Diversity, host, and habitat specificity of oomycete communities in declining reed stands (*Phragmites australis*) of a large freshwater lake

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ABSTRACT

We studied the diversity of oomycetes in a declining reed belt (*Phragmites australis*) of Lake Constance, Germany, using conventional baiting with specific reed and standard oak baits, accompanied by molecular techniques. Apart from an *Aphanomyces* sp. and a *Phytophthora* sp., baiting from reed rhizosphere samples from flooded, as well as drier, littoral sites revealed only *Pythium* spp. A total of 67 oomycete isolates was classified according to PCR-RFLP banding patterns and ITS sequencing, and 18 different sequence types could be separated. The majority of these seemed previously unknown species, as indicated by the degree of similarity to those deposited in nucleotide databases. Species communities in both flooded and drier habitats or both reed and oak baits clearly differed from one another, and only few species occurred in both dry and flooded sites, or in both oak and reed baits. A frequently occurring group of related *Pythium* species appeared to be specifically associated with reed, and these were the only species that proved pathogenic towards this host *in vitro*. Our study proved that unexplored natural ecosystems harbour diverse communities of oomycete species with specific habitat and host preferences within close-by, but ecologically contrasting, sites. Among the species isolated, those associated with the predominating plant might accumulate and thus may be reed pathogens of considerable importance.

Introduction

The oomycetes (*Peronosporomycetes*) is a group of fungus-like organisms that, although ecologically and physiologically similar to true fungi is considered to belong to the kingdom of *Straminipila*, unrelated to the *Eumycota* (Cavalier-Smith 1998; Dick 2001). Several oomycete genera are known as economically significant pathogens of numerous agricultural or horticultural plants, with the most important being the downy mildews, *Pythium*, *Phytophthora*, and *Aphanomyces*. Other taxa infect fish, insects and mammals (Hendrix & Campbell 1973;

van der Plaats-Niterink 1981; Dick 2001). By contrast, many oomycete species are not known to be pathogens, either due to the lack of knowledge on the respective taxa, or due to their strictly saprotrophic lifestyle (van der Plaats-Niterink 1981; Martin 1992; Dick 2001).

In the past decade, a significant increase in interest in soil- and waterborne oomycete pathogens of plants other than agricultural crops has been observed. In particular, *Phytophthora* communities of diverse natural ecosystems were investigated in several studies (e.g. Brasier & Jung 2003). Much knowledge has also accumulated on the involvement

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of *Pythium* spp. in plant diseases or seedling losses of 'wild' plants, or their potential influence on natural plant community composition (e.g. Mills & Bever 1998; Packer & Clay 2000, 2004; Nechwatal & Osswald 2001). Host-specific accumulation of *Pythium* species was shown to occur in the soil beneath certain plant species and, due to negative feedback effects on growth, *Pythium* soil pathogens are believed to contribute to species diversity within plant communities (Packer & Clay 2000; Mills & Bever 1998). Supported by molecular evidence, several new oomycete species, mostly *Pythium* and *Phytophthora*, from natural and non-agricultural ecosystems have been described recently (e.g. Brasier & Jung 2003; Nechwatal & Osswald 2003; Paul 2003). Accordingly, the current estimates of ca 120 *Pythium* and 70 *Phytophthora* species (Dick 2001) by far underestimate the true diversity within these genera. Studies of largely unexplored non-agricultural ecosystems, and the accompanying use of molecular identification tools, such as PCR-RFLP and sequencing, are likely to reveal large numbers of 'new', undescribed oomycete species, with yet unknown functions, capabilities or host ranges.

The common reed (*Phragmites australis*), a large perennial grass with worldwide distribution, is a major component of wetland and littoral plant communities of many freshwater ecosystems. It usually forms large natural monocultures at the land-water interface with several important ecological functions (Haslam 1972). European freshwater reed belts have been suffering from major decline events during the past 50 y (Brix 1999), and due to their aquatic or semi-aquatic nature are likely to harbour large numbers of yet unknown oomycete species, as these are often associated with moist, flooded, or water-logged soil conditions. They may play a major role in the breakdown of plant litter, but may also be reed pathogens of considerable significance, such as the recently described *Pythium phragmitis* (Nechwatal et al. 2005). We investigated the oomycete community, particularly the genus *Pythium*, in declining reed stands of Lake Constance, Germany, using a traditional baiting approach supplemented by molecular identification methods. This study describes diversity, habitat preferences, and pathogenicity of oomycetes in a natural ecosystem, largely undisturbed by agricultural activity, and adds new aspects to the discussion on possible causes and aetiology of reed decline.

