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Aquatic fungi and fungus-like organisms growing on driffing in water nuts of seven birch species

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Abstract. Aquatic fungi and fungus-like organisms, growing on nuts of seven birch species (*Betula gracilis, B. humilis, B. lutea, B. nana, B. papyrifera, B. pubescens* and *B. verrucosa*) found in the water of three limnologically and trophically different water bodies (spring, river and pond), were investigated. The total of 63 species, including 23 fungus-like organisms and 40 fungal species were found on the nuts of the investigated birches. The most common species were *Karlingia rosea, Nowakowskiella macrospora, Achlya americana, Aphanomyces laevis, Saprolegnia ferax, Acrodictys bambusicola, Angulospora aquatica, Arbusculina fragmentans, Canalisporium caribense, Heliscus lugdunensis, Pithomyces obscuriseptatus, Tetracladium marchalianum* and *Tripospermum camelopardus.* Most of the species were observed on the nuts of *Betula verrucosa* (49 species) and the fewest on the nuts of *Betula nana* (42). In Cypisek spring, the number of fungal species and fungus-like organisms on the nuts was closely associated with the concentration of chlorides. However, in Supraśl River and Dojlidy pond it was associated with the sulphates concentration (in both cases negative correlation).

Key words: aquatic fungi, fungus-like organisms, hydrochemistry, nuts of birch, Poland, water bodies

Introduction

The organic matter, occurring in water bodies, comes from two sources. Most of it is produced in the reservoir, whereas some part of it is formed on the dry land at a different distance from it. The matter, produced on the dry land, gets to the open water along with the rainwater or the streams of wind. A great amount of tree and plant pollen as well as spores of cryptogamic plants gets to the water in the spring period (Czeczuga & Muszyńska 2001, 2004a, b; Czeczuga & Orłowska 2001). This kind of terrigenous organic matter is assimilated by numerous groups of fungi and fungus-like organisms.

During the summer months in the Northern Hemisphere, when the birches bloom, the wind brings to open water a big amount of seeds of these trees. We have studyed the participation of some fungi in the mineralization of that kind of organic matter, which is typical for the forest lakes during the summer.

Material and Methods

The study included nuts of seven birch species: weeping birch (*Betula gracilis* Roth.), river birch (*B. humilis* Schrk.), yellow birch (*B. lutea* Michx.), dwarf birch (*B. nana* L.), canoe birch (*B. papyrifera* Marsh), downy birch (*B. pubescens* Ehrh.) and common birch (*B. verrucosa* Ehrh.) collected in August-September in Knyszynska Forest and Botanical Park of north-eastern Poland. The water for experiments was collected from three different water bodies; two running (spring Cypisek and river Supraśl) and one stagnant (pond Dojlidy):

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- Spring Cypisek, localized in the north part of Białystok: Limnokrenic type, width 0.41 m, depth 0.17 m, discharge 0.6 l/s, surrounded by cultivated fields but not by trees. The bed is covered with sand.
- River Supraśl, right-bank tributary of the middle part of the Narew River, flowing through the Knyszyńska Forest: Length 106.6 km. The samples were collected from the site above the municipal swimming pool, at the sluice of an arm of the Supraśl River, flowing just through the town of Supraśl. The sampling site is surrounded by meadows. The bed is muddy.
- Pond Dojlidy, localized in the near of Białystok: Area 34.2 ha, max. depth 2.85 m, its south shores border on coniferous woods and its western part on the town of Białystok. The samples were collected from the western part of this pond, which is used by the inhabitants of the town as a beach.

Nineteen water parameters of sampling sites, mentioned above, were determined (Table 1) according to the methods recommended by *Standard Methods for the Examination of Water and Wastewater* (Greenberg *et al.* 1995).

