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MICROSCOPIC FUNGI OF *PHRAGMITES AUSTRALIS* IN THE LITTORAL OF TWO LAKES IN DRAWA NATIONAL PARK (NW POLAND)*

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Abstract. Microscopic fungi accompanying lesions of leaves, stalks, and leaf sheaths of *Phragmites australis* were surveyed within the littoral of Lake Marta and Lake Sitno (Drawa National Park, Poland) in 2005–2006. The fungi were isolated from affected tissues by methods used in phytopathology. In all, 72 microfungi and 1 *Mycelia sterilia* species were found in the phyllo- and caulosphere of *P. australis*. This biodiversity was represented mostly by species belonging to Hyphomycetes (38; 52% of species), Ascomycota (10; 14%), and Coelomycetes (9; 12%). Species of Basidiomycota and Zygomycota (3 each, 8% together) *Incertae sedis* (5; 7%), and fungi-like organisms (FLOs) (4; 6%) had smaller shares. The similarity coefficient for microfungal species in the littoral of the two lakes was 54%. The following species caused chlorotic or necrotic lesions on reeds of both lakes: *Alternaria alternata* (Fr.) Keissl., *Deightoniella arundinacea* (Corda) S. Hughes, *Fusarium sporotrichioides* Sherb., *Gibberella avenacea* R. J. Cook, *Gaeumannomyces graminis* (Sacc.) Arx & D. L. Olivier, *Phaeosphaeria eustoma* (Fuckel) L. Holm, *Puccinia magnusiana* Körn., *Pythium phragmitis* Nechw., *P. ultimum* Trow, *Septoriella phragmitis* Oudem., *Stagonospora elegans* (Berk.) Sacc. & Traverso, and *S. macropycnidia* Cunnell. The last two of those, as well as *Stagonospora cylindrica* Gunnell, *Phaeosphaeria eustoma* and *P. nodorum* (E. Müll.) Hedjar., as causes of *P. australis* leaf and stalk lesions, were confirmed for the first time in Poland.

Key words: *Phragmites australis*, pathogenic and saprotrophic fungi, biodiversity, mycobiota

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INTRODUCTION

Common reed, *Phragmites australis* Trin. *ex* Steud. (= *Phragmites communis* Trin.), the species defining reed-bed associations, creates numerous communities of the alliance *Phragmition* Koch W. 1926, commonly found in lowlands. As an ecologically adaptable plant it draws the attention of not only biologists. From a naturalist's point of view, reed phytocoenoses can be treated as nuisance weeds threatening bodies of water, but also as a habitat for a number of cyprinid (Ciprinidae), esocid (Esoxidae), or silurid (Siluridae) fishes living in lakes. For fishes like these, reed provides an excellent spawning substrate and a nursing ground for larvae and fry. For a number of years, reed has been used as a component of soil-hydrophyte sewage treatment plants, a fashionable material for roof construction, or a source of biomass (Granéli 1984). As its importance has grown, the quality requirements of reed as a material have risen. The main quality criterion is health, which is influenced by microorganisms associated with the plant. Microscopic mycobiota, that is, fungi and fungi-like organisms (FLOs) of the reed, include phytopathogens, facultative pathogens, or saprotrophic organisms, which help maintain the ecological steady state in all plant communities and enrich the biodiversity of ecosystems.

To date, phytopathological studies on *P. australis* in Poland have been sporadic and narrowly focused (Durska 1970; Mazurkiewicz-Zapałowicz *et al*. 2005, 2006). Considering that the state of knowledge on this subject is still incomplete and that the economic importance of reed is increasing,

* This paper is dedicated to Professor Tomasz Majewski on the occasion of his 70th birthday.

a study was undertaken in order to describe the biodiversity of the reed mycobiota that influence the health of the plant.

Materials and methods

I studied the common reed of the *Phragmition* communities encircling Lake Marta (53.18°N, 16.06°E) and Lake Sitno (53.18°N, 16.02°E) in Drawa National Park (DNP) in two consecutive growing seasons (2005, 2006). The material was collected each year at the same 5 sampling sites along the banks of each lake (Fig. 1). Samples were collected 4 times in each growing season (June–October) at phenological stages of plant development: ear formation, early flowering, full flowering, and full seed formation. A total of 80 plant samples were collected from each lake in this study.