Materials and methods

Baiting

Soil samples for the isolation of oomycetes were taken from the rhizosphere of *Phragmites australis* growing in the reed belt of the Lake Constance littoral between April and September 2003. The sampling site is located on the shore of Bodan peninsula close to a nature reserve (9°11'20" E, 47°41'48" N), and lined by mixed alluvial forests. In total, 14 soil samples were taken during the sampling period from permanently flooded ($n=8$) and from drier, i.e. only occasionally flooded sites ($n=6$) along an approx. 50 m strip within the extension of the littoral reed belt. At each occasion (April, May, June, August, September), samples from both habitat types were taken. Samples were subjected to

a bait test using a presumably specific (young reed leaves, greenhouse plants) and a more standard bait (oak leaflets, *Quercus robur*, greenhouse plants). Oak baits have been previously shown to be able to isolate a large variety of oomycete genera from different natural soils (e.g. Jung et al. 1996; Newell & Fell 1996; Nechwatal & Osswald 2001, 2003; Nechwatal & Mendgen 2006a, b). Soil samples were flooded with deionised water, and several baits were spread over the water surface. Infected baits, showing discolouration after ca 3–5 d of incubation at 19 °C, were cut into small segments and plated onto a selective agar medium consisting of V8 agar (as given below), amended with 25 mg l⁻¹ Benomyl (Dupont, Bad Homburg, Germany), 50 mg l⁻¹ pentachloronitrobenzene, 10 mg l⁻¹ rifampicin, 200 mg l⁻¹ ampicillin (all from Sigma-Aldrich, Taufkirchen, Germany). Plates were incubated at 19 °C in the dark. Developing colonies were subcultured, purified from bacterial contaminants and transferred to V8 agar plates (V8A, 100 ml V8 juice, 16 g agar, 3 g CaCO₃, 900 ml distilled H₂O) for further identification and maintenance.

Species characterisation and identification

Preliminary identification and classification of the recovered isolates to genus or species level was based on mycelial growth rate, sporangial and oogonial shape with the aid of the keys and monographs of Waterhouse (1968), van der Plaats-Niterink (1981) and Dick (1990). For molecular identification, DNA from mycelial material scraped off from agar plates was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). PCR amplification of rDNA ITS regions was performed with primer pair ITS4 and ITS6 (White et al. 1990; Cooke & Duncan 1997). The enzymes *AluI*, *HinfI*, *MspI* and *TaqI* (Fermentas, St Leon-Roth, Germany) were used for digesting the amplicons in a total volume of 20 µl. DNA fragments were separated on 3 % agarose gels. The resulting banding patterns allowed the isolates to be separated into groups of identical patterns (RFLP types). Direct sequencing of the PCR products of selected isolates of each of the RFLP types was carried out by MWG Biotech (Martinsried). Forward and reverse strand sequences were aligned, ambiguous signals checked manually, and sequences trimmed to comprise complete ITS1, 5.8S and ITS2 regions only. BLAST searches of the GenBank nucleotide database were performed to find the most closely related species. In cases where several isolates from a given soil sample turned out to be identical according to RFLP banding patterns and ITS sequences, these were counted as one isolate only.

Selected isolates of each of the RFLP types were characterised in detail as to their morphology. Isolates were grown on V8A plates for at least 6 d, and a minimum of 25 diagnostic structures (oospores, oogonia, sporangia, hyphal swellings etc.) were measured at ×400 magnification using a light microscope. Data were confirmed in random checks within all isolates of a particular RFLP type. For the assessment of growth rates all isolates were subcultured onto V8A plates (20 ml), and incubated at 25 °C after the onset of hyphal growth. Growth was recorded daily for 3 d along two straight axes drawn from the inoculation plug, and values averaged. Colony patterns were assessed after at least 6 d growth on 2 % malt

extract agar (MEA). All isolates are stored at the Konstanz University/Phytopathology culture collection.

