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Zoosporic Fungi Growing on Gymnosperm Pollen in Water of Varied Trophic State

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Abstract

The authors investigated zoosporic fungi developing on the pollen grains of 36 taxons of gymnosperm plants in three bodies of water of various trophic state. A total of 83 zoosporic fungus species were noted, with predominance by the Peronosporales (39) and Chytridiales species (29). Twelve fungus species were recorded for the first time in Polish waters.

Keywords: zoosporic fungi, aquatic fungi, gymnosperm, pollen grains, hydrochemical study

Introduction

In the majority of forest complexes in northeastern Poland conifers are the predominant species, particularly pine, spruce and common juniper.

In spring months during the pollen season these plants produce vast amounts of pollen which are carried by the wind, cover the ground and end up in different types of water reservoirs. At the end of May and the beginning of June (i.e. during pine pollen time) concentrations of pollen can be observed on pool margins after rain. Pollen is a substratum for the growth of numerous zoosporic fungus species, especially Chytridiomycetes representatives [1]. Since until now studies have been conducted only on the pollen of a few species of the genus *Pinus,* our aim is to investigate a number of other gymnosperm species growing in parks and producing as much pollen as pine.

Material and Methods

Thirty-six gymnosperm taxons collected in gardens and parks of Bialystok and the Knyszynska Forest during their pollen time were subjected to the investigation (Table 1).

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Table 1. Chemical composition (in mg l^{-1}) of water from the different sites (n=3).

Specification	Spring Jaroszówka	River Supraśl	Pond Dojlidy
Temperature, °C	6.2	13.0	13.2
pH	7.45	7.50	6.82
O_2	8.4	11.2	11.2
BOD_5	2.2	4.8	5.2
COD	7.30	13.58	7.30
CO_2	17.6	8.8	11.0
Alkalinity in $CaCO_3$ (mval l^{-1})	4.4	3.7	0.8
$N\text{-}NH_3$	0.61	0.07	0.0
$N\text{-}NO_2$	0.010	0.035	0.026
$N\text{-}NO_3$	0.08	0.08	0.07
$P\text{-}PO_4^{3-}$	3.500	1.350	0.070
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	15.30	13.76	0.0
Fe	0.30	0.25	0.0
Dry residue	398	208	48
Dissolved solids	396	155	32
Suspended solids	2	49	16

The water for experiments was collected from three different water bodies:

(I) Jaroszowka Spring, limnokrenic type, width 0.65 m, depth 0.12 m, discharge 2.4 l/s, is in the northern part of Białystok.

(II) Suprasl River, length 106.6 km; the right-bank tributary of the middle part of the Narew river, flowing through Knyszyńska Forest.

(III) Dojlidy Pond, area 34.2 ha, max. depth 2.85 m., while the south shore borders a coniferous woods and western part of this pond with the town of Biarystok, this part of pond is used as a beach.

Nineteen water parameters of the above sampling sites were determined (Table 1) following the methods of Greenberg et al. [2].

For the determination of the presence of aquatic fungal species on the pollen grains, the following procedure was employed: a certain number of pollen grains (1000-2000) of each plant species was transferred to two samples of water representing each site, in an 1.0 dm^3 vessel (altogether six vessels for each species) and placed in the laboratory at ambient temperature. A part of pollen grains from each vessel was observed under a microscope and the mycelium (zoosporic, antheridia and oogonia) of aquatic fungi on the pollen grains was recorded. The methods are described in detail by Sparrow

Table 2. Coniferous species investigated and fungi found on the pollens.