The phytopathological samples were lesion-struck reed leaves, stalks and inflorescences, surface-disinfected (70% C_2H_5OH). Samples (3–5 cm long fragments) were incubated in humid chambers at 20±2°C for 2–14 days. Microorganisms occurring on chlorotic, necrotic or decomposing tissues were successively inoculated on agarose CDA, PDA, SA, and MEA media in Petri dishes. Passaging and single spore isolation was done according to standards adopted in phytopathology (Király *et al*. 1977; Waller *et al*. 1998). Some pathogens (obligatory pathogens) were identified directly from the herbarium material. My basic criterion for microscopic fungi identification was the manner of mitosporic spore or sporocarp formation, and their morphometrics. In this paper the term 'mycobiota' is used for both FLOs and fungi.

The following keys were used for identification of FLOs and fungi: Skirgiełło (1954), Ellis (1971), Barron (1972), Kochman and Majewski (1973), Majewski (1979), Sutton (1980), Brandenburger (1985), Borowska (1986), Kwaśna *et al.* (1991), Ellis and Ellis (1997), and Riethmüller (2000). Kirk's system (Kirk *et al*. 2008) was adopted as the basis for classification; author's epithets were verified according to *Index Fungorum* (http://www.indexfungorum.org/Names/Names.asp). The Department of Hydrobiology of the West Pomeranian University of Technology in Szczecin holds the documentation of the material, in the form of dried and described *P. australis* plants showing disease symptoms and etiological signs, as well as identified fungal isolates on agar slants in

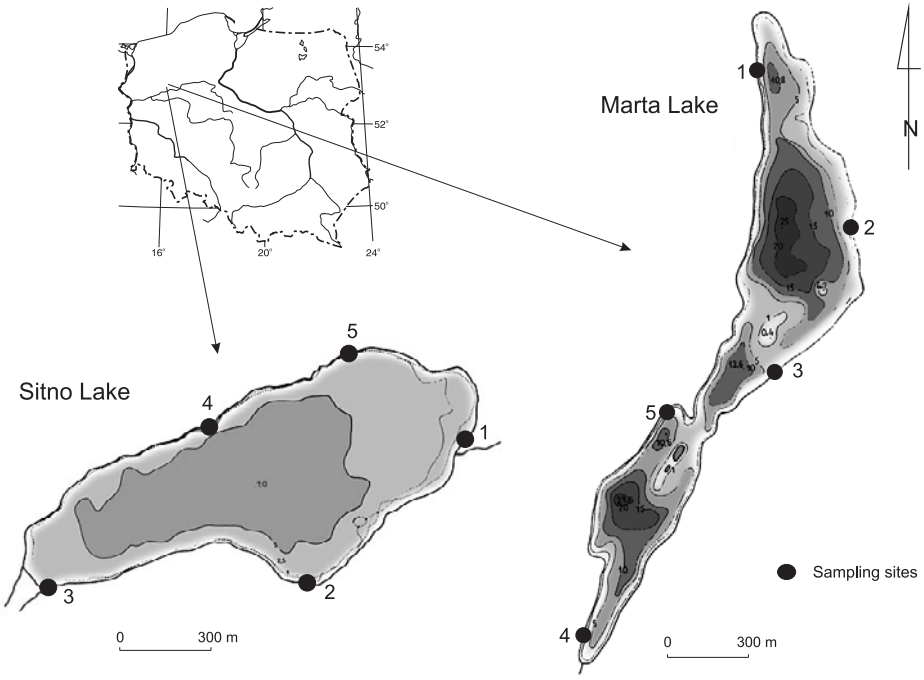

Fig. 1. Location of sampling sites with *Phragmites australis* in littoral associations of Lake Marta and Lake Sitno (Drawa National Park) in 2005–2006.

test tubes, and microscope slides with preserved fungi diagnostic forms. Most of the identified species are also archived as digital microscopy images.

