Aquatic zoosporic fungi from baited spores of cryptogams

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Zoosporic fungi on spore baits of 24 taxa of cryptogams from three water bodies were investigated. A total of 61 zoosporic fungal taxa were noted, with predominance of the *Chytridiales* (27) and *Peronosporales* (14) species. The largest number of species (46) was recorded in the stagnant water at Dojlidy pond, with fewer (31) from Supraśl River and 34 species from Jaroszówka spring. Five species of *Pythium* were recorded for the first time from Poland.

Key words: aquatic fungi, hydrochemistry, spores, zoosporic fungi.

Introduction

According to Sparrow (1968), Karling (1977), Czeczuga and Muszyńska (2001), and Czeczuga and Orłowska (2001), the sporomorphs of land plants are an attractive substratum for the growth of certain aquatic fungi. Until now, aquatic fungi have mostly been investigated by using the pollen of various tree species as baits. Only three fungal species have been reported from spores of ferns (Batko, 1975).

The aim of our present study is to evaluate the suitability of spores of certain plant species as baits for the growth of aquatic fungus species.

Materials and methods

Twenty-three sporulating cryptogam species collected in the Knyszyńska Forest (N. Poland) and *Selaginella selaginoides* (L.) received from V. Cogt, Mongolia (Table 1) were used in this study. Mature fruiting bodies of macromycetes and a fragment of the spore producing part of the plant (a part of sporophyte in moss, clubmoss and horsetail, and a leaf from sporangium in pteridophytes) were taken to the laboratory, placed in paper and after a few hours spores were collected for study in test tubes.

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Table 1. Cryptogamic spores investigated and fungi found.

Taxa		Fungi (seeTable3)	No. taxa
Mycota			
1	*Amanita muscaria* (L. ex Fr.) Pers.	22,40,43,45,61	5
2	*Calvatia excipuliformis* (Pers.) Perd.	17,22,23,24,32,36,43	7
3	*Langermannia gigantea* (Batsch) Rostk.	1,2,5,8,14,25,27,31,35,56,58	11
4	*Paxillus involutus* (Batsch ex Fr.) Fr.	22,24,43,47	4
5	*Pleurotus ostreatus* (Jac:Fr.) Kummer	4,22,23,32,36,43	6
Bryophyta			
6	*Polytrichum attenuatum* Menz.	2,5,8,9,11,16,18,30,31,35,37,51,53, 57,60	15
7	*Polytrichum commune* L.	1,2,5,9,11,19,30,35,40,55,57,58	12
Pteridophyta			
Lycopediaceae			
8	*Lycopodium annotinum* L.	3,7,22,27,29,32,57	7
9	*Lycopodium clavatum* L.	7,15,19,22,23,27,29,32,59	9
10	*Lycopodium complanatum* L.	1,2,5,8,9,11,18,31,35,55	10
11	*Lycopodium selago* L.	2,22,27,29,32,35,55	7
12	*Selaginella selaginoides* (L.)	2,8,22,24,34,35,48	7
Equisetaceae			
13	*Equisetum arvense* L.	11,22,24,27,29,32,36,38,40,4143,46	12
14	*Equisetum hyemale* L.	2,5,9,11,15,27,30,35,37,54,55,58	12
15	*Equisetum limosum* L.	2,8,11,27,35,37,39,42,44,52,54,55	12
16	*Equisetum palustre* L.	1,2,5,8,11,21,35,37,40,54,56	11
17	*Equisetum pratense* Ehrh.	1,2,5,11,29,35,37,40,49,50,58	11
18	*Equisetum sylvaticum* L.	4,5,8,11,18,19,23,25,26,31,35,49,55	13
Polypodiaceae			
19	*Dryopteris filix-femina* (L.) Roth.	2,6,11,22,29,55	6
20	*Dryopteris filix-mas* (L.) Schott	3,10,13,28,29,30,59	7
21	*Dryopteris spinulosa* Watt.	2,11,19,20,29,30,35,55,57	9
22	*Phegopteris dryopteris* (L.) Fée	6,11,12,22,29,32,36,43,61	9
23	*Pteridium aquilinum* (L.) Kahn	3,14,22,29,32,35,47	7
24	*Thelipteris palustris* Schott	2,5,8,11,16,29,33,35,37,40,42,44	12

