

Phyllosphere mycobiota on garden pond plants

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Investigations were conducted on calamus, common cattail, soft rush, yellow iris and white water lily plants in twenty ponds in Malopolska and Podkarpacie Regions. Mycobiota existing in the phyllosphere caused discolouring and necroses of leaves and shoots. 88 species of mycobiota were identified and isolated from the diseased tissues. Dominant were *Alternaria alternata*, *Epicoccum nigrum* and *Isaria farinosa*. Fungi of genera: *Aspergillus*, *Botrytis*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Ilyonectria*, *Mortierella*, *Mucor*, *Penicillium*, *Phialophora*, *Phoma*, *Pleustomophora*, *Sordaria*, *Trichoderma* and *Umbelopsis* were also numerous. The monophagous and the polyphagous were identified.

Key words: garden pond, water plants, phyllosphere, fungi, chromistan fungi, plant diseases

INTRODUCTION

Garden pond is a specific system functioning on the border of water and land ecosystem. Plant phyllosphere is a natural place of mycobiota existence. In a pond environment mycobiota pressure on various water plant species is heightened, which results in the occurrence of disease symptoms.

The problems of water plants' health state, as results from the literature of the subject, has been still a rarely addressed issue, therefore presented investigations aimed at determining the species composition of the mycobiota settling the phyllosphere of ponds and causing diseases visible as colour changes and necrosis.

Presented work is a recapitulation of research results showing taxonomic and species diversity of the mycobiota existing in the phyllosphere of five plants: calamus, common cattail, soft rush, yellow iris and white water lily – invariable components of pond ecosystem (Kowalik, Maik 2010; Kowalik 2011a, b; Kowalik, Cwynar 2011; Kowalik 2012).

MATERIALS AND METHODS

Field investigations were conducted during four vegetation seasons (2006, 2008-2010) in twenty ponds situated in house gardens in the Malopolska and Podkarpacie Regions. The investigations covered forty plants from each species: calamus *Acorus calamus* L., common cattail *Typha latifolia* L., soft rush *Juncus effusus* L., yellow iris *Iris pseudoacorus* L. and white water lily *Nymphaea alba* L. Each year, five observations were carried out from April to October at four-six-weekly long intervals. Mycobiota were determined on the basis of etiological symptoms and microscopic analyses of mycelium, sporodochia, pycnidia, conidophores and conidia forming on diseased plants. 3200 infected fragments of necrotic plant tissues, including 600 fragments of calamus, soft rush and common cattail, each and 700 fragments of yellow iris and white water lily, each were collected for mycological analyses. Isolation and culturing of mycobiota were conducted using the standard methods practised in mycology. Mycobiota were classified to appropriate species using mycological keys: Booth (1971); Batko (1975); Domsch et al. (1980), Sutton (1980); Ho (1981); Ellis and Ellis (1987); Ramirez (1982); Sivenesan (1984).

RESULTS

In result of mycological analysis of the diseased tissues 2675 colonies of *Fungi* and fungi-like organisms *Chromista* were obtained, which belong to the following genera: *Alternaria*, *Aspergillus*, *Botrytis*, *Chaetomium*, *Cladosporium*, *Davidiella*, *Drechslera*, *Epicoccum*, *Fusarium*, *Humicola*, *Ilyonectria*, *Isaria*, *Khuskia*, *Mortierella*, *Mucor*, *Penicillium*, *Phaeosphaeria*, *Phialophora*, *Phoma*, *Phyllosticta*, *Phytophthora*, *Rhizopus*, *Sordaria*, *Trichoderma*, *Umbelopsis* and other (Tab. 1).

The mycobiota were represented by 88 species, which evidences species diversity in pond phyllosphere. The least number of species – 27 settled calamus and the highest – 43 common cattail. 37 were isolated from yellow iris tissues, 34 from soft rush, whereas 40 fungi species and 2 species of fungus-like organisms were isolated from white water lily.

