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Observations on vertical distribution of fungi associated with standing senescent *Acanthus ilicifolius* stems at Mai Po Mangrove, Hong Kong

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Abstract

The present study was carried out to investigate the higher fungi colonizing the herbaceous mangrove associate *Acanthus ilicifolius*. This paper reports part of an investigation to determine if there is vertical distribution of fungi on the standing plant.

The Mai Po Mangrove, Hong Kong is estuarine with great variations in salinity mainly due to the influence of the Pearl River. Senescent and dead stems of standing *Acanthus ilicifolius* were collected from mangroves in Mai Po from April to December 1992. The maximum tidal range observed was 2.6 m. A stratified sampling strategy was employed to assess the vertical distribution on the standing plant. A total of 44 fungi were collected: 32 Deuteromycotina, 11 Ascomycotina and 1 Basidiomycotina. Very frequent species were *Acremonium* sp.(55%), *Colletotrichum gloeosporioides* cf.(42.5%), *Phoma* sp. (42.5%) *Fusarium* sp.,(25%), *Tubercularia* sp. (24.2%) and *Phialophora* sp. cf. (19.2%). *Agerita* sp., *Corynespora cassiicola*, *Stachybotrys chartarum*, *Trichoderma* sp. and D82 were frequent, while the remaining species were recorded at less than 10%. Vertical zonation of fungi colonizing the standing stems was observed. The apical portions were colonized by typical terrestrial fungi and the basal portions by marine species. This can be attributed to both the nature of the substratum and the degree of exposure due to tidal inundation.

Introduction

There has been a considerable increase in information on mangrove fungi since they were first reported from mangrove roots in Australia by Cribb & Cribb (1955). It is now recognized that mangrove fungi constitute the second largest group of marine fungi (Hyde & Jones, 1988). However, the bulk of the data generated from various areas in the world has concentrated on the description of new species on hosts not previously investigated (Hyde, 1992; Hyde & Nakagiri, 1992; Hyde *et al.*, 1992; Jones & Agerer, 1992; Volkmann-Kohlmeyer & Kohlmeyer, 1993). Only a few systematic ecological studies have been carried out (Hyde & Jones, 1988; Kohlmeyer, 1969; Tan & Leong, 1989). Moreover, these studies were mostly limited to fungi on bark and decorticated wood, roots, pneumatophores (Kohlmeyer & Vittal, 1986; Hyde *et al.*, 1986; Hyde & Jones, 1988), and on leaves either partially immersed or totally submerged in high salinity areas (Anastasiou & Churchland, 1969; Fell & Master, 1973, 1980; Newell, 1976). Little is known of the fungi colonizing herbaceous mangrove plants or their associates (e.g. *Acrostichum speciosum* Willdenow, *Acanthus ilicifolius* L.).

Mangrove fungi on lignocellulose substrata and leaves have been studied recently in Hong Kong (Vrijmoed, 1990; Vrijmoed & Tam, 1990). The present study was undertaken to investigate the higher fungi colonizing *Acanthus ilicifolius* since little is known about fungi colonizing herbaceous substrata in mangroves.

Materials and methods

The study area

The Mai Po Mangrove is situated along the northtwestern coast of the New Territories facing the Pearl river estuary (22°29′N, 114°02′E). This is the largest mangrove community in Hong Kong, covering an area of approximately 0.9 km^2 (Lee, 1990) within the Mai Po marshes along the shoreline of Deep Bay. The dominant mangrove species include *Kandelia candel* (L.) Druce, *Aegiceras corniculatum* (L.) Blanco, *Avicennia marina* (Forsk.) Vierh. and the prickly herbaceous plant *Acanthus ilicifolius*.

Table 1. Hydrographical data* of the test sites in the Mai Po mangrove, Hong Kong (April - December 1992).

