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FUNGI OF THE BAY OF FUNDY V: FUNGI FROM LIVING SPECIES OF SPARTINA SCHREBER

J. DAVID MILLER
*Chemistry and Biology Research Institute**
Agriculture Canada
Ottawa, Ont.
K1A 0C6

and

NORMAN J. WHITNEY
Department of Biology
University of New Brunswick
Fredericton, N.B.
E3B 6E1

Some 26 species of fungi were isolated from living *Spartina* species in the Bay of Fundy. Most (22 species) were Deuteromycetes, 3 were Ascomycetes. One Basidiomycete was identified. Most of these fungi have been reported before on marine angiosperms. This is the first report of *Pleospora spartinae* outside the United Kingdom. The fungi reported are discussed in terms of their possible relationship to terrestrial fungi.

Quelques 26 espéces de champignons furent isolées à partir d'espéces vivantes de *Spartina* de la baie de Fundy. La plupart (22) sont des Deutéromycètes, 3 des Ascomycétes. Un Basidiomycète fut identifié. La présence, sur des Angiospermes marins, de la plupart de ces champignons a déjà été signalée auparavant. Cependant, c'est la première fois que *Pleospora spartinae* est signalé en dehors du Royaume Uni. La possible relation entre les champignons rapportés ici et les champignons terrestres est discutée.

Introduction

There have been a number of studies of fungi associated with *Spartina* species in eastern North America (Boland & Grund, 1979; Gessner, 1977; Gessner & Goos, 1973; Gessner & Kohlmeyer, 1976). These studies have involved collections of living and dead material of *Spartina* species, but none have attempted to examine the fungi associated with leaves, stems and roots of living plants in the manner of several studies of marine angiosperms in the United Kingdom (Dickinson, 1965; Lindsey & Pugh, 1976; Pugh & Williams, 1968; Sivaneson & Manners, 1970). No studies have been reported of fungi associated with *Spartina* species in New Brunswick.

The Bay of Fundy is noted for its high tides and other unusual hydrographic factors, details of which are given by Bailey (1957) and Hachey (1975). There are extensive salt marshes along the Bay of Fundy in New Brunswick and Nova Scotia (Thomas, 1983).

This paper reports on fungi found on living plants of several species of *Spartina* from the Bay of Fundy.

Materials and Methods

Samples from healthy *Spartina* species were taken from sites noted in Table I in July and early August, 1980 and treated in two ways: (1) Seawater incubation: 12 screw-capped bottles each containing 100 mL sterile artificial seawater (ASW)

**C.B.R.I. contribution No. 1372*

Table I Details of sampling sites.

Station	Name	Location (N.W.)	High Tide Salinity (‰)	Tidal Range vertical (m)	Tidal Range horizontal (m)
1	Alma Beach	45° 36′ 64° 57′	28-31	9.1	1000
2	Point Wolfe	45° 32′ 65° 01′	20-25	9.0	500
3	St. Martins	45° 21′ 65° 33′	28-31	7.8	100
4	Taylor's Peninsula	45° 13′ 66° 08′	28-31	6.7	500
5	St. Andrews Harbour	45° 04′ 67° 03′	30-32	6.0	25

NOTE: Surface temperature of Bay of Fundy annual range 3-12°C.

(Lyman & Fleming, 1940) adjusted to 28‰ salinity and with 100 mg/L chloramphenicol were inoculatd with pieces of the roots, stems and leaves respectively, of *Spartina* species (with no nitrogen compounds or phosphate added). These were incubated at 12°C for 4 months. At the end of that period, one-ml aliquots of the fluid were plated on seawater Potato Dextrose Agar (SPDA) and Seawater Agar (SA) (Johnson & Sparrow, 1961) and the fungi isolated. In addition, the remaining fluid and the piece of plant were poured into Petri plates 100 x 15 mm and allowed to dry at 20°C for 6 wk. As fungi developed on the plant and/or the surface of the seawater, they were isolated and/or examined directly.

(2) Moisture-Chamber incubation: Samples of the roots, stems and leaves of *Spartina* species were collected in sterile plastic bags and transferred to the laboratory under cold conditions. These were placed individually in Petri plates containing a piece of Whatman No. 1 filter paper moistened with ASW adjusted to 28°/oo salinity and with 100 mg/L chloramphenicol (with no nitrogen compounds or phosphate added). These were incubated for four months at 12°C with periodic remoistening with sterile distilled water. As fungi developed on the plant or the filter paper, they were isolated on SA and/or examined directly.