The following were prevailing in the identified mycobiota community: *Alternaria alternata* constituting 33.23% of the whole community, *Epicoccum nigrum* – 10.24% and *Isaria farinosa* – 9.35%. In the influent group (making up from 1 to 5% of the mycobiota community) identified were: *Aspergillus niger* f. *niger*, *Botrytis cinerea*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Ilyonectria radicularis*, *Mortierella alpina*, *Mucor hiemalis*, *Penicillium aurantiogriseum*, *P. expansum*, *P. hirsutum* var. *hirsutum*, *P. verrucosum*, *Phoma medicaginis* var. *medicaginis*, *Pleustomophora richardsiae*, *Sordaria fimicola* and *Umbelopsis isabellina*.

Polyphagous species, such as: *A. alternata*, *A. niger* f. *niger*, *E. nigrum*, *Fusarium poae*, *I. farinosa*, *M. alpina*, *S. fimicola* and *U. isabellina* were identified among fungi existing in water plant phyllosphere. These fungi were settling necrotic tissues of all five plant species. *B. cinerea*, *Ch. globosum*, *C. cladosporioides*, *P. aurantiogriseum*, *P.*

expansum, *P. verrucosum*, *Phialophora cyclaminis*, *Ph. medicaginis* var. *medicaginis*, *Pleustomophora richardsiae* and *Rhizopus stolonifer* existed on four plant species in ponds.

The monophagous were less numerous: *Ascochyta acori* was isolated from calamus, *Phaeosphaeria eustoma* (syn. *Leptosphaeria typhae*), *Ph. typharum* (syn. *L. typharum*) and *Periconia typhicola* from common cattail, whereas *Physalospora scirpi* (syn. *Arthrimum curvatum* v. *minus*), *Phaeosphaeria juncina* (syn. *Leptosphaeria juncina*), *Morenoina paludosa*, *Paraphaeosphaeria michotii* and *Septoriella junci* from soft rush.

Phytophthora citricola and *Ph. undulata* chromistan fungi were isolated from diseased petioles of white water lily.

Disease symptoms were visible on pond plants in all years of the investigations.

Broad spots, yellow in colour visible on calamus in May and June were located in top leaf parts, in the late summer season the colour changed to brownish-brown. The border of spots was changing colour from yellow through reddish to light brown. The spots were situated all over the leaf surface. Another symptom were narrow spindle-shaped brown smudges and small, round dots with red-russet rim located along the vein. Tissue in places of the spot underwent necrosis and the heaviest intensity of necroses occurred in October. The following were most frequently isolated from the diseased tissues: *A. alternata*, *I. farinosa*, *E. nigrum*, *B. cinerea* and *A. niger* f. *niger*.

Common cattail plants revealed first disease symptoms in May, visible as yellowish discolorations on top and base of leaves. In September the symptoms were visible on the surface of leaf blades as brown-red spots and smudges. By the end of vegetation necrotic spots covered the whole leaf blade area. Beside *A. alternata*, *I. farinosa* and *E. nigrum*, the most numerous fungi isolated from the necrotized tissues included *P. expansum*, *S. fimicola* and *P. richardsiae*.

The first symptoms of leaf spot disease were visible on soft rush in April as delicate chlorotic discolourings in leaf top parts. With time the leaves were yellowing and browning. Streaks of chlorotic tissue changing its colour to brown-black were noticeable on stems. These symptoms sometimes led to dieback of plant clusters in a pond. The species dominant on necrotised soft rush tissues were also *A. alternata*, *I. farinosa* and *E. nigrum*, *B. cinerea*, *M. alpina* and *S. fimicola* were numerous, too.

Disease symptoms and their exacerbation on yellow iris leaves were diversified in individual vegetation periods. Generally, first disease symptoms were observed in May and the greatest intensification occurred in September or October. Small elongated or oval yellowish spots were visible on leaf blade, which in time changed their colour to orange or brown. The spots were surrounded by a clear reddish or brown rim. Initially top leaf parts were dying back, whereas by the end of vegetation a full necrosis of leaf blades occurred. Spots and necrosis of yellow iris leaves were most frequently caused by: *A. alternata*, *E. nigrum*, *I. farinosa*, *Ph. medicaginis* var. *medicaginis* and *Phyllosticta pseudoacori*.