The water for experiments was collected from different water body types at three locations:

1. Jaroszówka Spring, limnokrenic type, width 0.65 m, depth 0.12 m, discharge 2.4 l/s, (northern part of Białystok).

2. Supraśl River, length 106.6 km, on the right-bank tributary of the Narew river, flowing through the Knyszyńska Forest

3. Dojlidy Pond, area 34.2 ha, max. depth 2.85 m., its south shores border with coniferous woods while its western part borders with the town of Białystok and this part of pond is used as a beach.

Table 2. Chemical composition (in mg l^{-1}) of water (the mean of three sites in water body).

Specification	Jaroszówka Spring	Supraśl River	Dojlidy Pond
Temperature (C)	6.2	13	13.2
pH	7.45	7.5	6.82
O_2	8.4	11.2	11.2
BOD_5	2.2	4.8	5.2
COD	7.3	13.58	7.3
CO_2	17.6	8.8	11
Alkalinity in $CaCO_3$ (mval l^{-1})	4.4	3.7	0.8
N-NH_3	0.61	0.07	0
N-NO_2	0.01	0.035	0.026
N-NO_3	0.08	0.08	0.07
P-PO_4	3.5	1.35	0.07
Sulphates	70.76	28.21	4.11
Chlorides	33	43	36
Total hardness in Ca	90.00	67.68	19.44
Total hardness in Mg	15.3	13.76	0
Fe	0.3	0.25	0
Dry residue	398	208	48
Dissolved solids	396	155	32
Suspended solids	2	49	16

Nineteen water parameters were determined at the above sampling sites (Table 2) following the methods of Greenberg *et al*. (1992).

For the determination of the presence of aquatic fungal species in the water samples, the following procedure was employed: 1000-2000 spores of each plant species were transferred in two water samples (800 ml) representing each site in an 1 L vessel (all together six vessels for each plant species) and placed in the laboratory at the ambient temperature between 12º to 14 C. Some spores (50-100) from each vessel were transferred to a glass slide with a micropipette and observed under a microscope. Fungal structures (zoospores, antheridia and oogonia) and spores were recorded. General methods are detailed by Sparrow (1960) and Seymour and Fuller (1987). The spores of the various plant species were observed under a microscope every 3-4days. The size of the fungal structures was measured using light-microscopy at 600×. The duration of the experiments was three weeks. The following keys aided identification of the fungi: Johnson (1956), Sparrow (1960), Waterhouse (1968), Seymour (1970), Batko (1975), Dick (1990) and Pystina (1998).

Colonies of some species of *Pythium* originated from pure cultures. To obtain a single species culture, a hyphal tuft was taken from the gross culture of spores of cryptogams, the tuft was washed several times with distilled and sterilized water, and plated onto cornmeal agar (CMA). Autoclaved hemp seed halves were used as bait (Seymour and Fuller, 1987). Germinating single-spores culture were taken with a sterilized needle together with a piece of agar and transferred to Petri-dishes containing sterilized distilled water and incubated at 20 C for 7-10 days. Repeating this procedure resulted in bacteria-free cultures.

The water chemistry data and the aquatic fungal communities of these investigations were determined by cluster analysis (McGarigal *et al*., 2000). The method of group combination was applied to assess the correlation between phisico-chemical parameters and species numbers. The method based on the similarity of parameters expressed by means of Euclidean distance. Smaller distances correlated with a higher the association between the parameters.

Results

Chemical analysis of water used for the experiment has revealed differences in water trophic states among the sampling places. The waters of Supraśl River had the most abundant in biogenes, followed by the Jaroszówka Spring and Dojlidy pond being the poorest (Table 1).