In the next stage of analysis, the Jaccard index as modified by Sørensen (Krebs 1997) was calculated for the resulting data in order to estimate the between-lake similarity of microscopic fungal species on reed.

Results and discussion

The two-year survey on the banks of Lake Marta and Lake Sitno yielded a collection of 458 isolates of FLOs and fungi, representing 72 species in 44 genera, and 1 *Mycelia sterilia.* There were more species on reed at Lake Marta (61 taxa) than at Lake Sitno (38). Species richness at particular sampling sites ranged from 4 taxa (site 1 at Lake Sitno) to 38 taxa (site 4 at Lake Marta) (Table 1). The differences in number of species of the phyllo- and caulosphere probably are due to differences in habitat conditions between the lakes. The phytocoenoses of *P. australis* around Lake Marta form a narrow, rather loose littoral. At Lake Sitno the communities form a 2–3 times wider zone of thick, dense vegetation, which may have blocked migration of spores, especially those of polyphagous fungi, from adjacent bodies of water; this would be expected to reduce the species richness of the fungi.

The reed mycobiota were represented mostly by Hyphomycetes (38 species; 52% of total species), Ascomycota (10; 14%) and Coelomycetes (9; 12%). Basidiomycota and Zygomycota (3 each, 8% together), *Incertae sedis* (5; 7%), and FLOs (4; 6%) had smaller shares, below 10%. Irrespective of qualitative differences, the composition of particular fungal taxonomic groups on reed at the two lakes was similar: fungi of Hyphomycetes were dominant at both lakes (32 taxa at Lake Marta and 21 at Lake Sitno, in both cases more than half of all the isolated fungal species). Species of Ascomycota and Coelomycetes formed a smaller group, slightly more than 10% of the species (Fig. 2). Where reed grows in brackish water, Ascomycota and Coelomycetes species predominate (Van Ryckegem & Verbeken 2005a, b). This suggests that the microhabitat provided by the tissues of the host

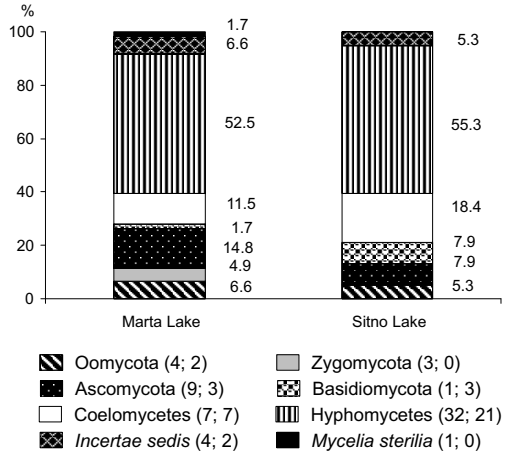

Fig. 2. Percentage share of microscopic fungi on *Phragmites australis* in littoral associations of Lake Marta and Lake Sitno in Drawa National Park. In parentheses: number of fungal species on *Phragmites australis* around Lake Marta and Lake Sitno.

plant (*P. australis*) differs between freshwater and brackish environments, and that largely determines the biodiversity of microorganisms inhabiting the phyllosphere and caulosphere of the plants. Domination of Ascomycota and Coelomycetes (Van Ryckegem & Verbeken 2005a, b) in brackish habitats implies that conidia and sporocarps can provide protection for these fungi, which are thus better adapted to the environment. Such conditions are more difficult to tolerate Hyphomycetes species, for which the direct impact of saline water on the microhabitat seems to be a limiting factor for sporulation.