Taxa	Fungi (see Table 3)	Number of species
Taxaceae		
1. *Taxus baccata* L.	10,15,19,24,26,27	6
2. *Taxus cuspidata* Sieb. et Zucc.	3,7,13,14,21,22,26,30,38,49,68	11
Pinaceae		
3. *Abies concolor* Lindl. ex Hildebr.	1,3,11,14,15,25,27,60,63	9
4. *Abies koreana* Wils.	3,14,15,22,24,28,38,41,42,75	10
5. *Abies nordmanniana* Spach	1,21,24,38,44,47,50,58,72,81	10
6. *Pseudotsuga menziesii* var. *caesia* Franco	3,6,13,14,22,24,34,40,41,45,52,69	12
7. *Pseudotsuga menziesii* var. *glauca* Franco	1,3,7,12,14,22,24,30,35,38,41,47,52	13
8. *Pseudotsuga menziesii* var. *viridis* Franco	1,3,7,15,22,26,27,59,64	9
9. *Tsuga canadensis* Carr.	1,3,7,11,13,14, 22,24,26,27,38,57	12
10. *Tsuga diversifolia* Mast.	3,7,14,17,24,27,38,51,52,54,67,71	12
11. *Picea abies* (L.) Karst.	3,7,24,28,38,61,65,69,79	9
12. *Picea abies "Falcato-viminalis"*	1,14,24,27,48,52,63,68,71,78	10
13. *Picea bicolor* Mayr	3,12,14,24,26,30,38,41,46,52,59,77,80	13
14. *Picea engelmannii* Engelm.	3,7,12,14,22,24,26,40,41,64	10
15. *Picea glauca* var. *albertiana* Sarg.	2,4,5,11,13,19,27,29,38	9
16. *Picea jezoensis* Carr.	3,7,14,22,24,38,73,74	8
17. *Picea omorika* Purkyne	4,8,9,11,12,17,22,26,27,36,39	11
18. *Picea pungens* Engelm.	11,22,24,27,29,41,45,50,52,54,55,64,69,75	14
19. *Picea sitchensis* Carr.	7,14,22,24,27,38,41,47,69	9
20. *Larix decidua* Mill.	5,10,13,15,27,33,38,39	8
21. *Larix kaempferi* Sarg.	1,3,14,24,26,30,59,66,72	9
22. *Pinus mugo* var. *mughus* Zenari	3,4,5,10,12,13,22,26,27	9
23. *Pinus mugo* var. *pumilio* Zenari	5,10,11,13,26,27,30,34,37	9
24. *Pinus nigra* Arnold	4,11,15,27,29,34,37	7
25. *Pinus ponderosa* Dougl. ex Laws.	7,12,13,14,16,20,29,31,38	9
26. *Pinus silvestris* L.	8,18,22,24,27,29,34,35	8
27. *Pinus strobus* L.	5,11,22,26,27,34,36,37,83	9
28. *Pinus uncinata* Ramond ex DC	1,3,12,13,14,15,27,38,61,62	10
Cupressaceae		
29. *Chamaecyparis pisifera* Endl.	3,11,21,24,26,28,52,53,56	10
30. *Juniperus chinensis* L.	1,3,13,14,21,30,31,38,76	9
31. *Juniperus communis* L.	5,11,22,23,27,34,35,36,41	9
32. *Juniperus horizontalis* Moench	7,11,12,14,26,30,38,47,56,59,69	11
33. *Juniperus procumbens* Miq.	3,7,11,14,24,26,29,38,44,46,69,70,82	13
34. *Juniperus sabina* L.	3,7,11,14,15,31,38,43,50,69	10
35. *Juniperus virginiana* L.	1,7,12,13,14,21,27,38	8
36. *Thuja occidentalis* L.	1,3,13,14,15,21,22,24,38,51,59	11

[3] and Fuller and Jaworski [4]. The pollen grains of the various plant species were observed under a microscope for one and a half weeks. The duration of the experiments was three weeks. Identification of the fungi was aided by the following keys: Skirgiełło [5], Johnson [6], Sparrow [3], Seymour [7], Batko [8], Karling [1], Dick [9], and Pystina [10].

Results

Chemical analysis of water used for the experiment has revealed differences in water trophicity. The water of Dojlidy pond was the least abundant in biogenes, Jaroszowka spring the richest (Table 1).

Eighty-three zoosporic fungus species were found on the pollen grains of 36 gymnosperm taxons in the water of three various aquatic reservoirs, with the predominance of Peronosporales (39) and Chytridiales (29). The most common were *Olpidium longicollum, Blyttiomyces heliscus, Chytriomyces mamillifer, Karlingia rosea, Rhizophydium carpophilum, Rhizophydium globosum, Rhizophydium keratinophilum, Rhizophydium pollinis-pini* and *Myzocytium microsporum.* Of the Peronosporales representatives, *Pythium papillatum* was found on the pollen grains of 7 taxons, *Pythium catenulatum* on 6, the remaining species of this order on the grains of single species. Of the 83 species 12 appeared new to Polish waters (Table 3). The fewest fungus species were observed on pollen grains of yew *Taxus baccata* (6), the most on the pollen of spruce *Picea pungens* (14).

Forty-nine zoosporic fungus species were encountered on gymnosperm pollen in the Suprasl river, 50 in Jaroszowka spring and 57 in Dojlidy pond (Table 4). Several fungus species were found on pollen only in the water of one reservoir.