Sixty-one zoosporic fungal taxa belonging to seven orders were identified from the spores of 24 cryptogamic plants baited in water of the three different water bodies (Table 2, 3, Fig. 1). The most numerous were the *Chytridiales* (27 species) and followed by the *Peronosporales* (14). The most common species were: *Olpidium longicollum, Karlingia rosea, Rhizophydium carpophilum, R. sphaerotheca, Myzocytium microsporum* and *Pythium periplocum*.

The least species were found on the spores of *Amanita muscaria* (5), while the greatest number were found on the spores of moss *Polytrichum attenuatum* (15 species).

The largest number of fungal taxa (46) occurred on cryptogam spores in the stagnant water in the Dojlidy pond, there were fewer in the running waters of the Supraśl River (31) and the Jaroszówka Spring (34) (Table 4). A relatively large number of *Pythium* species (13) were observed, of which five appear new to Polish waters, i.e. *P. arrhenomanes, P. marsipium*, *P. megalacanthum, P. salinum* and *P. vexans*.

Cluster analysis correlating water body parameters to zoosporic taxa revealed that in Jaroszówka Spring the number of taxa was closely associated with the concentration of chlorides, in Supraśl River with sulphates and in Dojlidy pond with dry residue.

Table 3. Aquatic fungi found on the spores of particular cryptogam species.

	Taxa	Species of cryptogams (see Table 2)	No. of species
	Chytridiomycetes		
	Olpidiales		
1	*Olpidium appendiculatum* Karling	3,7,10,16,17	5
2	*Olpidium longicollum* Uebelmesser	3,6,7,10,11,12,14,15,16,17,19, 21,24	13
3	*Olpidium pendulum* Zopf	8,20,23	3
4	*Synchytrium macrosporum* Karling	18	1
	Chytridiales		
5	*Blyttiomyces helicus* Sparrow	3,6,7,10,14,16,17,18,24	9
6	*Chytridium xylophilum* Cornu	19,22	2
7	*Chytriomyces mammilifer* Persiel	8,9	2
8	*Chytriomyces poculatus* Willoughby & Townley	3,6,10,12,15,16,18,24	8
9	*Chytriomyces vallesiacus* Persiel	6,7,10,14	4
10	*Entophlyctis rhizina* (Schenk) Minden	20	1
11	*Karlingia rosea* (de Bary & Woronin) Johanson	6,7,10,13,14,15,16,17,18,19, 21,22,24	13
12	*Nowakowskiella elegans* (Nowakowski) Schröeter	22	1
13	*Nowakowskiella macrospora* Karling	20	1
14	*Phlyctochytrium biporosum* Couch	3,5,23	3
15	*Phlyctochytrium circulidentatum* Umphlett	9,14	2
16	*Phlyctochytrium furcatum* Sparrow	6,24	2
17	*Phlyctochytrium hydrodictyi* (Braun) Schröeter	2	1
18	*Phlyctochytrium indicum* Karling	6,10,18	3
19	*Phlyctochytrium papillatum* Sparrow	7,9,18,21	4
20	*Phlyctochytrium reinboldtiae* Persiel	21	1
21	*Rhizophydium bullatum* Sparrow	16	1
22	*Rhizophydium carpophilum* (Zopf) Fischer	1,2,4,5,8,9,11,12,13,19,22,23	12
23	*Rhizophydium globosum* (Braun) Rabenhorst	2,5,9,18	4
24	*Rhizophydium keratinophilum* Karling	2,4,12,13	4
25	*Rhizophydium laterale* (Braun) Rabenhorst	3,18	2
26	*Rhizophydium macrosporum* Karling	18	1
27	*Rhizophydium pollinis-pini* (Braun) Zopf	3,8,9,11,13,14,15	7
28	*Rhizophydium polysiphoniae* (Cohn) Peters	20	1
29	*Rhizophydium sphaerotheca* Zopf	8,9,11,13,17,19,20,21,22,23,24	11
30	*Rhizophydium subangulosum* (Braun) Rabenhorst	6,7,14,20,21	5
31	*Rhizophydium utriculare* Uebelmesser	3,6,10,18	4
	Hyphochytriomycetes		
	Hyphochytriales		
32	*Rhizidiomyces apophysatus* Zopf	2,5,8,9,11,13,22,23	8
33	*Rhizidiomyces hirsutus* Karling	24	1
	Oomycetes		
	Lagenidiales		
34	*Lagenidium pygmaeum* Zopf	12	1

Table 3 continued. Aquatic fungi found on the spores of particular cryptogam species.