Leaf spot diseases, called brown leaf spot disease was visible on white water lily every year and in all ponds. The symptoms of disease, visible as tiny chlorotic discolourings of leaf blade, appeared in May. The symptoms were intensifying gradually and by the end of vegetation brown spots surrounded with yellowish or reddish rim covered a considerable part of leaf. Necrotic tissue was covered by a coating of a concentrically zoned mycelium. The tissue was crumbled and leaf perforation

Table 1
Mycobiota isolated from affected tissues of garden pond plants

Fungus	Calamus <i>Acorus calamus</i>	Common cattail <i>Typha latifolia</i>	Soft rush <i>Juncus effusus</i>	Yellow iris <i>Iris pseudocacorus</i>	White water lily/ <i>Nymphaea alba</i>	Total	Percentage [%]
<i>Alternaria alternata</i> (Fr.) Keissl.	277	183	110	215	104	889	33.23
<i>Arthrinium sphaerospermum</i> Fuckel		1				1	0.04
<i>Arthrinium sporophleum</i> Kunze		5				5	0.19
<i>Arthrinium urticae</i> M. B. Ellis		1				1	0.04
<i>Arthroderma tuberculatum</i> Kuehn			1			1	0.04
<i>Ascochyta acori</i> Oudem.	3					3	0.11
<i>Aspergillus alutaceus</i> Berk. et M. A. Curtis			1			1	0.04
<i>Aspergillus nidulans</i> (Eidam) G. Winter var. <i>nidulans</i>				5		5	0.19
<i>Aspergillus niger</i> f. <i>niger</i> Tiegh.	20	16	9	2	7	54	2.02
<i>Aspergillus versicolor</i> (Vuill.) Tirab.		6		7	8	21	0.79
<i>Boeremia exigua</i> var. <i>exigua</i> (Desm.) Aveskamp, Gruyter et Varkley		1		1		2	0.07
<i>Boeremia hedericola</i> (Durieu et Mont.) Aveskamp, Gruyter et Verkley	1	6				7	0.26
<i>Botryotinia convoluta</i> (Drayton) Whetzel				6		6	0.22
<i>Botrytis cinerea</i> Pers.	23		22	10	2	57	2.13
<i>Chaetomium cochlioides</i> Palliser			1	3		4	0.15
<i>Chaetomium elatum</i> Kunze		1		7		8	0.30
<i>Chaetomium globosum</i> Kunze	23	14	6		3	46	1.72
<i>Cladosporium cladosporioides</i> (Fresen) G. A. de Vries		1	13	1	12	27	1.01
<i>Cladosporium herbarum</i> (Pers.) Link			1			1	0.04
<i>Cladosporium sphaerospermum</i> Penz.		4			9	13	0.49
<i>Clonostachys rosea</i> f. <i>rosea</i> (Link) Schroers, Samuels, Seifert et W. Gams		5				5	0.19
<i>Coleophoma empetri</i> (Rostr.) Petr.		1	2		6	9	0.34
<i>Davidiella macrocarpa</i> Crous, K. Schub. et U. Braun		7		6		13	0.49
<i>Diplodia melaena</i> Lév.					3	3	0.11
<i>Drechslera poae</i> (Baudys) Shoemaker			16			16	0.60
<i>Epicoccum nigrum</i> Link	33	108	31	90	12	274	10.24
<i>Fusarium culmorum</i> (W. G. Sm.) Sacc.	9	4	1			14	0.52
<i>Fusarium oxysporum</i> Schldtl.					17	17	0.64
<i>Fusarium poae</i> (Peck) Wollenw.	4	1	4	2	7	18	0.67
<i>Fusarium sporotrichioides</i> Sherb.	9				2	11	0.41
<i>Gibberella tricineta</i> El-Gholl, McRitchie, Schoult et Ridings				4	1	5	0.19
<i>Humicola fuscoatra</i> Traaen	3	4		5		12	0.45
<i>Humicola grisea</i> Traaen var. <i>grisea</i>	2			7		9	0.34
<i>Ilyonectria radiciala</i> (Gerlach et L. Nilsson) Cheverri et C. G. Salgado			3		28	31	1.16
<i>Isaria farinosa</i> (Holmsk.) Fr.	38	44	82	45	41	250	9.35
<i>Khuskia oryzae</i> H. J. Huds.	8			3		11	0.41
<i>Mammaria echinobotryoides</i> Ces.		1		2		3	0.11
<i>Morenoina paludosa</i> J. P. Ellis			6			6	0.22
<i>Mortierella alpina</i> Peyronel	11	4	19	1	10	45	1.68
<i>Mortierella parvispora</i> Linnem.	2				1	3	0.11
<i>Mucor hiemalis</i> Wehmer		1		9	22	32	1.20
<i>Mycosphaerella iridis</i> (Auersw.) J. Schröt.				2		2	0.07
<i>Myrothecium cinctum</i> (Corda) Sacc.		10				10	0.37