Discussion

The present study has revealed 29 zoosporic fungus species of the class Chytridiomycetes on the pollen of 36 gymnosperm taxons examined. Most of them have already been shown on the pollen of pine or other plants [1, 8, 11]. Some of them, however, have been known to occur on other substrata. For instance, *Olpidium endogenum* is reported in monographs as a parasite of algae of the genus *Spirogyra* and representatives of Desmideacea. In our study this fungus was found to grow on the pollen of a few gymnosperm species. *Achlyella flahaultii* was observed by Lagerheim [12] on pollen grains of *Typha* found in water and since that time only this substratum has been mentioned in connection with that fungus [1, 5]. We observed *Achlyella flahaultii* on the pollen of 6 gymnosperm species. *Chytridium xylophilum* has been reported as a saprophyte of higher plants [5]. We found it on the pollen of *Picea omorika* and *Pinus silvestris. Chytriomyces annularus* and *Chytriomyces aureus* have been observed on flower plant pollen, the latter also being found on chitin-containing substrata [14-16]. *Phlyctochytrium circulidentatum* and *Phlyctochytrium multidentatum* were first described from the pollen of a plant of the genus *Liquidambar* [17]. *Phlyctochytrium laterale, Rhizophydium contractophilum* and *Zygorhizidium willei* are known as alga parasites. *Phlyctochytrium laterale* is reported as a parasite of algae of the genus *Spirogyra, Zygorhizidium willei* parasites on various species of green algae [8], while *Rhizophydium contractophilum* is known as a parasite of algae of the genus *Eudorina* [18]. *Myzocytium vermicolum,* a representative of the Oomycetes is described as a parasite of nematodes. We found this fungus on the pollen of spruce *Picea omorika* and larch *Larix decidua.*

Worth noting is the finding of a large number of fungus species of the genus *Pythium* growing on gymnosperm pollen. In his monograph Sparrow [3] mentions only that fungus species of the genus *Saprolegnia* and even *Pythium* can grow at a large pollen concentration, yet no *Pythium* species are mentioned in the chapter "List of Substrata". Of 32 species of the genus *Pythium* found on gymnosperm pollen for the first time, 12 are also new to Poland. *Pythium akanense* was found on the pollen of *Pseudotsuga menziesii* var. *caesia* and *Picea pungens* in the river Suprasl. It was first described in Japan by Tokunaga [19] as a parasite of *Aegagropila sauteri. Pythium angustatum,* also new to Polish waters, was described by Sparrow [20] as a parasite of some green algae. In our study it grew on the pollen of *Picea bicolor* and *Juniperus procumbens* in the river Suprasl and pond Dojlidy. *Pythium araiosporon* was found to grow on the pollen of *Picea abies "Falcato-viminalis",* while *Pythium ascophallon* on the pollen of yew *Taxus cuspidata,* both in Jaroszowka spring. These two species were first reported in North America by Sideris [21] as plant root parasites. *Pythium conidiophorum,* described as a parasite of green algae of the genus *Spirogyra* [22], in our study was observed on the pollen of *Chamaecyparis pisifera* in Jaroszowka spring. *Pythium coloratum* reported from South Australia as a soil saprophyte [23], was found to grow on the pollen of *Tsuga diversifolia* and *Picea pungens* in the Suprasl river. *Pythium debaryanum* var. *viticola,* known as a parasite of vine-plant roots [24] was found on the pollen of spruce *Picea pungens* in Jaroszowka spring. *Pythium nagae* was encountered on the pollen grains of *Picea abies* in the Suprasl river and Dojlidy pond. It was first described in Japan as a fungus causing damage to rice roots [25]. *Pythium nelumbii,* also described in Japan as a parasite of lotus roots [26] was found on the pollen grains of larch *Larix kaempferi* in the water of all three reservoirs involved in the analysis. *Pythium splendens,* described by Braun [27] as a parasite of plants of the genus *Geranium,* in our study was found on the pollen grains of two species - fir *Abies koreana* and spruce *Picea pungens* in Jaroszowka spring and Dojlidy pond. *Pythium undulatum* var. *litorale,* found on the pollen of fur *Abies nordmanniana* in pond Dojlidy, was first described in German estuaries as a cellulose-decomposing saprophyte [28]. *Pythium vexans,* first described as a saprophyte of potato bulbus by de Bary [29], was observed on the pollen grains of *Juniperus procumbens* in Jaroszowka spring.

Most zoosporic fungus species were found to grow on gymnosperm pollen grains in Dojlidy pond. We observed a similar phenomenon when studying angiosperm pollen [30]. Among the three water reservoirs included in the study, the water of Dojlidy pond has the smallest

Table 3. Aquatic fungi found on the pollens of particular coniferous species.