	Taxa	Species of cryptogams (see Table 2)	No. of species
35	*Myzocytium microsporum* (Karling) Sparrow	3,6,7,10,11,12,14,15,16,17, 18,21,23,24	14
36	*Olpidiopsis saprolegniae* (Braun) Cornu	2,5,13,22	4
	Saprolegniale**s**		
37	*Achlya colorata* Pringsheim	6,14,15,16,17,24	6
38	*Achlya debaryana* Humphrey	13	1
39	*Aphanomyces amphigynus* Cutter	15	1
40	*Aphanomyces irregularis* Scott	1,7,13,16,17,24	6
41	*Aphanomyces parasiticus* Coker	13	1
42	*Aplanes androgynus* (Archer) Humphrey	15,24	2
43	*Saprolegnia ferax* (Gruith.)Thuret	1,2,4,5,13,22	6
44	*Saprolegnia mixta* de Bary	15,24	2
45	*Saprolegnia monoica* Pringsheim	1	1
	Leptomitales		
46	*Leptomitus lacteus* (Rolh) Agardh	13	1
	Peronosporales		
47	*Pythiogeton nigrescens* A. Batko	4,23	2
48	*Pythium aquatile* Höhnk	12	1
49	*Pythium aristosporum* Vanterpool	17,18	2
50	*Pythium arrhenomanes* Drechsler	17	1
51	*Pythium hydnosporum* Schröter	6	1
52	*Pythium intermedium* de Bary	15	1
53	*Pythium marsipium* Drechsler	6	1
54	*Pythium megalacanthum* de Bary	14,15,16	3
55	*Pythium periplocum* Drechsler	3,7,10,11,14,15,18,19,21	9
56	*Pythium rostratum* Butler	16	1
57	*Pythium salinum* Höhnk	6,7,8,21	4
58	*Pythium sylvaticum* Campbell & Hendrix	3,7,14,17	4
59	*Pythium ultimum* Trow	9,20	2
60	*Pythium vexans* de Bary	6	1
	Zygomycetes		
	Zoopagales		
61	*Zoophagus insidians* Sommerstorff	1,22	2

Discussion

The chemical analysis of water revealed that in Dojlidy pond where the largest number of zoosporic fungal taxa occurred, there is a lower concentration of nitrates and phosphates. We observed a similar phenomenon when studying gymnosperm pollen (Czeczuga and Muszyńska, 2001). It is likely that higher concentrations of these compounds in water limit the growth of certain zoosporic fungus species (Czeczuga and Mazalska, 2000; Czeczuga