Month	Temperature Air (°C)	Water (‰)	Salinity	pH
April	22.9±1.3	22.2±1.0	2.7±1.3	7.1±0.1
June	24.7±0.1	24.7±0.1	6.3±0.4	7.3±0.0
July	32.3±0.3	28.8±0.5	1.8±1.5	7.2±0.2
August	30.0±2.9	32.8±0.3	5.8±0.8	7.4±0.1
September	29.4±1.4	31.7±1.2	12.3±0.4	7.3±0.0
October	28.3±2.3	26.3±2.2	12.0±0.0	7.6±0.1
November	21.5±0.7	18.2±0.4	20.0±0.0	7.2±0.0
December	19.9±1.5	19.1±0.3	21.0±1.0	7.1±0.0

* Average values from four sites

The host: Acanthus ilicifolius

Acanthus (Acanthaceae) is the only genus of the family with representatives in mangrove communities. *Acanthus ilicifolius* is a low woody herb up to 2 m high, which occurs on the inland side of channels in mangroves. It is a characteristic associate of mangroves with a known distribution from India to the Western Pacific, tropical Australia, and the Philippines (Tomlinson, 1986). Locally it is found in great abundance at Mai Po. *Acanthus* is a perrenial plant which spreads largely by vegetative means (Tomlinson,1986). The mature stem is largely herbaceous consisting of soft tissue, but the lower portions are woody (soft wood). This is largely due to the growth pattern of *Acanthus*, older portions will be lignified gradually. *Acanthus* therefore offers a range of non-lignified and lignified tissues for colonization, possibly by different ecological groups of fungi.

In Mai Po, *Acanthus* plants grow well above the banks of the major water channel. Thus the lower parts of the plants are submerged only during high tide and are well exposed during low tides. However, the apical portions of tall plants are exposed to the air even at high tide.

Sampling sites within the study area

Stem samples (1.5–2.0 m high) were collected within one to two meters on both sides of the major water channel which runs through the mangrove. The four sampling sites chosen for the determination of selected hydrographical characteristics were just below the wooden boardwalk running along the channel. Water samples were collected in plastic vials and kept in an ice box and brought to the laboratory for pH and salinity measurements. Air and water temperatures were determined *in situ*.

Collection of samples

To determine whether there is vertical stratification of fungi along the standing stem of *Acanthus*, samples were collected from apical, middle and basal portions of the senescent plant. Apical portions include those portions approximately 1.5–2.0 m from the soil surface while the middle about 0.5–1.0 m. The basal samples were collected directly above the soil surface. No lateral branches were included as they would not provide information relating to vertical stratification of fungal occurrence of the *Acanthus*. All stems collected were approximately 5–10 mm in diameter. Five stems from each level were collected in each month from April to December 1992.

Treatment of samples

Samples were treated following the procedures of Hyde & Jones (1988). The fungi colonizing on the samples were identified on retrieval and after incubation. Voucher slides of all fungi collected were prepared and are held in the first author's collection. Based the frequency of occurrence of the collected fungi, they are classified as 'very frequent' (>20%), 'frequent' (10–20%) and infrequent (<10%) as adopted by Tan and Leong (1989).

Table 2. Frequency of occurrence (%)* of fungi associated with stratified samples of *Acanthus ilicifolius* stems collected in Mai Po between April and December 1992.