Taxa	Species of coniferous (see Table 2)	Total number
Chytridiomycetes Olpidiales 1. *Olipdium appendiculatum* Karling	3,5,7,8,9,12,21,28,30,35,36	11
2. *Olpidium endogenum* (Braun) Schroeter	15	1
3. *Olpidium longicollum* Uebelmesser	2,3,4,6,7,8,9,10,11,13,14,16,21,22,28,29,30,33,34	19
3. *Olpidium pendulum* Zopf	15,17,22,24	4
Chytridiales 5. *Achlyella flahaultii* Lagerheim	15,20,22,23,27,31	6
6. *Blyttiomyces aureus* Booth	6	1
7. *Blyttiomyces heliscus* Sparrow et Barr	2,7,8,9,10,11,14,16,19,25,32,33,34,35	14
8. *Chytridium xylophilum* Cornu	17,26	2
9. *Chytriomyces annularus* Dogma	17	1
10. *Chytriomyces aureus* Karling	1,20,22,23	4
11. *Chytriomyces mamillifer* Persiel	3,9,15,17,18,23,24,27,29,31,32,33,34	13
12. *Chytriomyces poculatus* Willoughby et Townley	7,14,17,22,25,28,32,35	8
13. *Chytriomyces vallesiacus* Persiel	2,6	2
14. *Karlingia rosea* (de Bary et Woronin) Johanson	2,3,4,6,7,9,10,12,13,14,16,19,21,25,28,30,32,33,34,35	20
15. *Phlyctochytrium biporosum* Couch	1,3,4,8,20,24,28,34,36	9
16. *Phlyctochytrium circulidentatum* Umphlett	25	1
17. *Phlyctochytrium indicum* Karling	10	1
18. *Phlyctochytrium laterale* Sparrow	26	1
19. *Phlyctochytrium multidentatum* Umphlett	1,15	2
20. *Phlyctochytrium papillatum* Sparrow	25	1
21. *Rhizophydium bullatum* Sparrow	2,5,29,30,35,36	6
22 . *Rhizophydium carpophilum* (Zopf) Fisher	2,4,6,7,8,9,14,16,17,18,19,22,26,27,31,36	16
23. *Rhizophydium contractophilum* Canter	31	1
24. *Rhizophydium globosum* (Braun) Rabenhorst	1,4,5,6,7,9,10,11,12,13,14,16,18,19,21,26,29,33,36	19
25. *Rhizophydium halophilum* Ubelmesser	3	1
26. *Rhizophydium keratinophilum* Karling	1,2,8,9,13,14,17,21,22,23,27,29,32,33	14
27. *Rhizophydium pollinis-pini* (Braun) Zopf	1,3,8,9,10,12,15,17,18,19,20,22,23,24,26,27,28,31,35	19
28. *Rhizophydium racemosum* Gaertner	4,11,29	3
29. *Rhizophydium sphaerotheca* Zopf	15,18,24,25,26,33	6
30. *Rhizophydium subangulosum* (Braun) Rabenhorst	2,7,13,21,23,30,32	7
31. *Rhizophydium utriculare* Uebelmesser	25,30,34	3
32. *Rhizophlyctis harderi* Uebelmesser	7,31	2
33. *Zygorhizidium willei* Lowenthal	20	1
Hyphochytriomycetes Hyphochytriales 34. *Rhizidiomyces apophysatus* Zopf	6,23,24,26,27,31	6
35. *Rhizidiomyces bivellatus* Nabel	17	1
36. *Rhi zidiomyces hirsutus Karling*	17,27,31	3
Oomycetes Lagenidiales 37. *Lagenidium pygmaeum* Zopf	23,24,27	3
38. *Myzocytium microsporum* (Karling) Sparrow	2,4,5,7,9,10,11,13,15,16,19,20,25,28,29,30,32,33,34,35,36	21
39. *Myzocytium vermicolum* (Zopf) Fischer	17,20	2
40. *Olpidiopsis karlingiae* Karling	6,13	2
Saprolegniales 41. *Aphanomyces irregularis* Scott	4,6,7,13,14,18,19,31	8
42 *Saprolegnia turfosa* (Minden) Gaumann	4	1
Leptomitales 43. *Leptomitus lacteus* (Roth) Agardh	34	1
Peronosporales 44. *Pythium afertile* Kanouse et Humphrey	33	1
45.* *Pythium akanense* Tokunaga	6,18	2
46.* *Pythium angustatum* Sparrow	13,33	2
47. *Pythium aquatile* Hohnk	5,7,19,32	4
48.* *Pythium araiosporon* Sideris	12	1
49.* *Pythium ascophallon* Sideris	2	1