Phylogenetic analysis

Sequences of each *Pythium* taxon from this study and those of each of their closest relatives (reference taxa from GenBank) were aligned using ClustalW implemented in the BioEdit package (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Complete ITS1, 5.8S, and ITS2 sequences were used for phylogenetic studies, *Aphanomyces* was not included in the alignment. Genetic distances among isolates and NJ trees were calculated using DNADIST and NEIGHBOR from the PHYLIP package (v3.57c, Felsenstein 1995). Tree topology was confirmed in 1K BS replicates using SEQBOOT and CONSENSE (Felsenstein 1995). Trees were drawn using Njplot (Perrière & Gouy 1996).

Community comparison

To compare species diversity in bait-derived oomycete communities, the Shannon–Wiener and Simpson indices were calculated and compared using the program Species Diversity and Richness v. 3.03 (Pisces Conservation Ltd., Lymington). Average sequence divergence (θ_{π}) was calculated using Arlequin 2.000 (Schneider et al. 2000). Aligned complete ITS1, 5.8S, and partial ITS2 sequences for each taxon obtained during this study and their relative frequencies were used for analysis, without the addition of reference taxa. The degree of differentiation between communities from dry and flooded sites, or from reed and oak baits, respectively, was estimated by computing F_{st} statistics using Arlequin. Methods for molecular diversity calculation have been described by Martin (2002) and Arcate et al. (2006).

Pathogenicity

Mature, greenhouse-grown reed (*Phragmites australis*) leaves were used to test the potential pathogenicity of the oomycete isolates towards this host. For each isolate, seven leaves of approximately the same age (i.e. the same position on the culm) were collected, clipped on base and apex to a length of ca 10–12 cm, and placed in glass Petri dishes containing moist filter paper. Leaf blades were inoculated centrally with a disc (4 mm diam) taken from the margin of an actively growing culture of each of the RFLP types/oomycetes tested. A drop of a 0.05 % skimmed milk solution was applied to the agar disc to facilitate adhesion to the leaf surface. Controls received uncolonised V8A plugs. Leaf lesion development was recorded after 6 d of incubation at 19 °C. Experiments were conducted in duplicate for each species.

Results

Baiting, species characterisation, and identification

Baiting from 14 reed soil samples with oak and reed baits revealed a total of 54 *Pythium* isolates, 11 isolates of an undescribed, presumably sterile *Phytophthora* sp. (Nechwatal &

Mendgen 2006b), and two isolates of an *Aphanomyces* with spiny oogonia. One to seven individual oomycete isolates were obtained from a single soil sample. RFLP and sequencing analysis of the ITS1 and ITS2 regions suggested that the oomycete isolates belonged to 18 different taxa, of which a majority of 16 were *Pythium* (Table 1). After restriction analysis with up to four enzymes and subsequent sequencing of one or more RFLP type representatives all isolates could unambiguously be assigned to a given taxon. Despite only minor sequence differences between some of the proposed taxa and low BS support for some of the outer branches (Fig 1), morphological studies confirmed our molecular classification scheme and taxa were tentatively given ‘species’ rank for all subsequent analyses. Morphological data of the species isolated are summarised in Table 2. Multiple isolations of the same taxon per soil sample could subsequently be eliminated, resulting in 48 different oomycete isolates. From oak baits, 33 isolates (13 species) were obtained, whereas reed baits caught 15 isolates (eight species). The most abundant species from dry sites was *Pythium* type III (22 % of all isolates), in flooded sites *Pythium* type X-c and *Phytophthora* sp. were predominant (17 %).

BLAST searches with the ITS sequences generated during this study revealed that most of the oomycetes were undescribed species or not available in public sequence databases. Only four of the 18 taxa could unambiguously be assigned to known species, i.e. were 100 % identical to one or more respective database entries. Sequence identities of the other isolates to known taxa ranged from 88–99 % (Table 1).

The majority of *Pythium* taxa from flooded sites (73 %) belonged to species from clades A and B of Lévesque & De Cock (2004), i.e. had filamentous sporangia, whereas from dry sites, only 44 % had sporangia of this type. *Pythium* isolates from clade B1 (types X-a, X-b, X-c, XIV) all were members of the grass-associated clusters B1d and B1e (Lévesque & De Cock 2004), and were among the dominating in both habitat types (dry: 22 %, flooded: 24 % of all isolates). Unlike all other species, these types were exclusively isolated from reed baits, not from oak baits (Table 1). Type X-c was recently described as *P. phragmitis* sp. nov. (Nechwatal et al. 2005), and type X-b is considered to be a hybrid between *P. phragmitis* and a species close to *P. arrhenomanes* (Nechwatal & Mendgen 2007). Most isolates from clade B2 (types I, I-a, I-b, VI, VII-a) were closely related and close to the *P. dissotocum*/*P. lutarium* complex, with identities of ca 97 %. The frequently occurring type III, as well as types V, VIII and XII from clade K were only distantly related to any other species in that clade. Type III was recently described as *P. litorale* sp. nov. (Nechwatal & Mendgen 2006a). Phylogenetic positioning and clade affiliations of the *Pythium* species from this study and their closest relatives as indicated by ITS sequences is shown in Fig 1.