	Apical	Middle	Basal
ASCOMYCOTINA (11 species)			
Aniptodera chesapeakensis Shearer & Miller	–	5.0	12.5
Gnomonia sp. *cf.*	2.5	–	–
Halosarpheia retorquens Shearer & Crane	–	–	2.5
Halosarpheia marina cf.	–	–	2.5
Leptosphaeria sp.	–	2.5	5.0
Lignincola laevis Höhnk	–	2.5	2.5
Marinosphaera mangrovei Hyde	–	2.5	–
Nectria sp. 1	5.0	2.5	–
Nectria sp. 2	10.0	2.5	–
Ophioceras sp.	10.0	–	–
Tirispora unicaudata Jones, Vrijmoed & Read	10.0	2.5	12.5
BASIDIOMYCOTINA (1 Species)			
Agerita sp.	5.0	15.0	27.5
DEUTEROMYCOTINA (32 species)			
Acremonium sp.	37.5	70.0	90.0
Annellophora sp. *cf*	2.5	12.5	5.0
Cirrenalia basiminuta Raghu-kumar, Zainal & Jones	–	5.0	25.0
Clavatospora bulbosa (Anast.) Nakagiri & Tubaki	–	–	2.5
Colletotrichum gloeosporioides cf.	65.0	25.0	37.5
Coniothyrium sp. 1	7.5	15.0	–
Coniothyrium sp. 2	2.5	–	–
Corynespora cassiicola (Berk. & Curt) Wei	27.5	12.5	–
Fusarium sp.	12.5	30.0	35.0
Graphium sp. 1	–	10.0	15.0
Graphium sp. 2	–	5.0	22.5
Graphium sp. 3	–	7.5	7.5
Periconia prolifica Anastasiou	–	10.0	10.0
Phaeoisaria sp.	–	7.5	2.5
Phialophora sp. *cf.*	55.0	2.5	–
Phoma sp. 1	30.0	–	55.0
Phoma sp. 2	–	–	5.0
Phomopsis sp.	2.5	2.5	–
Stachybotrys chartarun (Ehrenb. ex Link) Hughes	10.0	22.5	17.5
Stachylidium bicolor Link ex S.F. Gray	25.0	10.0	–
Trichocladium achrasporum (Meyers & Moore) Dixon in Shearer & Crane	–	–	7.5
Trichoderma sp.	15.0	17.5	15.0
Tubercularia sp.	40.0	7.5	25.0
Zelosatchmopsis sp. *cf.*	–	–	5.0

Results

Hydrographical data

Table 1 shows the hydrographical characteristics of the study area as determined at the four sampling points between April and December 1992.

Fungi associated with stratified samples of Acanthus ilicifolius stems

Forty-four higher fungi were identified from 120 samples collected from the field and the pooled results are presented in Table 2. In general, the domi-

Table 2 cont..

	Apical	Middle	Basal	
D28 (globose, verrucose, purplish or brown conidia)	10.0	2.5	12.5	
D71 (truncate at one end and rounded on the other, hyaline one-celled conidia)	2.5	–	–	
D82 (fusiform, hyaline, one-celled conidia)	25.0	20.0	17.5	
D36 (filiform, hyaline, one-celled conidia)	–	5.0	2.5	
D6 (ellipsoidal, hyaline, one-celled conidia)	–	5.0	2.5	
D34 (ellipsoidal, 1–3 septate. hyaline conidia)	–	5.0	2.5	
D90 (filiform, septate, hyaline, ends with chambers?, straight or curved)	–	2.5	–	
D43 (cylindrical, hyaline, one-celled conidia)	–	–	2.5	
Summary:	Apical	Middle	Basal	Total
No. of Ascomycotina	4	7	6	11
No. of Basidiomycotina	1	1	1	1
No. of Deuteromycotina	17	24	24	32
Total collections	154	151	194	499
Total no. of species	22	32	31	44
No. of samples examined	40	40	40	120

* Frequency of occurrence (%)= $\frac{\text{No. of collections of a species}}{\text{No. of samples examined}} \times 100$

nant group was the Deuteromycotina with 32 species. The Ascomycotina and Basidiomycotina were represented by 11 and 1 species respectively. The same pattern was observed on the different portions surveyed (Deuteromycotina > > > Ascomycotina > > Basidiomycotina). Based on the overall frequency of occurrence, very frequent species were *Acremonium* sp.(55%), *Colletotrichum gloeosporioides* cf. (42.5%), *Phoma* sp. (42.5%), *Fusarium* sp. (25%), *Tubercularia* sp. (24.2%) and *Phialophora* sp. cf. (19.2%). *Agerita* sp. *Corynespora cassiicola* (Berk. & Curt) Wei, *Stachybotrys chartarum* (Ehrenb. ex Link) Hughes, *Trichoderma* sp., and D82 were frequent (10–20%), while the remaining species were recorded at less than 10%.

Ten species were common to all levels of the stem portions (Table 2). However, their percentage occurrence differed at the three levels. They showed a comparatively higher affinity to a particular level on the stems (Table 2). Thus, *Agerita* sp., *Acremonium* sp., *Fusarium* sp., and D28 had a higher affinity towards the basal portions. *Colletotrichum gloeosporioides* cf:, *Tubercularia* sp. and D82 were more frequently found in the apical portions, whereas *Annellophora* sp. cf. and *Stachybotrys chartarum* were generally restricted to the middle portions. *Trichoderma* sp. was evenly distributed at all three levels.