The presence of aquatic fungi and fungus-like organisms on the birch nuts was determined by the folowing procedure: a certain number of nuts (about 2 mg of each birch species) was transferred to two samples of water, representing each site, in a 1.0 dm^3 vessel (all together six vessels for each species) and placed in laboratory at room temperature. A part of the nuts from each vessel was examined under a light microscope and the mycelium of aquatic fungi and fungus-like organisms found on the nuts was recorded. The methods were described in detail by Seymour & Fuller (1987). The nuts of the various birch species were observed under a microscope for one and a half weeks. The duration of experiments was four weeks. The identification of fungi and fungus-like organisms was based on the morphological and biometric data of antheridia and oogonia and conidiophores and conidia of the anamorphic fungi.

Identification of the fungi was aided by the following keys: Johnson (1956), Seymour (1970), Batko (1975), Karling (1977), Dick (1990), Pystina (1998), and for anamorphic fungi – Dudka (1974), Ingold (1975), Carmichael *et al.* (1980), Matsushima (1993), and the works, where the studyed species were firstly described.

The chemical parameters of the water as well as the fungal flora were investigated by numerical analysis (McGarical *et al.* 2000).

Results

Chemical analysis of the water, used for the experiment, revealed some differences in water trophic states among the sampling places. The water of Cypisek spring was most abundant of biogenes, followed by the Dojlidy pond as the poorest (Table 1).

Table 1. Chemical composition (in mg l^{-1}) of water from different sampling sites

Specyfication	Water from		
	Cypisek Spring	Dojlidy Pond	Supraśl River
Temperature (°C)	14.0	19.0	17.0
pH	7.19	8.10	7.92
O_2	4.2	13.6	14.8
BOD_5	4.2	12.4	5.2
Oxydability	16.89	15.74	6.52
CO_2	11.0	6.6	4.4
Alkalinity in $CaCO_3$ (mval l^{-1})	2.9	3.1	4.3
$N\text{-}NH_3$	0.870	0.075	0.051
$N\text{-}NO_2$	0.094	0.006	0.012
$N\text{-}NO_3$	0.120	0.020	0.190
$P\text{-}PO_4$	1.500	0.061	1.300
Sulphates	20.98	46.89	43.61
Chlorides	24.0	13.1	13.0
Total hardness in Ca	53.28	62.64	74.88
Total hardness in Mg	3.87	11.18	6.45
Fe	2.05	0.60	0.50
Dry residue	270	265	223
Dissolved solids	130	213	176
Suspended solids	140	52	47

Table 2. Aquatic fungi and fungus-like organisms found on nuts*

	Taxa	*Betula*						
		gracilis	*humilis*	*lutea*	*nana*	*papyrifera*	*pubescens*	*verrucosa*
	Chromista							
	Oomycetes							
	Saprolegniales							
1	*Achlya americana* Humphrey	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s
2	*Aphanomyces laevis* de Bary	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s
3	*Aplanes androgynus* (W. Archer) Humphrey	d		d	d		d	d
4	*Saprolegnia ferax* (Gruith.) Thur.	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s
5	*S. monoica* Pringsh.		d, s			d, s	d, s	
6	*S. parasitica* Coker	d, s		d, s	d, s			d, s
7	*S. pseudocrustosa* A. Lund	d	d		d		d	
	Peronosporales							
8	*Pythium afertile* Kanouse et T. Humphrey		d		d	d		d
9	*P. anandrum* Drechsler	c	s	d	s	c, s	d, s	c, s
10	*P. aphanidermatum* (Edson) Fitzp.	s		c			c, s	d
11	*P. aquatile* Höhnk	c	s	s	c, d	s	c, d	c
12	*P. aristosporum* Vanterp.		c, s			c, s		c, s
13	*P. inflatum* V.D. Matthews	c, s		c, s			c, s	
14	*P. intermedium* de Bary	d	c		c, d	d	s	s
15	*P. multisporum* Poitras	c	s	d	s	s	c	c, d, s
16	*P. myriotylum* Drechsler		d		d		d	
17	*P. palingenes* Drechsler	s	d		d		d, s	
18	*P. papillatum* V.D. Matthews	c, s	s	c, d	s	c, d, s	c	c, s
19	*P. paroecandrum* Drechsler	c	s	d	s	s	c	c, d, s
20	*P. periplocum* Drechsler	c, s		c, s		c, s	c, s	c, s
21	*P. rostratum* E.J. Butler		c, d		c, d	c, d		
22	*P. tardicrescens* Vanterp.	c	s		c, s	s		c, s
23	*P. ultimum* Trow var. *sporangiiferum* Drechsler	d, s		d, s			d	d, s
	Fungi							
	Chytridiomycetes							
	Chytridiales							
24	*Cylindrochytridium johnstonii* Karling		d, s		d, s		d, s	
25	*Dangeardia mamillata* Schröd.	c		c		c		c
26	*Karlingia lacustris* Hassan		d		d		d	d
27	*K. polonica* Hassan	d		d		d		
28	*K. rosea* (de Bary et Woronin) A.E. Johanson	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s
29	*Nowakowskiella macrospora* Karling	c, s	d, s	c, s	c, d, s	c	s	d, s