Community comparison

Of the 18 species recovered from soil samples, only four occurred in both habitats, four were recovered exclusively from dry, ten from flooded sites. Similarly, the community baited with reed was different from that baited with oak leaves, with only three species isolated from both bait types (Tables 1 and 3). Comparing species diversity indices, bait communities from flooded sites and from oak baits were

Table 1 – Composition, clade affiliation, habitat, and bait type of oomycete species communities from reed sites as revealed by baiting techniques

Type/ species ^a	Genus/ <i>Pythium</i> clade ^b	Sporangial type ^c	No. of isolates		No. of isolates		Closest BLAST hit (ITS)	Identity (%)	GenBank accession no.
			From dry sites ($\Sigma = 18$)	From flooded sites ($\Sigma = 30$)	From oak ($\Sigma = 33$)	From reed ($\Sigma = 15$)			
I	B2	F	2	3	4	1	<i>Pythium dissotocum/lutarium</i>	97.3	DQ230902
I-a	B2	F	1	0	1	0	<i>P. dissotocum/lutarium</i>	97.3	DQ230903
I-b	B2	F	0	1	1	0	<i>P. dissotocum/lutarium</i>	97.2	DQ230904
II	F	HS	2	0	2	0	<i>P. intermedium</i>	100	DQ232765
III	K	G	4	3	7	0	<i>P. litorale^d</i>	100	DQ144637
IV	J	G	3	0	3	0	<i>P. perplexum</i>	100	DQ232766
V	K	G	0	1	1	0	<i>P. helicoides</i>	88.5	DQ232768
VI	B2	F	0	2	2	0	<i>P. dissotocum/lutarium</i>	97.6	DQ232767
VII-a	B2	F	0	3	2	1	<i>P. aquatile</i>	96.7	DQ235226
VIII	K	G	0	1	1	0	<i>P. helicoides</i>	92.6	DQ235227
X-a	B1e	F-I	1	0	0	1	<i>P. arrhenomanes</i>	100	AY743661
X-b	B1e	F-I	3	1	0	4	<i>P. phragmitis^d</i>	99.4	AY594258
X-c	B1e	F-I	0	5	0	5	<i>P. phragmitis^d</i>	100	AY594259
XII	K	G	0	1	1	0	<i>P. citrinum</i>	88.4	DQ237932
XIII	A	F	0	1	0	1	<i>P. porphyrae</i>	99	DQ237933
XIV	B1d	F-I	0	1	0	1	<i>P. inflatum</i>	99.6	DQ237934
A	<i>Aphanomyces</i>	–	0	2	1	1	<i>A. cladogamus</i>	93.8	DQ861921
Ph	<i>Phytophthora</i>	–	2	5	7	0	<i>P. taxon 'salixsoil'</i>	100	AY762973

a As revealed by analysis of the ITS1, 5.8S and ITS2 region of the isolates (RFLP, sequencing).

b *Pythium* clades after Lévesque & De Cock (2004).

c Sporangial types in *Pythium*: F, filamentous; F-I, filamentous-inflated; G, globose, ovoid or pyriform; HS, hyphal swellings.

d Species recently described (Nechwatal et al. 2005; Nechwatal & Mendgen 2006a).

significantly more diverse than those from dry sites, or reed baits, respectively.

Molecular diversity indices (mean sequence divergence θ_{π}) of bait communities were largely similar in dry and flooded sites, and according to F_{st} statistics, communities did not differ significantly. In contrast, the community from oak baits was more diverse than from reed, and both were significantly different as expressed by F_{st} statistics (Table 3).

