewed under an OLYMPUS Bx4 type optical microscope. After the preparation of specimens, materials from moist chambers were dried and viewed under an OLYMPUS SZx9 type stereoscopic microscope in order to carry out a thorough analysis of the tested material. Genera of the fungi were identified based on morphological characteristics of the asexual and sexual stages. Identification keys by the following authors were used: Brandenburger [11], Braun [12], Braun [13], Ellis [14], Sałata [15] and Wołczańska [16]. The isolated fungi were starting material for further detailed research.

## Results

A total of 560 fungal isolates were isolated from the two lakes, of which 40 were fungi of the *Fusarium* genus (7.14%). 340 were isolated from Lake Skanda, and 220 from Lake Kortowskie: fungi of the *Fusarium* genus accounted for 21 and 19 isolates (6.18% and 8.64%), respectively (Table 1).

Most fungal isolates of the *Fusarium* genus were noted on *Phragmites australis* – 16 isolates were isolated from two bathing lakes. This was the only plant on which both micro- and macroconidia of

these fungi were observed under an optical microscope. However, more than half of them, i.e. 11 isolates were noted from Lake Kortowskie. Fungi of the *Fusarium* genus are more frequently noted from Lake Skanda stations than from Lake Kortowskie stations, as they were isolated from a greater number of plants.

## Discussion

The obtained results confirmed that the phyllosphere may be an interesting object of research to fungal disease epidemiologists due to the presence of phytopathogens capable of causing lesions, including in the human body. According to Richardson and Lass-Flörl [6], fungi of the *Fusarium* genus are among the most important etiological factors of hyalohyphomycoses caused by mould fungi living in the human environment and the species most frequently isolated from patients include *F. solani*, *F. moniliforme*, *F. oxysporum* and *F. proliferatum* [5,7,17,18].

Fungi of the *Fusarium* genus are facultative parasites of both cultivated and free-living plants and soil saprobionts developing on plant residues or other organic substrates. On the surfaces of the infected plants they form a well-developed mycelium whose sac-like clusters are either white or intensely yellow, light-brown or in shades of red and brown. All species produce sickle-shaped, multicellular macroconidia and bi- or single-cellular microconidia, and some of them also produce vegetative spores known as chlamydospores.

Most species of the *Fusarium* genus prefer temperate climates but are found in the biosphere of all climatic zones, from extreme Arctic areas and desert sands to green areas of tropical climate. They have physiological mechanisms allowing them to rapidly change their metabolism and reproduction systems, the consequence of which is perfect adaptation that is proven *inter alia* by their capacity for attacking human and animal bodies [10,19,20]. Fungi of the *Fusarium* genus primarily attack weakened bodies with decreased resistance and the risk of attack and development of a systemic fungal disease is directly proportional to the disturbances of the resistance. In extreme cases, fungemia or even death may occur [9,21]. Therefore, the presence of *Fusarium* mycelium and spores on coastal vegetation of the bathing lakes under study poses a potential risk to bathing area users of infections of the skin and its appendage,

inflammatory and proliferative conditions in the nasal cavities and sinuses as well as throughout the respiratory and gastrointestinal tracts [6,8,17]. Over the surface of a bathing lake, small and light *Fusarium* microconidia are dangerous – when inhaled by people with hypersensitivity to fungi, they are capable of causing allergic reactions and symptoms of bronchial asthma [20].

Fungi of the *Fusarium* genus produce very biochemically active mycotoxins and pigments of importance to enzymatic transformations (rubrofusarin, coumarin), which have a bacteriostatic effect. The latter remark appears to be particularly important in the context of inhibiting the development of bacteria that are natural microbiological shields of the skin which is most vulnerable to *Fusarium* infections.

*Fusarium* mycotoxins act in a variety of ways: they cause skin necroses, inhibit the synthesis of DNA and proteins, cause the “hemorrhagic syndrome” with symptoms including extravasations due to the damage to the capillary vessels and they cause the occurrence of the “vomitory syndrome” and a loss of appetite. Some of them stimulate the occurrence of the “estrogenic syndrome”, manifesting itself in infertility. Most *Fusarium* mycotoxins exhibit affinity for the bone marrow and cause alimentary toxic aleukia and panmyelophthisis with a set of symptoms resembling the initial phase of leukemia. Certain mycotoxins (e.g. fusarin) in model systems with bacteria (mainly with *Salmonella*) are characterised by strong mutagenic effects [22–24]. It should be stressed that mycotoxins penetrate into the body by the inhalation and oral routes and through the skin, and their subtoxic doses accumulate in the liver and other parenchymatous organs [5,6]. Considering the danger of mycotoxin poisoning and the highest number of *Fusarium* isolates being noted in the phyllosphere of *Phragmites australis*, it is recommended that toxicological analyses should be carried out on this plant community.

The microbiological purity of bathing waters underlies the measures aimed at the protection of health of the users of these waters by means of an assessment of sanitary and epidemiological safety, monitoring environmental conditions determining their cleanliness, and the indication of hazards associated with the presence of specific pathogens. These include fungi of the *Fusarium* genus, isolated from the phyllosphere i.e. a rarely investigated reservoir of fungi potentially pathogenic to humans.

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Received 10 May 2018

Accepted 10 July 2018