Results

Fungi isolated by both methods above and the part of the plant yielding the fungi are listed in Table II. Virtually all of the Deuteromycetes isolated have been reported previously on marine angiosperms (Dickinson, 1965; Gessner & Goos, 1973; Lindsey & Pugh, 1976; Pugh & Williams, 1968; Sivaneson & Manners, 1970). The Ascomycetes *Buergenerula spartinae* and *Leptosphaeria albopunctata* have been reported on *Spartina* in Nova Scotia (Boland & Grund, 1979) and eastern Canada (Gessner & Kohlmeyer, 1976). *Buergenerula spartinae* was reported on *Spartina* in New Brunswick (Kohlmeyer & Kohlmeyer, 1979). *Leptosphaeria albopunctata* may be a first report on *Spartina* from New Brunswick (cf. Kohlmeyer & Kohlmeyer, 1979; Miller & Whitney, 1981a). The collection of *Pleospora spartinae* is thought to be the first report for this fungus outside the U.K. *Sporobolomyces roseus* has been reported on *Hippophaë rhamnoides* L. from coastal England (Pugh & Lindsey, 1975).

Discussion

Consistent with other studies of the fungal flora of living marine angiosperms, there are many 'terrestrial' Deuteromycetes on the *Spartina* species reported in this paper. A number of common microfungi on living marine angiosperms are listed in Table III. Of the 14 species from six studies of four marine angiosperms about half were common on the plants. This included such fungi as *Cladosporium (C. herbar-*

Table II Fungi isolated from living *Spartina* species.

Deuteromycetes	Leaves	Stems	Roots
Alternaria alternata[1] (Fr.) Keissler	xx[2]	xx	x
Botrytis cinerea Persoon	xx		
Cladosporium cladosporioides (Fresen.) de Vries	xx		xx
C. herbarum (Pers.) Link ex Gray	xx	x	x
C. macrocarpum Preuss	x		
C. resinae (Lindau) de Vries			x[3]
Dendryphiella salina (Suth.) Pugh & Nicot	xx	xx	
Fulvia fulvum (Cooke) Ciferri	xx	x	
Fusarium oxysporum Schlecht.	x		x
Gliocladium catenulatum Gilman & Abbot	x		
G. roseum (Link) Thom	xx	xx	xx
Monodictys castaneae (Wallr.) Hughes	x		
Mucor sp.			x
Penicillium brevi-compactum Dierckx			x
P. lividum Westling	x	x	
P. notatum Westling			x
P. puberulum Bainier	x		
Phialophora melinii (Nannf.) Conant	x		
Stemphyllium sp.	x		
Trichoderma album Pers.	x		
pink yeast-like organisms	x	x	x
sterile sp.	x		x
Ascomycetes			
Buergenerula spartinae Gessner & Kohlm.			xx
Leptosphaeria albopunctata[1] (Westend.) Sacc.		x	
Pleospora spartinae[1] (Webster & Lucus) Apinis & Chesters		x	
Basidiomycetes			
Sporobolomyces roseus Kluyver & Van Niel	x		
isolates with clamp connections	x		x

NOTES:
[1]see taxonomic part
[2]x refers to 1-5 isolations, xx>5
[3]oil-contaminated marsh

um and *C. cladosporioides* are closely related; de Vries, 1952) and *Aureobosidium pullulans*[1] which are considered benign endophytes, and species of *Alternaria, Botrytis* and *Fusarium* which are pathogenic in the terrestrial phylloplane (Dickinson, 1976).

In the present study, *Dendryphiella salina* and *Gliocladium roseum* were frequently isolated. *D. salina* is a common marine fungus in salt marshes (Pugh, 1974) and wood (Miller & Whitney, 1981a), and *G. roseum,* also common in salt marshes, is considered by Pugh (1974) and Miller & Whitney (1981b) to be amphibious (i.e., terrestrial and marine).

It appears that the phylloplane fungi of marine angiosperms are similar to their terrestrial counterparts, with the addition of some marine species. It is possible that

[1]Meyers, *et al.* 1967, state that there can be a pink yeast-like stage of *A. pullulans* (DeBary) Arnaud in marine systems. Such isolates were found in this study.

Table III Common microfungi on living marine angiosperms.