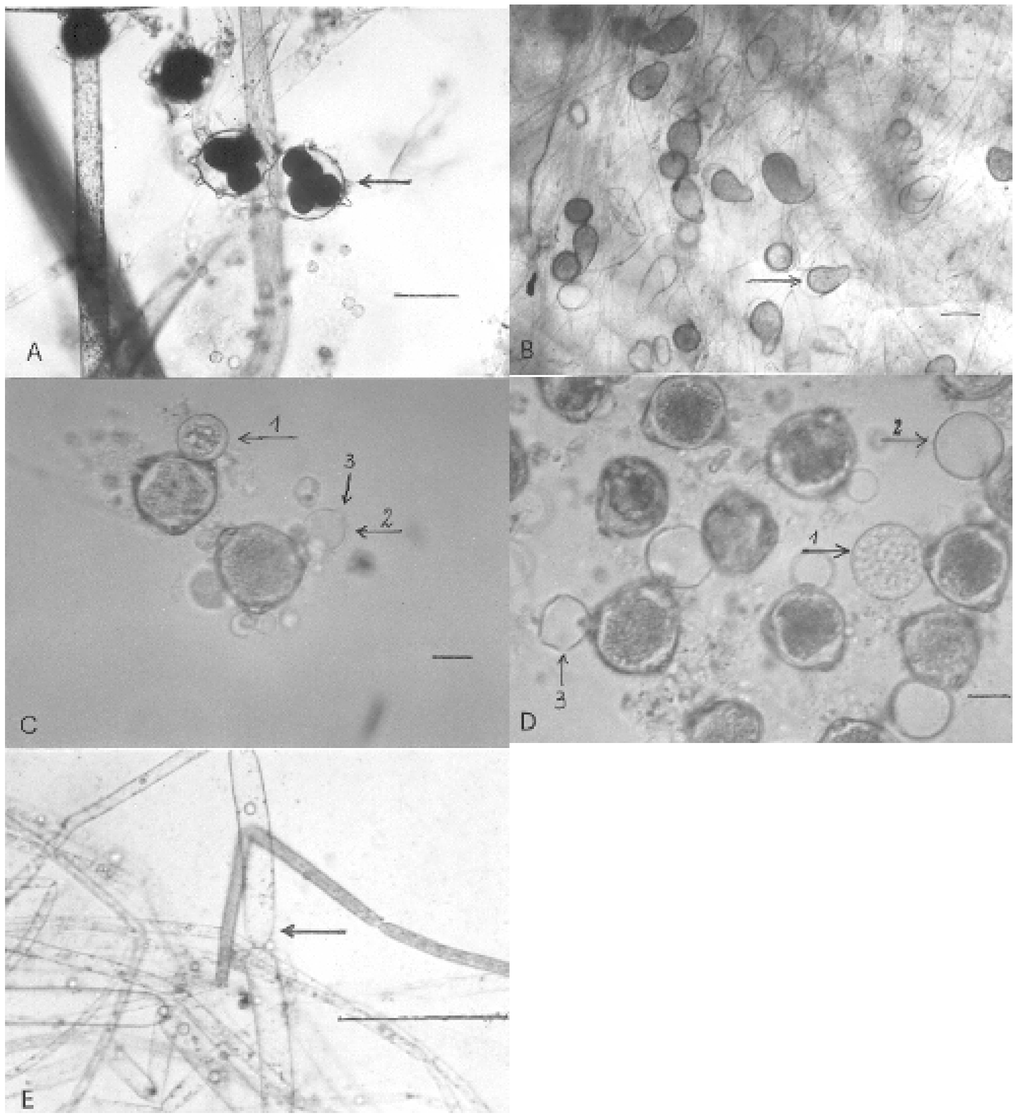

Fig. 1. Some aquatic fungi growing on the spores of cryptogam representatives. **A.** *Achlya colorata* - hyphae from oogonia (30-110 µm) with characteristic papillas and thorny outgrowths. **B.** *Nowakowskiella elegans* - hyphae from sporangia (14-42 µm); the sporangia formed are spherical, oval, rarely elongated, pearshaped, frequently with apophase, sometimes with tubular outlet. **C.** *Rhizophydium globosum* - mature (1) and empty (2) sporangia (21-45 µm); sporangia are regularly spherical, thick-walled, with 2-4 distinct papillas (3) in the upper part. **D.** *Rhizophydium sphaerotheca* - mature (1) and empty (2) sporangia (34-50 µm); sporangia are regularly spherical, unlike *R. globosum*, thin-walled, with 1, larger with 2-5 distinct papillas (3). **E.** *Leptomitus lacteus* - vegetative hyphae (40 × 300 µm); tangled mass of hyphae with numerous characteristic narrowings and cellulin rootlets. Bars = 50 µm.

Table 4. Aquatic fungi found on the spores in the different water.