Table 2. (continued)

	Taxa	*Betula*						
		gracilis	*humilis*	*lutea*	*nana*	*papyrifera*	*pubescens*	*verrucosa*
30	*N. methistemichroma* A. Batko et Hassan	c	d	d	s	c, d	d, s	c, s
31	*N. moubasherana* Hassan	s	c	c, s		d, s		c, d
32	*Siphonaria petersenii* Karling	c			c	c		c
	Anamorphic fungi							
33	*Acrodictys bambusicola* M.B. Ellis	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s
34	*Anguillospora pseudolongissima* Ranzoni		c			c	c	
35	*Angulospora aquatica* Sv. Nilsson	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s
36	*Antipodium arecae* Matsush.		c		c		c	c
37	*Arbusculina fragmentans* Marvanová	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s
38	*Canalisporium caribense* (Hol.-Jech. et Mercado) Nawawi et Kuthub.	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s
39	*Colispora elongata* Marvanová	c	c		c		c	
40	*Cordana musae* (Zimm.) Höhn.	d		d		d		d
41	*Corynesporella simpliphora* Matsush.	c, d, s	c, s		c, d		c, d, s	
42	*Dactylella submersa* (Ingold) Sv. Nilsson			c, d		c, d		c, d
43	*Didymobotryum verrucosum* I. Hino et Katum		c		c		c	
44	*Dimorphospora foliicola* Tubaki	d	d			d		d
45	*Haplographium* sp.	s		s	s		s	
46	*Helicosporina globulifera* G. Arnaud ex Rambelli	s		s		s		s
47	*Heliscus lugdunensis* Sacc. et Therry	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s
48	*Lemonniera aquatica* De Wild.	d, s	d, s	d, s	d, s	d, s	d, s	d, s
49	*Mirandina corticola* G. Arnaud ex Matsush.		s	c, s	c, d		s	s, d
50	*Paraarthrocladium amazonense* Matsush.		s		s		s	s
51	*Phialogeniculata multiseptata* Matsush.		d	d			d	d
52	*Pithomyces obscuriseptatus* Matsush.	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s
53	*Pseudohansfordia dimorpha* Matsush.	s	d	s	s	d	d	s
54	*Pseudospiropes lotorus* Morgan-Jones	c	s	d	d	d	s	d, s
55	*Stenella novae-zelandiae* Matsush.		d	d			d	d
56	*Sterigmatobotrys uniseptata* H.S. Chang	s		s	s	s		
57	*Tetracladium marchalianum* De Wild.	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s
58	*T. maxilliforme* (Rostr.) Ingold	c, s	s	c	c, s	s	s	c, s
59	*T. setigerum* (Grove) Ingold	d, s	d	s		d, s		d
60	*Tricellula aquatica* J. Webster	c		c		c		c
61	*Trinacrium subtile* Riess	s	c		c, s		c, s	d
62	*Tripospermum camelopardus* Ingold, Dann & P.J. McDougall	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s	c, d, s
63	*Ypsilina graminea* (Ingold, P.J. McDougall & Dann) Descals, J. Webster & Marvanová		d, s	d, s		d, s	d, s	s
	Total number of species	47	48	43	42	43	47	49