The two lakes had 54% of their mycobiota species in common. The more important obligate and facultative phytopathogens found on the reeds of both lakes included *Alternaria alternata* (Fr.) Keissl., *Deightoniella arundinacea* (Corda) S. Hughes, *Fusarium sporotrichioides* Sherb., *Gibberella avenacea* R. J. Cook, *Gaeumannomyces graminis* (Sacc.) Arx & D. L. Olivier, *Phaeosphaeria eustoma* (Fuckel) L. Holm, *Puccinia magnusiana* Körn., *Pythium phragmitis* Nechw., *P. ultimum* Trow, *Septoriella phragmitis* Oudem., *Stagonospora elegans* (Berk.) Sacc. & Traverso, and *S. macropycnidia* Cunnell (Table 1). The last two species, as well as *S. cylindrica* Cunnell,

Table 1. Diversity of microscopic fungi on *Phragmites australis* in littoral associations of Lake Marta and Lake Sitno (Drawa National Park) in 2005–2006; occurrence on leaves (L), leaf sheaths (P) and stalks (S).

No.	Taxa	Marta Lake					Sitno Lake				
		Stand									
		1	2	3	4	5	1	2	3	4	5
1	*Achlya racemosa* Hildebr.			L							
2	*Acremoniella atra* (Corda) Sacc.									L	
3	*Alternaria alternata* (Fr.) Keissl.	L	L	L,P,S	L,P,S	L,S	L	L,S	S	L,P,S	L,P
4	*Alternaria* sp.					L					
5	*Alternaria tenuissima* (Kunze) Wiltshire				L,P						
6	*Apodachlya pyrifera* Zopf			L	L,P						
7	*Arthrinium phaeospermum* (Corda) M. B. Ellis	L		L	L,P,S			L,S	L,S	L	
8	*Arthrinium* sp.	L	L								
9	*Aspergillus niger* Tiegh.			L							
10	*Aspergillus* sp.		L								
11	*Bipolaris pedicellata* (A. W. Henry) Shoemaker					L	L				
12	*Botryotrichum piluliferum* Sacc. & Marchal				L				L		
13	*Cephalosporium* sp.				L,S		L	S			
14	*Chaetomium elatum* Kunze				L,S			L			
15	*Chaetomium globosum* Kunze			L	L,P						
16	*Chaetomium* sp.					L,P					
17	*Chalaropsis* sp.		L		L,P						
18	*Chloridium chlamydosporum* (J. F. H. Beyma) S. Hughes								S		
19	*Chloridium* sp.							L			
20	*Cladosporium cladosporioides* (Fresen.) de Vries	L	L		S	L					S
21	*Cladosporium herbarum* Pers. Link	L			L,P	L				L,P	
22	*Clonostachys rosea* f. *rosea* (Link) Schroers, Samuels, Seifert & W. Gams				S	S					S
23	*Deightoniella arundinacea* (Corda) S. Hughes	L				L		L		L	
24	*Dictyosporium elegans* Corda			L	L						
25	*Epicoccum nigrum* Link	L			L,P			L	S		L
26	*Fusarium incarnatum* (Desm.) Sacc.								Z		
27	*Fusarium oxysporum* Schltdl.		Z								
28	*Fusarium poae* (Peck) Wollenw.								L		
29	*Fusarium solani* (Mart.) Sacc.										L
30	*Fusarium* sp.		L							L	
31	*Fusarium sporotrichioides* Sherb.			L	L,P		L	L,S	L,S		
32	*Gaeumannomyces graminis* var. *graminis* (Sacc.) Arx & Olivier				S				S		
33	*Giberella avenacea* R. J. Cook			L				S			
34	*Gliomastix luzulae* (Fuckel) Mason *ex* S. Hughes	L	L	L							
35	*Helminthosporium* sp.				L,S						
36	*Hendersonia culmiseda* Sacc.			L	L,P						
37	*Hendersonia phragmitis* Desm.	L		L	L,P						L
38	*Humicola fuscoatra* Traaen		L		L						
39	*Humicola grisea* Traaen				L						
40	*Hypomyces chrysospermus* Tul. & C. Tul.					L					
41	*Lophiostoma semiliberum* (Desm.) Ces. & De Not.		S		L						
42	*Lophiostoma* sp.					L					
43	*Microascus brevicaulis* S. P. Abbott		L								
44	*Mucor hiemalis* Wehmer				L						