Site	Fungi (see Table 3)	Occurred restrictedly (only in one water)	No. of taxa
Jaroszówka Spring	1,2,3,5,8,9,11,13,14,16,18,19,22,23, 24,27,29,30,31,32,35,36,40,43,49,50, 51,52,56,57,58,59,60,61	13,49,50,56,60	34
Supraśl River	2,3,4,5,6,8,11,12,22,23,24,25,26,29, 30,32,33,35,37,39,40,42,44,45,47,48, 52,53,55,59,61	4,6,26,33,39,45,48,55	31
Dojlidy Pond	1,2,3,5,7,8,9,10,11,12,14,15,16,17,18, 19,20,21,22,23,24,25,27,28,29,30,31, 32,34,35,36,37,38,40,41,42,43,44,46, 47,51,53,54,57,58,61	7,10,15,17,20,21,28,34, 38,41,46,54	46

and Snarska, 2001). Dojlidy pond also had comparatively lowest pH, alkalinity, sulphates, calcium, dry residue and dissolved solids. When comparing the hydrochemical data of these water bodies, it can be seen that only the water of the Dojlidy pond did not contain detectable amounts of ammonium, magnesium and iron.

Only *Karlingia rosea* has been found on horsetail spores, as well as other plant substrata (Batko, 1975). The growth of *Rhizophydium sphaerotheca* has been observed on the microspores of *Isoëtes lacustris* (Karling, 1977).

Most of the zoosporic taxa in the class *Chytridiomycetes* found on cryptogamic spores have been also observed on pine pollen. This includes three species of *Olpidium* and most species of the *Chytridiales* (Karling, 1977). However, species such as *Chytridium xylophilum, Entophlyctis rhizina*, *Synchytrium macrosporum,* two species of the genus *Nowakowskiella*, *Phlyctochytrium hydrodictyi, Rhizophydium laterale* and *R. polysiphoniae* have never been observed on plant spores or pollen. *Synchytrium macrosporum* is known as a parasite of plants of the genus *Ambrosia* (Karling, 1942). In our study it was found to grow on the spores of horsetail *Equisetum sylvaticum* in the Supraśl River. *Chytridium xylophilum* is known as a saprobe of higher plants (Batko, 1975). Czeczuga and Muszyńska (2001) observed this fungus on gymnosperm pollen. *Entophlyctis rhizina*, a parasite of filiform green algae, mainly of the genus *Spirogyra* and *Vaucheria* (Domjan, 1936; Batko, 1975), was found to grow on the spores of a fern, *Dryopteris filix-mas* in Dojlidy pond. *Nowakowskiella elegans* and *N. macrospora* have been described from cellulose-containing substrata (Batko, 1975; Karling, 1977). In the present study they were observed on the spores of two fern species. *Phlyctochytrium hydrodictyi* is known as a parasite of algae of the genus *Hydrodictyon* (Braun, 1855), while *Rhizophydium laterale* as a parasite of green algae of the genus

Ulothrix and *R. polysiphoniae* of other algae, mostly of the genus *Polysiphonia* (Batko, 1975; Karling, 1977). Two species of the class *Hyphochytriales* we found on spores are *Rhizidiomyces apophysatus* and *R. hirsutus*. The former, known as a parasite of the oogonium of the *Saprolegnia*, *Achlya* and algae of the genus *Vaucheria* (Zopf, 1884), also has been found on pine pollen (Karling, 1944). We observed this fungus on the spores of a few plant species examined. This fungus has been found on grass leaves (Karling, 1945, 1968) and on squash pollen (Batko, 1975). In our study this fungus grew on the spores of a fern *Thelipteris palustris* in Supraśl River.

Among the species of oomycetes growing on the spores subjected to analysis, only *Myzocytium microsporum* has been found on pollen, although it usually grows on living (Batko, 1975) and dead rotifers (Czeczuga *et al*., 2000). *Leptomitus lacteus* and a few species of the genus *Pythium* are new species to Polish waters. Interesting is also the presence of *Zoophagus insidians* known as a predacious fungus (Sommerstorff, 1911). The remaining oomycete species have been commonly encountered in the waters of northeastern Poland either as plant saprobes (Czeczuga, 1991a,b); all the three species of the genus *Saprolegnia* have been reported as also saprobes on avian excrements in various types of water bodies (Czeczuga and Mazalska, 2000).