Table1– cont.

<i>Neottiospora caricina</i> (Desm.) Höhnelt				3		3	0.11
<i>Nowakowskiella elegans</i> (Nowak.) Schröt.	1					1	0.04
<i>Paecilomyces variotii</i> Bainier					1	1	0.04
<i>Paraconiothyrium minitans</i> (W. A. Campb.) Vėrkley			2			2	0.07
<i>Paraphaeosphaeria michotii</i> (Westend.) O. Erikss.			1			1	0.04
<i>Paraphoma chrysanthemicola</i> (Hollós) Gruyter, Aveskamp et Varkley			3		2	5	0.19
<i>Penicillium aurantiogriseum</i> Dierckx	16		9	3	8	36	1.35
<i>Penicillium citrinum</i> Thom		6			2	8	0.30
<i>Penicillium expansum</i> Link	19	34		1	32	86	3.21
<i>Penicillium hirsutum</i> var. <i>hirsutum</i> Dierckx	15			10	15	40	1.50
<i>Penicillium lanosum</i> Westling					12	12	0.45
<i>Penicillium verrucosum</i> Dierckx	12	19		1	79	111	4.15
<i>Penicillium waksmanii</i> K. M. Zalesky		1			12	13	0.49
<i>Periconia typhicola</i> E. W. Mason et M. B. Ellis		2				2	0.07
<i>Pestalotiopsis sydowiana</i> (Bres) B. Sutton					1	1	0.04
<i>Phaeosphaeria eustoma</i> (Fuckel) L. Holm		1				1	0.04
<i>Phaeosphaeria juncina</i> (Auersw.) L. Holm			4			4	0.15
<i>Phaeosphaeria typharum</i> (Desm.) L. Holm		4				4	0.15
<i>Phialophora cinerescens</i> (Wollenw.) J. F. H. Beyma			10	2		12	0.45
<i>Phialophora cyclaminis</i> J. F. H. Beyma	3	5		10	4	22	0.82
<i>Phoma leveillei</i> Boerema et G. J. Bollen var. <i>leveillei</i>		2	17			19	0.71
<i>Phoma medicaginis</i> var. <i>medicaginis</i> Malbr. et Roum.	7	7	5	24		43	1.61
<i>Phyllosticta pseudacori</i> (Brunaud) Allesch.				11		11	0.41
<i>Physalospora scirpi</i> (Gutner) Arx			6	5		11	0.41
<i>Phytophthora citricola</i> Savada					3	3	0.11
<i>Phytophthora undulata</i> (H. E. Petersen) M. W. Dick					12	12	0.45
<i>Pleustomophora richardsiae</i> (Nannf.) L. Hostert, W. Gams et Crous		21	3	6	4	34	1.27
<i>Rhizopus arrhizus</i> A. Fisch. var. <i>arrhizus</i>	10					10	0.37
<i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.	9	6	1	1		17	0.64
<i>Scopulariopsis koningii</i> (Oudem.) Vuill.		1				1	0.04
<i>Septoriella junci</i> (Desm.) B. Sutton		1	9			10	0.37
<i>Sordaria fimicola</i> (Roberge et Desm.) Ces et De Not.	5	25	18	9	24	81	3.03
<i>Talaromyces wortmannii</i> C. R. Benj.					7	7	0.26
<i>Thielavia terricola</i> (J. G. Gilman et E. V. Abbott) C. W. Emmons					1	1	0.04
<i>Trichoderma aureoviride</i> Rifai					16	16	0.60
<i>Trichoderma harzianum</i> Rifai		5	2			7	0.26
<i>Trichoderma koningii</i> Oudem.					2	2	0.07
<i>Trichoderma pseudokoningii</i> Rifai					14	14	0.52
<i>Trichoderma viride</i> Pers.					8	8	0.30
<i>Trichothecium roseum</i> (Pers.) Link		3		2		5	0.19
<i>Ulocladium botrytis</i> Preuss		2				2	0.07
<i>Umbelopsis isabellina</i> (Oudem.) W. Gams	2	3	5	9	15	34	1.27
<i>Umbelopsis nana</i> (Linnem.) Arx					4	4	0.15
<i>Volutella arundinis</i> Desm.			7			7	0.26
Total	565	577	430	530	573	2675	100.00