Table 1. *Continued.*

No.	Taxa	1	2	3	4	5	1	2	3	4	5
45	*Mucor* sp.		L,S								
46	*Mycelia sterilia*					S					
47	*Myrothecium masonii* Tulloch				L,P						
48	*Myrothecium roridum* Tode				L,P						
49	*Papulaspora byssina* Hotson				L						
50	*Papulaspora sepedonioides* Preuss.					P					
51	*Penicillium* sp.			L							
52	*Periconia hispidula* (Pers.) Mason & M. B. Ellis				S			L,S		L	
53	*Periconia minutissima* Corda				L,P			L			
54	*Phaeosphaeria eustoma* (Fuckel) L. Holm				L			L			
55	*Phaeosphaeria nodorum* (E. Müll.) Hedjar.							L	L,S		
56	*Phoma arundinacea* (Berk.) Sacc.										L
57	*Puccinia coronata* Corda										L
58	*Puccinia magnusiana* Körn.			L					L		
59	*Puccinia phragmitis* (Schumach.) Körn.									L	
60	*Pythium phragmitis* Nechw.				L,S					L	
61	*Pythium ultimum* Trow			L,P,S	L,P,Z			L,P,S			
62	*Rhizopus stolonifer* (Ehrenb.) Vuill.		L, S								
63	*Rutola graminis* (Desm.) Crane & Schokn.	L	L		S	L		L,S	S		
64	*Scirrhia rimosa* (Alb. & Schwein.) Fuckel										L,S
65	*Septonema secedens* Corda		L								
66	*Septoriella phragmitis* Oud.	L		L,P,S	L,S			L,S	L,S		
67	*Stagonospora cylindrica* Cunnel.				L,P						
68	*Stagonospora elegans* (Berk.) Sacc. & Traverso	L	L	L	L						L,P
69	*Stagonospora macropycnidia* Cunnell.	S	S	L,P,S				L,S	L,S		
70	*Trichothecium roseum* (Pers.) Link			L	L,P			S			
71	*Ulocladium atrum* Preuss				S						
72	*Ulocladium botrytis* Preuss				L,P						
73	*Ulocladium chartarum* (Preuss) Simmons							L,S		L,S	
	Total	13	16	19	38	13	4	19	14	10	10

Phaeosphaeria eustoma (Fuckel) L. Holm and *P. nodorum* (E. Müll.) Hedjar., deserve more attention, since here they are confirmed for the first time as the factors causing leaf and stalk chloroses and necroses in *P. australis* in Poland. The great diversity of *P. australis* fungi found in DNP is surprising when compared with the findings from other studies done in Poland in the past. The reason for the difference is that previous reports on reed-associated fungi primarily concerned parasitic species. In the Masurian Lake District *P. australis* was found to be a host organism for 10 phytopathogens (Durska 1974). In the Łęczna-Włodawa Lake District, 6 parasitic fungi were found on reed (Mułenko 1989a). These include *Puccinia magnusiana*, *P. phragmitis* (Schumach.) Körn., *Claviceps purpurea* (Fr.) Tul., *Scirrhia rimosa* (Alb. & Schwein.) Fuckel, *Coniosporium arundinis* (Corda) Sacc. (current name *Apiospora montagnei* Sacc.), *Hadrotrichum phragmitis* Fuckel (current name *Scirrhia rimosa*) and *Ustilago grandis* Fr. (Mułenko 1989a, b). More recent reports on reed from Słowiński National Park list five phytopathogens: *Puccinia magnusiana*, *P. phragmitis*, *P. coronata* Corda, *Ustilago grandis* and *Cladosporium cladosporioides* (Fresen.) G. A. de Vries (Adamska *et al.* 1999; Adamska & Błaszkowski 2000; Adamska 2001). The most recent studies carried out in West Pomerania Province cover the general phytosanitary condition of *P. australis*, thus better characterizing the biodiversity of microfungi associated with this plant. There were 31