	Spartina	Halimone[4]	Salsola[5]	Hippophaë[6]
Alternaria alternata	1,2,3	x	x	x
Aureobasidium pullulans				x
Botrytis cinerea	1,2,3			x
Cephalosporium spp.	2,3	x	x	
Cladosporium cladosporioides	1			
C. herbarum	1,2,3	x	x	x
Dendryphiella salina	1	x		
Fulvia fulvum	1			
Fusarium spp.	2,3	x	x	x
Gliocladium roseum	1			
Penicillium frequentans West.	3			
Phoma spp.	2			
Septoria sp.	2			
Trichoderma koningii Oudem.	3			

NOTES:
1. this work
2. Gessner and Goos, 1973
3. Sivanesan and Manners, 1970
4. Dickinson, 1965
5. Pugh and Williams, 1968
6. Lindsey and Pugh, 1976

some of the terrestrial fungi might be marine-adapted strains (Miller & Whitney, 1981b; Pugh & Lindsey, 1975). The range of microfungi on the phylloplane of *Spartina* species is different from that isolated from littoral seaweeds from the same sampling stations (Miller & Whitney, 1981c).

Most of the microfungi were isolated from the leaves, with only a few from stems and roots (Table II). Sivanesan & Manners (1970) tested a number of *'Spartina'* microfungi for growth under conditions of low O_2 and high sulfate/sulfide to stimulate a salt marsh rhizosphere. *Gliocladium roseum* and two species of *Mucor* were found to tolerate these conditions and both fungi were found from *Spartina* roots in the present study. The fungi *Cladosporium* (four species) and *Alternaria alternata* were found on the roots of *Spartina* in this study, and were reported able to tolerate high sulfate/sulfide but not low 0_2. The fungus *Botrytis cinerea*, found only on leaves of *Spartina* (Table II), was reported unable to tolerate low 0_2 or high sulfate/sulfide. Thus, within the group of fungi found on living *Spartina*, there is a sub-group which is adapted to the salt marsh rhizosphere.

The low number (three) of Ascomycetes found in this study is consistent with the findings of other studies. This is in contrast to those which examined dead material, from which many Ascomycetes were found (e.g., Boland & Grund, 1979; Gessner & Kohlmeyer, 1976). *Buergenerula spartinae* was previously reported on living *Spartina* by Gessner (1977). *Leptospheria typharum* (Rabenh.) Karst. and *Pleospora herbarum* (Fr.) Rabenh. have been reported on living *Spartina* and *Halimione* respectively (Gessner & Goos, 1973; Dickinson, 1965).

Taxonomic Part

The following species from Table II require taxonomic explanation or are first reports for New Brunswick.

Alternaria alternata (Fr.) Keissler (Ellis, 1971). In the course of the present work and earlier studies of marine wood, seawater and seaweeds (Miller & Whitney, 1981a,b,c) approximately 50 isolates of an *Alternaria* species identified as *A. alternata* were isolated. Sutherland (1916) described a marine *Alternaria, A. maritima*. This species was accepted by many authors (e.g. Johnson, 1959; Jones, 1962; Neish, 1970; Curran, 1975). Several other authors have questioned the position of this species (e.g. Hughes, 1969; Joly, 1964). Johnson & Sparrow (1961) stated "It must be admitted that other than the fact that the fungus was originally described from, and has subsequently been found in, a marine habitat, there is little reason for considering it to be a true marine species. *Alternaria maritima* possibly could be assigned, if one were inclined to do so, to any one of several known terrestrial species in the genus". Sutherland (1916) described a green appearance of *A. maritima* that is not characteristic of *A. alternata*. *Alternaria alternata* is the most similar accepted species with respect to conidia size, shape and appearance to the marine isolates (Table IV). However, most of the marine isolates in these studies are definitely green in culture as opposed to the "usually black or olivaceous black or sometimes gray" of terrestrial isolates (Ellis, 1971; in the type description of *A. alternata*) even after several transfers on either saline or freshwater media. Prof. E.B.G. Jones (Portsmouth Polytechnic) also reports green *Alternaria* from marine sources (pers. com., 1980). The marine isolates discussed have consistently had rough conidia, whereas *A. alternata* from terrestrial sources may have either smooth or rough conidia (cf. Jones, 1962). In addition, about 10% of the marine isolates showed poor sporulation on freshwater media (Table IV). Kohlmeyer and Kohlmeyer (1979) reject *A. maritima*, considering it a *nomen dubium* (*cf. Bot. Mar.* 23: 622, 1980). However, pending further study it seems likely that there is an *Alternaria alternata* var. *maritima*.