happened. The most numerous species in the community of isolated fungi were: *A. alternata*, *P. verrucosum*, *I. farinosa*, *P. expansum* and *I. radicola*.

In two ponds *Ph. citricola* and *Ph. undulata* were isolated from white water lily tissues.

The symptoms on rhizomes, petioles and leaf blades pointed to an occurrence of a disease determined as a black rot on rhizomes.

DISCUSSION

A comparison of results of mycobiota isolation from phyllosphere of plants which are the fixed components of the analyzed ponds and information about the occurrence of diseases on their aboveground and underground organs (Westcott 1971; Van der Aa 1978; Sutton 1980; Ellis, Ellis 1987; Helberg 1998; Wagner 2000; Kowalik, Krasny 2009; Mazurkiewicz-Zapałowicz, Grajewski 2010), revealed the most serious pathogen threat from the polyphagous, such as: *A. alternata*, *B. cinerea*, *E. nigrum*, *S. fimicola*. These fungi, most numerous isolated by the end of vegetation period colonized plant tissues causing necrosis.

The fungi constantly accompanying pond plants included also those from *Mortierella* and *Umbelopsis* genera, identified as hydrophilous (Domsch et al. 1980) and fungi from *Aspergillus*, *Penicillium*, *Isaria*, and *Phialophora* genera existing in many ecological niches (Kowalik, Maik 2010; Kowalik 2011b; Kowalik, Cwynar 2011).

Slightly less numerous *Cladosporium* spp. isolated from the phyllosphere are regarded as causes of leaf spot disease, necroses and disturbances in many plants growth, not only in ponds (Westcott 1971; Van der Aa 1978; Płaskowska 2010; Kowalik 2011b).

A fact of settling the petioles and leaf blades by *Fusarium* spp. is noticeable in the presented investigations. These fungi, especially *F. culmorum*, *F. poae* and *F. sporotrichioides*, which beside *A. alternata*, *A. niger* f. *niger*, *P. aurantiogriseum*, *P. hirsutum* var. *hirsutum*, and *P. verrucosum* are counted to toxicogenous ones which intensify necrotization process considerably contributing to fast destruction of plant tissues (Płażek 2011; Chełkowski 2012).

Fungi from *Trichoderma* genus, which are well known for their antagonism towards pathogenic fungi from *Fusarium* and *Ilyonectria* genera, were relatively little numerous on the plant tissues (except for white water lily).

Intensified occurrence of necrotrophs from *Alternaria*, *Sordaria*, *Epicoccum*, *Chaetomium*, *Mucor* and *Rhizopus* genera was noticed by the end of vegetation period, (in October), which confirms their ability to develop in dead tissues of the diseased plants.

Biodiversity of mycobiota in plant phyllosphere is evidenced by isolation of incidental saprotrophs which may develop both on live and dead tissue. *Arthrinium*, *Boeremia*, *Diplodia*, *Mammaria*, *Nowakowskiella*, *Paraconiothyrium*, *Scopulariopsis*, *Thielavia*, *Ulocladium* and other fungi only occasionally present on diseased shoots and leaves may be also counted to this group.