Pathogenicity

Only species from the grass-associated clusters B1d and B1e were pathogenic towards mature reed leaves (Table 2). *Pythium phragmitis* and *Pythium* type X-b caused the most extensive lesions, those caused by *P. arrhenomanes* (X-a) and *Pythium* type XIV were somewhat smaller. The isolate of type VII-a (clade B2) caused minor lesions on some leaves. None of the other species tested or the control inoculations caused damage to the leaves.

Discussion

The past decade has brought much new insight into the role of oomycetes in natural and semi-natural ecosystems. Mainly *Phytophthora*, but also *Pythium* spp., were shown to be significant pathogens of numerous woody and non-woody plant species on a worldwide scale (e.g. Packer & Clay 2000; Brasier & Jung 2003). In this study, we investigated community structure and pathogenicity of oomycetes, particularly the genus

Pythium, in non-agricultural ecosystems, as exemplified by the analysis of the reed belt of a large pre-alpine lake in Germany, which has been reported to be in decline for about five decades.

As was expected from the (semi-)aquatic nature of the investigated sites, the study revealed large numbers of undescribed oomycete species, of which the majority were *Pythium* spp. These results contradict the common textbook view that, although debatable, considers non-cultivated sites to be relatively poor in *Pythium* species (van der Plaats-Niterink 1981). Out of 18 species isolated, only four could unambiguously be assigned to already described species. Two more taxa have recently been described as new species (Nechwatal et al. 2005; Nechwatal & Mendgen 2006a). As reed belts are largely unexplored ecosystems regarding their oomycete community, the high number of species new to science does not come as a surprise. A number of studies in previously unexplored natural ecosystems have also revealed several undescribed *Pythium* species (e.g. Nechwatal & Osswald 2003; Paul 2003; Romero et al. 2007), as well as oomycetes from the *Saprolegniaceae* (e.g. Dick 1971). Consistent with the close association of soilborne oomycetes with moist or flooded sites, oomycete communities from flooded soils were significantly more diverse than those from drier sites in our study (Table 3). As also shown before (Lévesque & De Cock 2004; Nechwatal & Mendgen 2006a), *Pythium* clade K, in particular, proved to be a source of new and highly divergent unknown species (Table 1).

Oak leaves have originally been used as baits in studies on the decline of oak, and highly diverse *Phytophthora* communities were consistently isolated using these baits (e.g. Jung et al.

Fig 1 – NJ phylogenetic tree of *Pythium* spp. detected by isolation from baits (Roman numerals), and their closest GenBank matches, constructed after distance-based analysis of ITS1, 5.8S and ITS2 regions of the rDNA. Numbers at the branches indicate the percentage of BS values after 1K replications (values below 50 % not shown). *Phytophthora* taxon 'Salixsoil' from this study was used as outgroup. Bar = number of nucleotide substitutions per site. The bars on the right margin indicate phylogenetic clades according to Lévesque & De Cock (2004).

1996). The choice was obviously based on the assumption that first evidence for the association of a particular oomycete with a specific host plant can be obtained using baiting material of the host under investigation. However, oak baits were also shown to be able to isolate large sets of *Phytophthora*, *Pythium*, *Saprolegnia*, and *Achlya* spp. from other natural ecosystems (Newell & Fell 1996; Nechwatal & Osswald 2001, 2003; Nechwatal & Mendgen 2006a, b; J.N. unpubl.). The diversity of species isolated in our study again confirmed that oak leaves are suitable as a standard bait, revealing highly diverse sets of oomycete genera and species (Table 3). However, oak baits were obviously not able to detect the set of grass-associated species. This indicates the specific bias brought about by the bait plants used in a particular study, and we cannot exclude the possibility that more taxa would have been found

with additional bait tissues/plants. However, we believe that by using an established standard and a specific (reed) bait, a significant fraction of the species present should have been recovered.