Thirteen species were collected only once from a particular level (Table 2). These were *Coniothyrium* sp. 2, *Gnomonia* sp. cf., D71 and *Ophioceras* sp. cf. from the apical portion; *Marinosphaera mangrovei* Hyde and D90 from the middle portion; and *Clavatospora bulbosa* (Anast.) Nakagiri & Tubaki, *Halosarpheia retorquens* Shearer & Crane, *Halosarpheia marina* cf., *Phoma* sp. 2, *Zelosatchmopsis* sp. cf., *Trichocladium achrasporum* (Meyers & Moore)Dixon in Shearer & Crane and D43 from the basal portion. The remaining 21 species occurred on more than one level along the stem.

Discussion

In comparing the data generated from this study with those reported by previous researchers, a number of factors require consideration: (1) Nature of the substratum – *Acanthus ilicifolius* comprises both soft and woody tissues and this is expected to affect the ability of the fungi to colonize them; (2) Ecosystem – the Mai Po mangrove is brackish to almost freshwater, depending on season, while other studies involve almost completely saline to brackish water habitats (e.g. Seychelles, Hyde & Jones, 1988). This will significantly affect the fungi recorded on the baits; (3) Vertical distribution – few studies have been undertaken

Table 3. Comparison of the vertical distribution of dominant fungi on *Acanthus ilicifolius* stems and *Rhizophora* spp. prop roots (after Hyde, 1988a).

Fungi on *Acanthus ilicifolius* (present study)	Frequency of occurrence[a] (%)	Fungi on *Rhizophora* spp. (Hyde, 1988a)	Frequency of occurrence[b]
APICAL (GROUP I)			
Colletotrichum gloeosporioides cf.	65	*Leptosphaeria* sp. 2	20
Phialophora sp. *cf.*	55	*Savoryella lignicola* Jones & Eaton	10
Corynespora cassiicola	27	*Halocyphina villosa*	8
Stachylidium bicolor	25	*Cytospora rhizophorae** Kohlm. & Kohlm.	6
Nectria sp. 2	10	*Lignincola tropica** Kohlm.	4
Ophioceras sp.	10		
MIDDLE (GROUP II)			
Stachybotrys chartarum	22	*Leptosphaeria avicenniae* Kohlm. & Kohlm.	6
Phaeoisaria sp.	8	*Massarina velatospora** Hyde & Borse	10
Marinospaera mangrovei	3	*Dactylospora haliotrepha* (Kohlm. & Kohlm.) Hafellner	6
D90	3	*Verruculina enalia* (Kohlm.) Kohlm. & Volkm.-Kohlm.	6
		*Aigialus parvus** Schatz & Kohlm.	4
BASAL (GROUP III)			
Acremonium sp.	90	*Trichocladium opacum**	40
Phoma sp. 1	55	*Cirrenalia pygmea* Kohlm.	20
Fusarium sp.	35	*Lulworthia* sp.	36
Agerita sp.	28	*Humicola alopallonella** Meyers & Moore	13
Cirrenalia basiminuta	25	*Bathyascus grandisporus* Hyde & Jones	6
Graphium sp. 2	23	*Antennospora quadricornuta** (Cribb & Cribb) T. W. Johnson	6
Aniptodera chesapeakensis	13	*Aniptodera* sp.	6
Tirispora unicaudata	13		
Trichocladium achrasporum	8		
Zelosatchmopsis sp. *cf.*	5		
Halosarpheia retorquens	3		
Halosarpheia marina	3		
Clavatospora bulbosa	3		
D43	3		

[a] Frequency of occurrence (%)= $\frac{\text{No. of collections of a species}}{\text{No. of samples examined at a specific level}} \times 100$

[b] No. of records

* Found only at this level.

on this aspect of mangrove fungal ecology (e.g. Hyde, 1988a).