Isolating from water plants the monophagous, which are strict parasites infesting calamus and soft rush in rush communities, Adamska 2005; Mazurkiewicz-Zapałowicz et al. 2006; Mazurkiewicz-Zapałowicz, Grajewski 2010) and wild sedge, testifies their expansiveness and proves that they sporulate both on wild plants and ornamental cultivars (Czerniawska, Adamska 2009).

In ponds particular attention should be paid to hazard provided by *Phytophthora*, because chromistan fungi which belong to these genera commonly colonize perennial plants, dwarf shrubs, coniferous and deciduous plants in their natural environment, whereas water is the agent spreading propagule (Orlikowski 2006). The hazard has been confirmed by isolation of *Ph. citricola* and *Ph. undulata* from white water lily plants.

It should be surmised that the composition of mycobiota settling the phyllosphere of plants depended on plant physiological properties and active substance content (ethereal oils, alkaloids, phytoncides and dyes), therefore the least number of fungi existed on calamus and soft rush. The role of fungi accompanying calamus vegetation and reducing the content of active substance were described by Mazurkiewicz-Zapałowicz and Grajewski (2010).

While comparing the frequency of diseases occurrence on pond plants, it was noticed that while water lily and common cattail plants were the most frequently infested, which confirmed previous reports (Kowalik, Krasny 2009).

Assessing the healthiness of all plants planted in ponds it should be emphasized that it was satisfactory, which does not change the fact that visible leaf spot diseases and necroses on leaves and shoots do not improve aesthetic values of a pond, on the contrary, they greatly diminish them.

CONCLUSIONS

1. Dominance of mycobiota of *Alternaria*, *Botrytis*, *Epicoccum*, *Fusarium*, *Isaria*, and *Penicillium* genera in the phyllosphere of pond plants: white water lily, yellow iris, common cattail, soft rush and calamus results from their common occurrence in the environment, ability for fast spreading on plants growing in close vicinity, abilities for facultative parasitism and considerable reproductive potential.
2. It was stated that mycobiota of *Botrytis*, *Ilyonectria*, *Fusarium*, *Phialophora*, *Pleustomophora*, *Phoma* and *Phytophthora* accompanying the plants in a pond during vegetation season caused diverse discolourings and necroses on leaves and shoots.
3. Intensified tissue necrotization process on all plants in ponds occurred by the end of vegetation process and was connected with the frequency of occurrence in the phyllosphere of necrotrophs of *Alternaria*, *Sordaria*, *Epicoccum*, *Chaetomium*, *Mucor* and *Rhizopus* genera.
4. While causing necroses, phyllosphere mycobiota of pond plants contributed to a destruction of tissues, which resulted in a diminishing of aesthetic values of ponds.

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Fyllosferowe mykobiota roślin oczek wodnych

Streszczenie

W badaniach terenowych i laboratoryjnych przeprowadzonych w latach 2006, 2008-2010, w dwudziestu oczkach wodnych usytuowanych na terenie Małopolski i Podkarpacia określono stan zdrowotny: tataraku zwyczajnego *Acorus calamus* L., pałki szerokolistnej *Typha latifolia* L., situ rozpierzchłego *Juncus effusus* L., irysa żółtego *Iris pseudoacorus* L. i grzybienia białego *Nymphaea alba* L. Mykobiota bytująca w fyllosferze roślin powodowały różnorakie przebarwienia i nekrozy.

Z porażonych tkanek roślin wyodrębniono 2675 kolonii mykobiota, należących do 88 gatunków. W zbiorowisku wyodrębnionych mykobiota dominowały: *Alternaria alternata*, *Epicoccum nigrum* i *Isaria farinosa*. Mniej licznie izolowano grzyby z rodzajów: *Aspergillus*, *Botrytis*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Ilyonectria*, *Mortierella*, *Mucor*, *Penicillium*, *Phialophora*, *Phoma*, *Pleustomophora*, *Sordaria*, *Trichoderma* i *Umbelopsis*.

Fyllosferowe mykobiota roślin oczek wodnych powodując nekrozy przyczyniały się do destrukcji tkanek, co skutkowało obniżeniem walorów dekoracyjnych oczek wodnych.