Leptosphaeria albopunctata (Westendorp) Saccardo, Syll. Fung. 2, 72, 1983.
Ascospores 26-30 (–40) x 8-10 μm, mostly seven-septate. Previously reported on wood in New Brunswick (Miller & Whitney, 1981a; Kohlmeyer & Kohlmeyer (1979 and Nova Scotia (Neish, 1970) and on *Spartina* in Nova Scotia (Boland & Grund, 1979). Collections on *Spartina* were similar to our collection on wood (Miller & Whitney, 1981a), except some ascospores were larger.

Pleospora spartinae (Webster & Lucus) Apinis & Chesters, Trans. Br. Mycol. Soc. 44, 1961, Figure 1, 2.

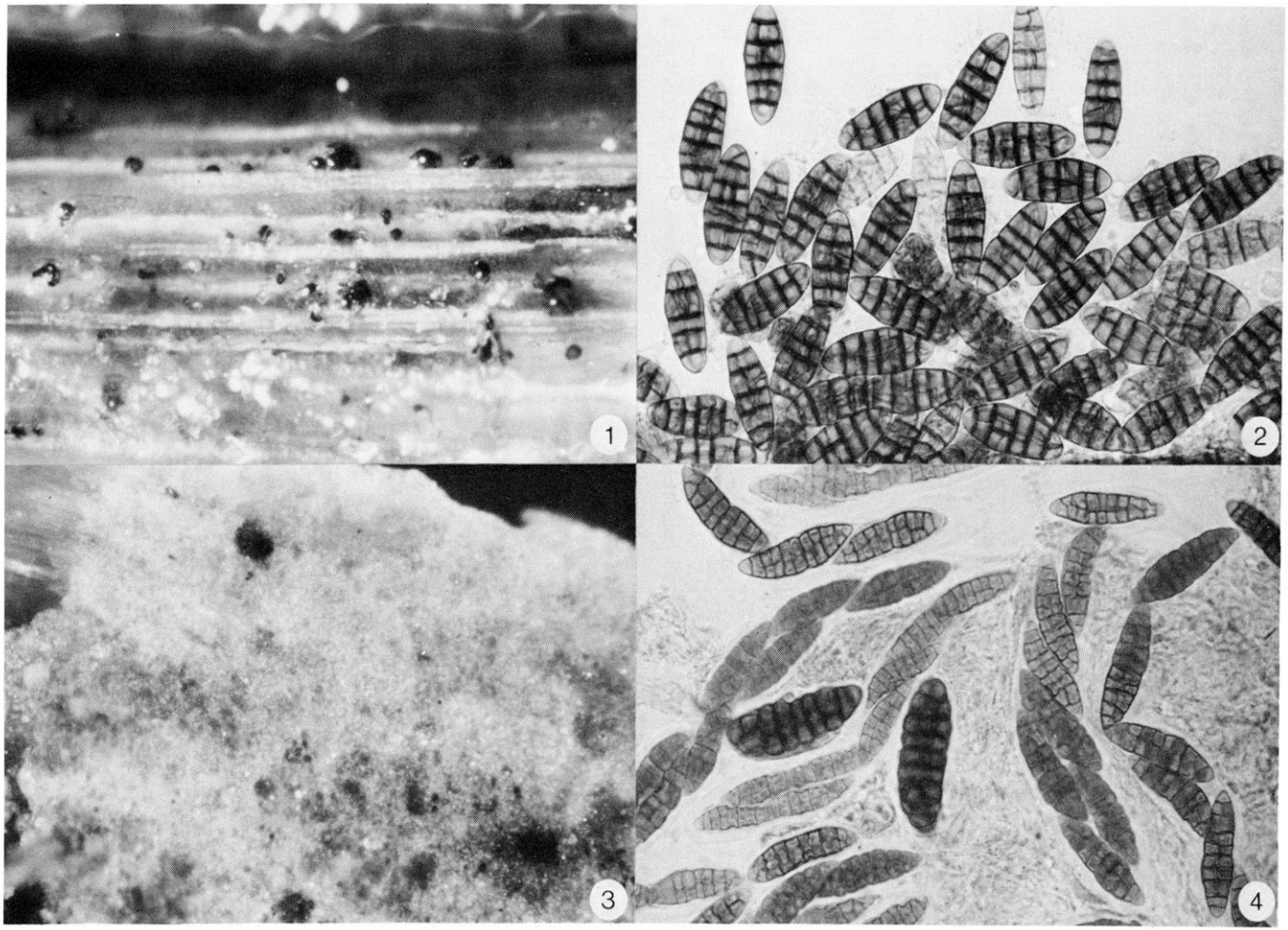
Ascocarps 350-370 μm in diameter, globose, immersed or slightly erumpent, black, solitary or gregarious. Pseudoparaphyses filiform. Asci 140-180 x 13-17 μm, eight-spored, cylindrical, apically rounded, bitunicate. Ascospores 24-28 x 9-11 μm, uniseriate, ellipsoidal, inequilateral, thick-walled, yellow-brown, muriform, usually 5 transverse septa, third cell wider than the others, occasionally 1 longitudinal septum in fourth cell. First report of this fungus outside the U.K. Collected at station 2. Voucher slide deposited in the Connell Memorial Herbarium (UNB). *P. spartinae* differs in several respects from *P. herbarm* which has been reported on a living marine angiosperm (Dickinson, 1965). *Pleospora herbarum* has longer and wider spores (26-50 x 10-20 μm), is mostly 7-septate and biserate (Wehmeyer, 1961).