Relatively quick changes in species dominance and composition have been observed for *Pythium* communities (e.g. Hardman & Dick 1987), rendering single-year, single-site studies somewhat uncertain. Nevertheless, although representing only a small fraction in both space and time, the approach applied in our study revealed well-defined habitat or substrate preferences for most of the species detected, consistent with the specific site properties, and indicating that the patterns observed were not merely by chance. Preferences of the taxa were clear-cut, with low levels of species overlap between communities occurring in both habitats or

Table 2 – Morphological characteristics, growth rates and pathogenicity of oomycete species from reed stands

Species/ group	Pythium clade	Sporangial type (Pythium) ^a	Oogonia diam (μm) ^b	Antheridia ^a	Oospore diam (μm) ^b	Oospore state	Oospore wall (μm)	Growth rate (25 °C, V8a) (mm d ⁻¹) ^b	Colony pattern (MEA)	Pathogenicity ^c	Voucher material ^d	Closest BLAST hit (ITS)
I	B2	F	20.5 (0.52)	m	17.5 (0.5)	a	<2	16.9 (2.4)	Faint rosaceous	–	UKN P1	<i>P. dissotocum</i>
I-a	B2	F	–	–	–	–	–	16.4 (n/a)	Faint radiate	–	UKN P9	<i>P. dissotocum</i>
I-b	B2	F	21 (n/a)	m/d	17 (n/a)	a	2.5	20.3 (n/a)	None	–	UKN P33	<i>P. dissotocum</i>
II	F	HS	–	–	–	–	–	30.5 (3.5)	None	–	UKN P2	<i>P. intermedium</i>
III	K	G	–	–	–	–	–	14.7 (0.7)	Rosaceous	–	CBS 118360	<i>P. litorale</i>
IV	J	G	21.5 (1.02)	h?	19.5 (1.1)	a	2	15.3 (1.5)	Faint radiate	–	UKN P5	<i>P. perplexum</i>
V	K	G	–	–	–	–	–	n.d.	n.d.	–	UKN P15	<i>P. helicoides</i>
VI	B2	F	–	–	–	–	–	20.5 (1.7)	Faint rosaceous	–	UKN P17	<i>P. dissotocum</i>
VII-a	B2	F	–	–	–	–	–	15.3 (2.6)	None	+	UKN P23	<i>P. aquatile</i>
VIII	K	G	30.5 (n/a)	d (m)	27.5 (n/a)	a	<2	n.d.	None	–	UKN P21	<i>P. helicoides</i>
X-a	B1e	F-I	–	–	–	–	–	34 (n/a)	None	++	UKN P54	<i>P. arrhenomanes</i>
X-b	B1e	F-I	26.5 (1.3)	m (d)	26.5 (1.3)	p	2.5	31.3 (1.5)	None	++	UKN P11	<i>P. phragmitis</i>
X-c	B1e	F-I	25.5 (1)	m (d)	25 (1)	p	2.5	29 (0.6)	None	++	CBS 117104	<i>P. phragmitis</i>
XII	K	G	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	Rosaceous	–	UKN P32	<i>P. citrinum</i>
XIII	A	F	–	–	–	–	–	8.5 (n/a)	Narrow rosaceous	–	UKN P43	<i>P. porphyrae</i>
XIV	B1d	F-I	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	None	++	UKN P41	<i>P. inflatum</i>
<i>Aphanomyces</i>	–	–	26 (n/a)	m	23 (n/a)	a	<2	15.4 (n/a)	None	–	UKN P24	<i>A. cladogamus</i>
<i>Phytophthora</i>	–	–	–	–	–	–	–	5.4 (0.5)	Rosaceous	–	UKN Ph1	<i>P. taxon salixsoil</i>

a F, filamentous; F-I, filamentous- inflated; G, globose, ovoid or pyriform; HS, hyphal swellings; m, monoclinal; d, diclinous; h, hypogynous; a, aplerotic; p, plerotic; n.d., not determined.

b If applicable, means and s.d. of isolate means are given.

c Pathogenicity towards mature reed leaves: – no lesions, + small lesions, ++ heavy lesions.

d UKN, culture collection Phytopathology Universität Konstanz, Germany; CBS Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

Table 3 – Comparison of diversity indices in oomycete communities from reed stands as revealed by baiting from dry and flooded sites using reed and oak baits

Community	Species diversity		Molecular diversity				
	Species	Shared species	Simpson's D	Shannon–Wiener	Usable loci	Theta (S.D.)	F _{st}
Bait (dry)	8	4	7.235 ^a	1.992 ^b	416	75.3 (38)	0.014 ($P > 0.05$)
Bait (flooded)	14		10.22 ^a	2.415 ^b	398	67.8 (33.5)	
Bait (oak)	13	3	8.292 ^a	2.277 ^b	419	81.6 (40)	0.276 ($P < 0.001$)
Bait (reed)	8		5.125 ^a	1.820 ^b	430	35.1 (18.2)	