FLOs and fungi found on reed around Lake Glinno (Mazurkiewicz-Zapałowicz *et al.* 2005), as compared with the 72 species and 1 *Mycelia sterilia* I found in DNP – more than twice as many. This biodiversity is much richer than that reported for reed fungi in England (18 taxa; Apinis *et al.* 1972). One possible explanation of the discrepancy is that the taxa were most often identified at the genus level only. The differences exist despite the use of similar methods involving isolation of fungi from lesion-afflicted plant samples collected in various periods through the growing season. This method not only better characterizes fungal biodiversity at each stage of tissue damage but also enables a preliminary evaluation of their pathogenicity. Among the obligatory phytopathogens, only *Puccinia magnusiana*, one of the factors causing rust in reed, was found in England (Apinis *et al.* 1972), Hong Kong (Poon & Hyde 1998a, b), Hungary (Bán *et al.* 1996, 2002) and Poland (Mułenko 1989a, b; Adamska *et al.* 1999; Adamska 2001). The species was also found in reed beds surrounding both studied lakes in DNP (Table 1). According to Majewski (1979), *P. magnusiana* develops its aecidial stage on various buttercups (*Ranunculus acris* L., *R. bulbosus* L., *R. flamula* L., *R. linqua* L., and *R. repens* L.); this has not been definitely confirmed. Reed rust in Słowiński National Park is also caused by two other *Puccinia* species, *P. coronata* and *P. phragmitis* (Adamska *et al*. 1999; Adamska 2001); this was also confirmed in the studied area of DNP, and may indicate that these pathogens have spread across the entire region of West Pomerania. Majewski (1979) suggested such a scenario for *P. phragmitis* but did not name the reed as host plant for *Puccinia coronata*, although this species has already been reported in Poland (Mułenko *et al*. 2008). The distribution of various rust-causing fungi in Poland is uneven, as confirmed by research by Mułenko (1989a, b), who did not observe *P. coronata* in the Łęczna-Włodawa Lake District. On the other hand, reed rust caused by *P. minutissima* or *P. phragmitis* has been reported from various places in the Masurian Lake District and West Pomerania (Durska 1970, 1974; Adamska & Błaszkowski 2000; Mazurkiewicz-Zapałowicz *et al.* 2005, 2006). The distribution range of *Puccinia magnusiana* and *P. phragmitis* seems confined to continental Europe; these reed-leaf specialized parasites have not been found on the British Isles yet (Taligoola *et al.* 1972; Apinis *et al.* 1972). That points to the regional importance of each pathogen, as well as the impact of the environmental and climatic conditions that determine outbreaks of fungal infections of varied etiology. For instance, in Hong Kong, where reeds coexist with mangrove vegetation of saltwater lagoons, dying leaves revealed other fungal species such as *Halosarpheia phragmiticola* Poon & H. D. Hyde, *Lignincola laevis* Höhnk, *Massarina phragmiticola* Poon & K. D. Hyde, *M. thalassiae* Kohlm. & Volkm.-Kohlm., *Nectria haematococca* Berk. & Broome [= *Haematonectria haematococca* (Berk. & Broome) Samuels & Rossman], *Phomatospora phragmiticola* Poon & K. D. Hyde, *Phragmitensis marina* M. K. M. Wong, Poon & K. D. Hyde, *Pleospora spartinae* (J. Webster & M. T. Lucas) Apinis & Chesters, *Pseudohalonectria falcata* Shearer, *Verruculina enalia* (Kohlm.) Kohlm. & Volkm.-Kohlm., *Zopfiella latipes* (N. Lundq.) Malloch & Cain, and *Cytoplacosphaeria phragmiticola* Poon & K. D. Hyde (Poon & Hyde 1998a). On the banks of Lake Balaton, a linear dependence was found between summer season temperatures and reed infection by *Puccinia magnusiana, Polythrinciopsis phragmitis* J. Walker, and *Stagonospora* sp. (Bán *et al.* 1996, 2002) A similar relationship was described between seasonal rainfall intensity and reed fungal infection by *Deightoniella arundinacea* (Corda) Hughes, *Puccinia phragmitis*, and *Deightoniella roumeguerei* (Cavara) Constant. (Bán *et al.* 1996, 2002). One may infer that specific etiological factors arise in each of the various climates, which at the optimal level of development determine the health status of plants. The activity of these etiological factors probably is also modified by the genetically-based susceptibility of common reed (Krzakowa & Judek 2009).