Continued of Table 3

Taxa	Species of coniferous (see Table 2)	Total number
50. *Pythium butleri* Subramaniam	5,34	2
51. *Pythium cactacearum* Preti	10,36	2
52. *Pythium catenulatum* Matthews	6,7,10,12,13,18,29	7
53.* *Pythium conidiophorum* Jokl	29	1
54.* *Pythium coloratum* Vaartaja	10,18	2
55.* *Pythium debaryanum* var. *viticola* Jain	18	1
56. *Pythium echinulatum* Matthews	29,32	2
57. *Pythium elongatum* Matthews	9	1
58. *Pythium epigynum* Hohnk	5	1
59. *Pythium globosum* Walz	8,13,32,36	4
60. *Pythium graminicola* var. *stagni* Hohnk	3	1
61. *Pythium helicandrum* Drechsler	11,28	2
62. *Pythium marchantiae* Nicolas	28	1
63. *Pythium megalacanthium* de Bary	3,12	2
64. *Pythium multisporum* Poitras	8,14,18	3
65.* *Pythium nagae* S. Ito et Tokunaga	11	1
66.* *Pythium nelumbii* Takahashi et Ohuchi	21	1
67. *Pythium oligandrum* Drechsler	10	1
68. *Pythium palingenes* Drechsler	2,12	2
69. *Pythium papillatum* Matthews	6,11,18,19,32,33,34	7
70. *Pythium periilum* Drechsler	33	1
71. *Pythium periplocum* Drechsler	10,12	2
72. *Pythium perniciosum* Serbinow	5,21	2
73. *Pythium proliferum* de Bary	16	1
74. *Pythium rostratum* Butler	16	1
75.* *Pythium splendens* Braun	4,18	2
76. *Pythium sylvaticum* Campbell et Hendrix	30	1
77. *Pythium tenue* Gobi	13	1
78. *Pythium torulosum* Coker et Patterson	12	1
79. *Pythium ultimum* Trow	11	1
80. *Pythium undulatum* Petersen	13	1
81.* *Pythium undulatum* var. *litorale* Hohnk	5	1
82.* *Pythium vexans* de Bary	33	1
Zygomycetes Zoopagales 83. *Zoophagus insidians* Sommerstorff	27	1

* Were recorded for the first time from Poland

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

Water from	Fungi (see Table 3)	Only in one water	Total number
Jaroszówka Spring	1,3,4,5,7,8,11,12,13,14,15,16,17,19,21,22,24,26,27,28,29,30,31,32, 34,36,37,38,44,47,48,49,51,52,53,55,56,59,60,63,64,66,69,71,74,75, 78,79,82,83	17,32,48,49,52,55,60,63,74,78,82	50
Supraśl River	1,3,4,5,6,7,10,11,12,13,14,15,19,20,21,22,23,24,26,27,28,30,31,33, 34,35,36,37,38,40,41,42,45,46,47,50,53,54,57,58,62,65,66,68,69,70, 71,72,79	20,23,33,40,42,45,54,57,58,62,70	49
Dojlidy Pond	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,18,21,22,24,25,26,27,28,29,30, 31,34,35,36,37,38,39,41,43,44,46,50,51,53,56,59,61,64,65,66,67,68, 69,72,73,75,76,77,80,81,83	2,9,18,25,39,43,61,67,73,76,77, 80,81	57

amounts of biogenes. It is likely that the higher content of biogenic salts in the other two reservoirs is a factor inhibiting the growth of certain zoosporic fungus species. As shown by the studies of Zemek et al. [31], Chandrashekar and Kaveriappa [32], Abdullah and Taj-Aldeen [33], aquatic fungi colonizing a given substratum secrete enzymes which break it down. They also possess enzymes which decompose plant cellular walls [34], including pectinases [35]. Pollen grains of seed-bearing plants have a complex structure. The cellular wall of pollen grains consists of two basic layers - external known as exine and internal - intine. The exine is made of sporopollenines (polymers and carotenoid esters) [36] which are very resistant to environmental factors. Similar substances are also found in the spores of algae, mushrooms and pteridophytes [37]. The intine is built of cellulose, pectin substances, calloses, polysaccharides and proteins [38].

Thus, in the spring time aquatic fungi, with a number of enzymes to break down plant substrata, use large quantities of plant pollen of such trees as pine, spruce or birch growing in mixed forests. During the dusting time a yellow coating of pollen covers forest water reservoirs.

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