Leptomitus lacteus is known as a typical mycobiont representative in the waters heavily polluted by municipal wastes as a nitrophilous fungus and also on gymnosperm pollen (Czeczuga and Muszyńska, 2001). We observed its growth on the spores of *Equisetum arvense*.

Of 13 species of the *Pythium*, whose growth was observed on the spores of cryptogamic plants in the present study, only *Pythium intermedium* has been mentioned in literature as a parasite of vascular cryptogam prothalia (Batko, 1975), the remaining species being saprobes or parasites of higher plants (Waterhouse, 1968; Plaats-Niterink, 1981; Yu and Ma, 1989). *Pythium arrhenomanes* new to Poland, was first described from rotten roots of maize *Zea mays* L. in Wisconsin, USA (Drechsler, 1928), was found on the spores of *Equisetum pratense* in Jaroszówka Spring. The water of the Jaroszówka Spring had the lowest content of oxygen, nitrite nitrogen, chlorides, suspended solids and a comparatively lowest of BOD_5. The water of this spring had a high content of CO_2, ammonium nitrogen, phosphates, sulphates, calcium, magnesium, iron, dry residue, dissolved solids and the highest alkalinity. *Pythium vexans* was observed on the spores of *Polytrichum attenuatum* in Jaroszówka Spring. It was first described by de Bary (1876) from potato tubers as saprobic. *Pythium marsipium* was noted in Jaroszówka Spring on the spores of *Polytrichum attenuatum*; first described as saprobic on leaves of *Nymphaeae* (Drechsler, 1941). *Pythium megalacanthum*, first described by de Bary (1881) was observed on the spores of *Equisetum hyemale* in Dojlidy pond, of *Equisetum limosum* and of *Equisetum palustre* in Jaroszówka Spring. The water of the Dojlidy pond is characterized by comparatively low contents of nitrite nitrogen, phosphates, sulphates and calcium and did not contain detectable amounts of ammonium nitrogen, magnesium and iron. *Pythium salinum* was found to grow on the spores of the two species of moss in Jaroszówka spring and Dojlidy pond. It was first described by Höhnk (1953) from estuaries near Kiel in Germany. These four species were also new to Polish hydromycology.

Using the method of similarities, a camparision of the chemical parameters of the waters examined in the number of fungus species growing on spores has shown that the number of fungal taxa was closely related to chemical factors such as chlorides, sulphates and dry residue. Both chlorides and sulphates occur in large amounts in polluted and polytrophic waters (Håkanson, 1999).

The monographs devoted to the aquatic fungi (Sparrow, 1960; Batko, 1975; Karling, 1977) mention only pollen grains as their substratum. No data concerning spores can be found. Our present results indicate that this type of substratum can also be colonized by a various group of zoosporic fungi.

The aquatic fungus species colonizing a given substratum secrete enzymes which break it down. They also possess enzymes which decompose plant cellular walls, including hydrolases, pectinases and proteinases (Unestam, 1966; Chamier, 1985). Our microscopic observations of germinating spores show that hyphae of zoosporic fungi pass through the pores in the external wall to the inside of the spore or pollen grain to the substrate where the fungi can grow further. The subsequent sporangia are also most frequently found outside the spore or pollen grain close to the pores.

Spores, like pollens (Clément *et al*., 1999), are enclosed in two walls - the external that contains pores and the internal wall. The external wall is solid with numerous patterned structures and comprises sporopollenins (Brooks and Shaw, 1968) which consist of polymers and carotenoid esters (Goodwin, 1980). This external wall of pollen or spores is very resistant to bacterial and fungal attacks. The internal wall of pollen is made of cellulose, pectin substances, calloses, polysaccharides, and proteins. Similar substances are also found of the spores of cryptogams (Dafni *et al*., 2000).

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