Level of significance of differences between communities from different habitats or baits, respectively:
a $P < 0.05$.
b $P < 0.001$.

baits. In addition, various diversity indices confirmed the differentiation between the communities from the different substrates (Table 3). However, the sequence divergence index θ_π did not reflect the clear differentiation of communities from dry versus flooded habitats (Table 3), due to the high frequency of closely related taxa (e.g. I, I-a, I-b, or X-a, X-b, X-c) that all have been given species rank in our study. Therefore, the index, although a useful tool (Martin 2002; Arcate et al. 2006), might underrate community diversity and differentiation in certain cases; as repeatedly shown for various oomycete genera, even low degrees of ITS sequence divergence can be used to delineate ecologically, morphologically and pathologically distinct species (e.g. Nechwatal et al. 2005).

According to our data, the oomycete species detected in the investigated sites can clearly be subdivided into two major functional categories: (1) species not or loosely associated with common reed; and (2) species closely associated with reed. The first group comprises a set of species that, under the conditions studied, can be considered at least facultatively saprobic, with the dominating representatives being species from clades B2 and K, which are generally considered to be non- or weakly pathogenic species (Lévesque & De Cock 2004). In the baiting campaign, nine different members of these two clades alone were found (Table 1). They were almost exclusively isolated from oak baits, and did not cause any considerable damage to mature reed leaves, indicating the absence of a close association with reed, the dominating plant in the investigated sites. Although pathogenicity towards other plants cannot be excluded, together with members of other less abundant types (e.g. II, IV, XIII), these species are obviously colonising substrates consisting of various types of plant debris (e.g. fallen tree leaves, dead reed material, submerged macrophytes) during different stages of decay. The second group consists of a set of grass pathogens, belonging to clusters B1d and B1e of Lévesque & De Cock (2004). In particular, *P. phragmitis*, among the most frequently occurring oomycetes, is considered an important factor in reed decline (Nechwatal et al. 2005). *P. arrhenomanes* (X-a), *P. aff. inflatum* (XIV), and the putative hybrid (X-b) are likely to be associated with reed. They are closely related to important grass pathogens (van der Plaats-Niterink 1981), and were shown to be pathogenic towards reed in our study. Accordingly, this group dominated the oomycete community caught with reed baits, indicating

their ability to act as pathogens on this host. Interestingly, *P. arrhenomanes* was frequently isolated from cornfields adjacent to reed stands during a separate isolation campaign (J.N. unpubl.).

Pythium spp. from cluster B1e (and B1d) were among the dominating species in the two habitat types investigated within the reed belt. Four different members of these groups were identified in our study, all of which are likely to be closely associated with reed. The prevalence of these species might be favoured by a particularity of the plant community studied here, i.e. the strong predominance of a single plant species, *Phragmites australis*. Thus, reed stands can be considered natural monocultures similar to agricultural systems, favouring the development and performance of host-specific pathogens. This is particularly true when the primarily clonal reproduction strategy of reed is taken into account, leading to low genetic diversity within a stand. It has been shown for natural (forest) ecosystems that accumulation of specific *Pythium* spp. can occur beneath a plant species, and due to negative feedback, favour the establishment of other species (Packer & Clay 2000). As littoral reed habitats are not suitable for the majority of plant species, negative feedback effects due to pathogen accumulation might become apparent as cycles of reed dieback (and progression), as observed for several European lakes for the past five decades (Brix 1999). These might correlate with the occurrence of water level fluctuations or flooding events, as shown in detail for *P. phragmitis* (Nechwatal et al. 2005, in press). According to the results of this study, a set of different, more or less closely related taxa rather than a single species can contribute to reed damage and decline phenomena in Lake Constance, with each species occupying a specific niche within the reed belt, and possibly being well-adapted to specific environmental conditions. Of these, *P. phragmitis* is the most abundant in flooded soils, while *P. arrhenomanes* seems to be an invading pathogen from adjacent agricultural sites, rather adapted to terrestrial sites. The putative hybrid, type X-b, thrives in flooded as well as in drier soils, and, according to recent field studies, seems to be a widely distributed, stable, and successful evolutionary unit (Nechwatal & Mendgen 2007). Further studies should elucidate the specific ecological prevalence of each of these species, their population dynamics over several consecutive years, and the correlation between their abundance, environmental factors in the littoral, and the extent of reed damage observed.

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