Apart from rust, the leaves and stems of reed are often covered by lesions caused by various etiological complexes. This study of reed growing on the littoral of Lake Marta and Lake Sitno in DNP, as well as research done around lake Glinno

(Mazurkiewicz-Zapałowicz *et al.* 2005), indicate that the diseases are caused primarily by *Stagonospora* and *Phaeosphaeria* species. In Poland these fungal genera can for the first time be confirmed as causes underlying chloroses and necroses of *P. australis* leaves. This observation is in accord with findings on the fungi of emerged and floating parts of reed in England, where *Leptosphaeria culmifraga* (Fr.) Ces. & De Not. and *Stagonospora elegans* (Berk.) Sacc. & Traverso predominated among the pathogenic factors causing lesions on the plants (Apinis *et al.* 1972; Taligoola *et al.* 1972). *Stagonospora* species are among the reed pathogens most often isolated, which cause physiological disorders leading to early mortality of the plants and thereby reduce the harvested biomass (Ernst *et al.* 2003). Those processes usually accelerate during further stages of succession by a range of species colonizing the submerged parts of the plants. Besides the discussed taxa, Taligoola *et al*. (1972) and Apinis *et al.* (1972) mention *A. alternata*, *Hendersonia* spp., and *Fusarium* spp., which I also found on reed (Table 1). The *Fusarium* species often isolated from stem and leaf lesions included *F. incarnatum* (Desm.) Sacc., *F. oxysporum* Schltdl., *F. poae* (Peck) Wollenw., *F. solani* (Mart.) Sacc., and *F. sporotrichioides*, as well as *Gibberella avenacea*. The *Fusarium* fungi are among the most expansive facultative phytopathogens (Kwaśna *et al.* 1991); in conditions that weaken the plants they can become the primary disease factors. The results of this survey indicate that fungi such as species of *Cephalosporium*, *Epicoccum*, *Alternaria* or *Hendersonia* also cause chlorotic and necrotic lesions to reed in DNP. The phytopathogenicity of these species was indicated decades ago (Apinis *et al.* 1972). This raises the status of these types of fungi as co-factors responsible for the health status of the reed. The heterogeneity of the observed symptoms of the disease justifies the continuation of studies on the taxonomic diversity of fungi occurring on diseased tissues. Extending these studies to successive growing seasons should confirm the already demonstrated differences in the associations of microorganisms colonizing *P. australis* and should also help identify trends in the dynamics of micromycete species succession on the studied plant parts. There have been similar studies of the diversity of micromycete associations and their succession, investigating vertically spaced microhabitats of *P. australis* leaves and stalks (Van Ryckegem & Verbeken 2005a, b).

Among pathogenic FLOs, two water molds (Oomycetes) found on reed in the presented study, *Pythium phragmitis* Nechw. and *P. ultimum* Trow, deserve particular attention (Table 1). Like other *Pythium* species, these are typically soil-borne, often polyphagous pathogens, widely distributed globally and very important economically. *Pythium phragmitis* isolated from reed growing on the shore of Lake Constance (Germany) by Nechwatal *et al.* (2005) is a pathogen much more aggressive on reed leaves and seedlings than any other species closely related to it. Its occurrence in areas with high ground water facilitates the spread of the pathogen's oospores, making it a very expansive species, as has been demonstrated here. This is the first observation of *P. phragmitis* in Poland, a warning signal about the possible health risk to reed communities. The pathogen causes decomposition of the host plant's roots, which in consequence leads to nutritional dysfunction and rapid death of the plant. Such effects in the muddy deposit layer of a lake accelerate decay processes in the rhizosphere through increased deposition of plant remains.

Expansion of the scope of the research to include the physiological properties of reed-associated fungi should yield a better understanding of the significance of these organisms, not only to the health status of plants but also to ecosystem functioning.

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