*c – Cypisek Spring, d – Dojlidy Pond, s – Supraśl River

Table 3. Aquatic fungi and fungus-like organisms found on nuts in different water bodies

Water from	Fungi and fungus-like organisms (see Table 2)	Occurred restrictedly (only in one body of water)	No of taxa
Spring Cypisek	1, 2, 4, 9, 10, 11, 12, 13, 14, 15, 17, 19, 20, 21, 23, 25, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 41, 42, 43, 47, 49, 52, 54, 57, 58, 60, 61, 62	25, 32, 34, 36, 39, 43, 60	40 (7)*
Pond Dojlidy	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15, 16, 17, 18, 19, 21, 22, 24, 26, 27, 28, 29, 30, 31, 33, 35, 37, 38, 40, 41, 42, 44, 47, 48, 49, 51, 52, 53, 54, 55, 57, 59, 61, 62, 63	3, 7, 8, 16, 26, 27, 40, 44, 51, 55	47 (10)
River Supraśl	1, 2, 4, 5, 6, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, 20, 22, 23, 24, 28, 29, 30, 31, 33, 35, 37, 38, 41, 45, 46, 47, 48, 49, 50, 52, 53, 54, 56, 57, 58, 59, 61, 62, 63	45, 46, 50, 56	44 (4)

*only in this body of water

On the nuts of seven birch species, found in the water of three different trophic reservoirs, 63 species of lower aquatic fungi have been established, of which 23 species are representatives of fungus-like organisms, whereas 40 species belong to fungal species (Table 2, Fig. 1). From those 63 species, 5 have not been found in water of Poland up to now. These are: the fungi *Siphonaria petersenii* and *Cylindrochytrium johnstonii* and the anamorphic fungi *Antipodium arecae, Didymobotrium verrucosum* and *Haplographium* sp. We can classify as a rare fungus *Dangeardia mamillata.* On the examined nuts of birch, most of the species were found in water of the Pond Dojlidy (47) and least in water of the Spring Cypisek (40) (Table 3). Those species of fungus-like organisms, as *Pythium aquatile* and *P. rostratum,* of Chytridiales, as *Karlingia rosea,* and of anamorphic fungi, as *Acrodictys bambusicola, Angulospora aquatica, Arbusculina fragmentans, Canalisporum caribense, Heliscus lugdunensis, Pithomyces obscuriseptatus, Tetracladium marchalianum* and *Tripospermum camelopardus,* grew on nuts in the water of all three reservoirs. The numerical method showed that the factor that had a big influence on the amount of fungal species in Spring Cypisek was the chlorides in water and the sulphates in River Supraśl and Pond Dojlidy. Analysis showed that the mycoflora of the spring Cypisek was affected by the considerable levels of chlorides, showing a negative correlation (-0.8012, level of significance 0.04). In the water of River Supraśl and pond Dojlidy, a negative correlation was revealed in the case of sulphates concentration (respectively, -0.7902 and -0.8004, level of significance 0.04).

Discussion

Cylindrical catkins of birch-tree occurs mainly in August, breaking down into single winged nuts, which may be carried by wind at considerable distances. The nuts have a hard wall, made up of scleroides constituting a specific type of mechanical tissue that forms a solid structure, like in other plant species. As shown by the current study, if the nut gets into water, it is colonized by fungi and, especially, by a group of anamorphic fungal species, which produce enzymes to decompose the nut structure. The study revealed a relatively large number of anamorphic species (31) and a small number of Saprolegniales (7). The latter, included only one species of *Achlya* and four of *Saprolegnia.* We observed a similar phenomenon when studying the growth of aquatic fungi and fungus-like organisms on the spores of cryptogams and pollen of seed plants (Czeczuga & Muszyńska 2001, 2004a, b). Both the spores and pollen, like birch nuts, contain a relatively thick and hard to degrade capsule. Representatives of anamorphic fungi grow abundantly on substrates that contain a relatively great amount of mechanical tissues. This may be the most crucial factor determining the growth of such group of anamorphic fungi on the birch nuts. They are likely to be found on plant debris, wood, dead branches and twigs, leaves, needles and roots (Bärlocher 1992).