Another marine *Pleospora*, *P. pelagica* Johnson (Fig. 3, 4) has been found on dead *Spartina* (Kohlmeyer & Kohlmeyer, 1979) and previously reported in New Brunswick on wood (Miller & Whitney, 1981a). The ascospores of *P. pelagica* are longer than *P. spartinae* (35-52 x 9.5-15 μm in the type description, Kohlmeyer & Kohlmeyer, 1979; 36-43(–45) x 10-13 μm, Miller & Whitney, (1981a) and also have 7-9 transverse septa and are biseriate. The collection of *P. pelagica* was marked by a deep red stain surrounding the ascocarps (Fig 3.) not seen in the collection of *P.*

Table IV Description of *Alternaria* isolates.

Species	Source	Conidia on SCZ[1]	Conidia on CZ[2]	Culture Colour
Alternaria alternata	damp plaster[3]	20-25 (-30) x 8-12 μm R[4]	20-25 x 8-12 μm R	black
A. alternata	wallpaper[3]	20-40 x 10-12 μm S[5]	20-32 x 10-12 μm slightly R	gray-black
A. maritima?	*Spartina* roots	20-40 (-60) x 10-12 μm R	25-40 x 10-18 μm sl. R	olive green
A. maritima?	seawater	25-30 (-45) x 10-15 μm R	25-30 (-40) x 8-15 μm R	gray-green
A. maritima?	seawater	23-30 (-40) x 19-12 μm sl. R	25-27 x 8-10 μm sl. R[6]	olive green
A. maritima?	*Fucus*	22-45 (-50) x 8-15 μm R	22-35 (-40) x 8-13 μm R	olive green
A. maritima?	*Ascophyllum*	22-45 (-48) x 9-13 μm R	22-40 x 8-14 μm R	olive green
		conidia		
Alternaria alternata type[7]		20-63 (x = 37) x 9-18 (x = 13) μm S and R		see text
A. maritima type[8]		30-50 x 12-18 μm R		olive green, brown
A. maritima[9]		16-71 x 8-15 μm		

NOTES:
1. seawater-Czapek's agar
2. Czapek's agar
3. Miller and Holland, 1981
4. rough conidia
5. smooth conidia
6. poor sporulation
7. Ellis, 1971
8. Sutherland, 1916
9. Johnson and Sparrow, 1961



1. Ascocarps of *Pleospora spartinae* on *Spartina alterniflora* (x ca. 20) stem
2. Ascospores of *P. spartinae* (x ca. 700)
3. Ascocarps (black) of *P. pelagica* on intertidal wood (x ca. 20)
4. Ascospores of *P. pelagica* (x ca. 600)

spartinae. Prof. J. Webster (Exeter) has observed *Pleospora* spp. causing a red stain in the substrate (pers. com., 1981). Wehmeyer (1961) lists *P. pelagica* as species *non vidi* although it was accepted by Kohlmeyer & Kohlmeyer (1979) and by Miller & Whitney (1981a).

Other marine species of *Pleospora* which have been reported from dead marine angiosperms include *P. gaudefroyi* Patouillard (ascospores 30-46.5 x 13.5-20.5 μm, with gelatinous appendages), *P. pelvetiae* Sutherland (ascospores 26.5-35 x 11.5-17 μm, with gelatinous sheath), *P. triglochinicola* Webster (ascospores 45-68 x 16.5-25 μm with a mucilaginous sheath) and *Pleospora* sp. 1 (ascospores 40-52 x 16-10 μm, with a gelatinous sheath) (Kohlmeyer & Kohlmeyer, 1979).

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