It was revealed (McLaughlin *et al.* 2001) that a few dozens of species of Saprolegniales, including several species of the genus *Achlya* and almost the same number of the genus *Saprolegnia* grow on "soft" plant substrate. A relatively large group of representatives of Peronosporales (16 species of *Pythium*) were found to grow on birch nuts. On plant substrates, the species of the genus *Pythium* colonize mainly root systems of free-growing and cultivated plants (Plaats-Niterink 1981; Yu & Ma 1989; Pystina 1998).

Sparrow (1960) mentiones only three species of fungus-like organisms which grow on branches of *Betula: Apodachlya pyrifera, Monoblepharis polymorpha* and *Rhipidium interruptum.*

On the leaves of birch Findlay *et al.* (1986) observed in aquatic conditions the growth of the anamorphic fungus *Arciculospora tetracladia* and Czeczuga & Orłowska (1994, 1995) detected several anamorphic fungi. However, lots of

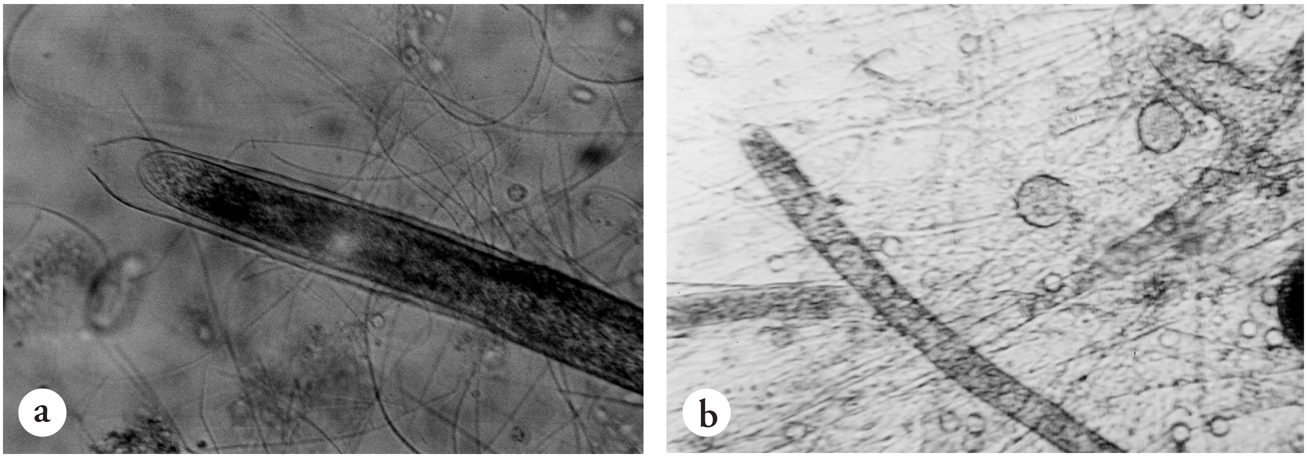

Fig. 1. Some fungus-like organisms growing on nuts of birch: **a** – *Saprolegnia ferax* – sporangium; **b** – *Pythium rostratum* – hyphae from sporangia

the fungus-like organisms (up to 17) and 15 anamorphic species, detected on pollen of some birch species, occur in Poland (Czeczuga & Orłowska 2001; Czeczuga & Muszyńska 2004b).

Our data show, the greatest number of species grew on birch nuts from the Pond Dojlidy, where 10 species were found, including four fungus-like organisms and six anamorphic fungi. In the other reservoirs the amount of species was smaller. Moreover, among the six species of fungi new for Poland's water, four grew only in water of the Spring Cypisek. One should stress the fact that water of the Spring Cypisek has the lowest temperature, as well as the lowest concentration of oxygen, BOD_5, alkalinite, sulphates, calcium and magnesium but the highest values of CO_2, oxidability, ammonium, nitrates, phosphate, chlorides and iron. The numerical method showed that chlorides (spring) and sulphates (river, pond) influenced the amount of species growing on birch nuts.

We observed the inhibitory effect of chlorides on the growth of fungi on spores of cryptogams in spring Jaroszówka as reported earlier (Czeczuda & Muszyńska 2004a). The effect of the sulphide on the number of anamorphic fungi was also investigated (Chandrashekar *et al.* 1991). The number of aquatic anamorphic fungal species in a sulphur spring in the Western Ghat region (India) was similarly greatly reduced. They were entirely absent in the spring proper, and far from the spring in the stream, which flows from it, as the amount of sulphide decreases and the number of anamorphic fungus species increases. As it is known (Häkanson 1999), chlorides and sulphates are pollution indicators for some water basins. Both of them increase with the eutrophication of the basins. It is well known that in waters, richer of biogens, the amount of fungi and fungus-like organisms is smaller (Czeczuga & Mazalska 2000; Czeczuga & Snarska 2001).

The rare Polish aquatic fungus *Dangeardia mamillata,* which was described for the first time by Schröder (1898), was found in the end eighteen century in Wroclaw Botanical Garden as a cell parasite of the green algae *Pandorina morum.* The water of Pond Dojlidy is a second locality of this fungus but birch nuts are new substrates for it, where it develops as a saprotroph. The water of Dojlidy pond containes a great amount of BOD_5, sulphates and magnesium, whereas nitrates, nitrites and phosphates occur in minimal amounts. The aquatic species *Cylindrochytrium johnstonii,* was described as a saprotroph on cellulose substrates (Karling 1941) and *Siphonaria petersenii* on substrates containing chitin and most often occurring in insects slough (Karling 1942). However, the new water anamorphic fungus *Antipodium arecae* was described by the Japanese mycologist Matsushima (1980) and was found on fallen palm leaves in Amazon basin (Matsushima 1993). *Didymobotrium verrucosum* was described also in Japan by Hino and Katumoto (1959 quote after Ellis 1971). It was found also in above ground conditions in the Amazon basin (Matsushima 1993). To the Amazonic group of anamorphic fungi growing on the birch nuts already belong *Corynesporella simpliphora, Paraarthrocladium amazonense, Phialogeniculata multiseptata, Pithomyces obscuriseptatus, Pseudohansfordia dimorpha* and *Stenella novae-zelandiae.* Up to the present, in water bodies of north-eastern Poland over 50 species, encountered in the Amazon River basin in South America (Matsushima 1993), were found (Czeczuga 2004). All species from the genus *Haplographium* were established in America up to now (Burnett & Hunter 1972). The water of River Supraśl is characterized with a maximum content of oxygen, alkalinity, nitrytes and calcium. Simultaneously it contains minimal amounts of oxidability, carbon dioxide, ammonium, chlorides and iron.

The aquatic fungal species, including chromistan organisms (Unestam 1966; Bodeumann *et al.* 1985) and anamorphic fungi (Chamier 1985), absorb a given substratum by production of enzymes which break it down. They also possess four groups of enzymes which decompose plant cellular walls. The cell-walls of dicotyledonous plants consist of polymers. The cell-walls contain pectic polysaccharides, cellulose and hemicelluloses in primary and secondary, lignin (the wheat straw). These substrates are decomposited by a group of enzymes produced by fungal species and fungus-like organisms. The peptic polysaccharides are decomposed by pectinases (Chamier & Dixon 1982), cellulose by a group of enzymes, cellulases (Singh 1982), and hemicelluloses by hemicellulases (Dekker & Richards 1976; Suberkropp & Klug 1980). The lignin is extremely difficult to assess chemically. Fisher *et al.* (1983) demonstrated the decomposition of lignin by some anamorphic fungi.

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