Effect of substratum

The effect of substratum, in terms of fungal colonization, can be demonstrated by comparing the data presented in this paper with those of Hyde (1988a), Table 3 being the only published information available on stratification of fungal colonization on mangrove wood. There are distinct differences in species composition in these two studies. In the present study, *Acanthus*, (both herbaceous and woody tissues), on the one hand, were colonized mainly by Deuteromycotina. However, the woody portions were also colonized by some marine Ascomycotina (e.g. *Halosarpheia retorquens*, *Clavatospora bulbosa*, *Aniptodera chesapeakensis* and *Lignincola laevis*). On the other hand, the woody *Rhizophora* supported more Ascomycotina such as *Hypoxylon oceanicum* Schatz, *Lignincola tropica* Kohlm., *Savoryella lignicola* Jones & Eaton, *Massarina velatospora* Hyde & Borse, *Dactylospora haliotrepha* (Kohlm. & Kohlm.) Hafellner and the common basidiomycete *Halocyphina villosa* Kohlm. & Kohlm. These differences could be attributed to host species and tissue types – *Rhizophora* has little non-lignified tissues. However, the ascomycetes *Aniptodera chesapeakensis*, *Halosarpheia retorquens* and *Lignincola laevis* have been collected from softer tissues like twigs and leaf petioles (Jones *et al.*, 1988).

Ecosystem

The dominant fungi recorded from a brackish site in this study are largely terrestrial Deuteromycotina. Investigations of fungal colonization on woody substrata in similar environments suggested similar results. Shearer (1972) noted that there were more ascomycetes on the balsa blocks submerged in brackish to saline parts of the Patuxent River but with more Deuteromycotina in the freshwater zone. A similar observation was made by Kirk and Brandt (1980), in their study of the fungi in lower Chesapeake Bay using the harder pine and birch wood with longer submergence time. Lastly, Gold (1959) reported no deuteromycetes in Newport River water estuary having a salinity higher than 28.9‰. The difference in species reported in this study could be due to salinity levels (freshwater to brackishwater) and substrata type.

Vertical distribution

In Table 3 the most abundant fungi at each zone or only recorded from one zone are listed. Group I comprises those fungi collected in the apical region of the plant and are typical terrestrial species, while some are known to be parasites e.g. *C. gloeosporioides* cf. Group III comprises largely marine species colonizing the basal part of the plant which was inundated by tidal water at each tidal cycle. Only a few species which colonized in the middle zone were not found at the upper or basal regions or were there in greater abundance. These were largely terrestrial forms.

Some fungi grew well at all levels, e.g. *Acremonium* sp. This may indicate its ability to tolerate varying salinity levels and colonise different tissue types. The isolate was also observed to grow and sporulate on 100% natural sea water corn meal agar (unpublished data). On the other hand, other species of the genus are commonly known to occur in soils which might explain their high frequency on stems close to the mangrove floor. *Fusarium* sp. also occurred on all stem portions of *A. ilicifolius* with increasing frequencies toward the basal portion. As with *Acremonium*, most *Fusarium* species are ubiquitous soil fungi (Domsch *et al.*, 1980) and are also active decomposers of cellulosic plant substrata such as *Spartina alterniflora* Loisel (Meyers *et al.*, 1970) and *Rhizophora mangle* L, seedlings (Newell, 1976).

The high affinity of *Colletotrichum gloeosporioides* cf. towards the apical portion appears to agree with its known distribution, that is, more on herbaceous and 'aerial parts' where it is often associated with the plant disease anthracnose (So, 1991). However, its frequent presence on other portions of the plant also indicates its ability to colonize substrata even in submerged conditions. Moreover, the isolates were observed to grow and sporulate on 100% natural sea water corn meal agar (unpublished data) and Newell (1976) also recorded *Colletotrichum* sp. on *Rhizophora mangle* seedlings. The sporodochial *Tubercularia* sp. was also collected at all levels but with the highest frequencies at the apical portions, indicating its ability to tolerate aquatic conditions with different salinity levels as well as substratum types.

The basal portions of *Acanthus* yielded Ascomycotina and Deuteromycotina commonly reported in mangroves either in brackish or marine waters (Hyde & Jones, 1988; Jones & Tan, 1987) (Table 3). *Halosarpheia retorquens* was originally described from balsa wood in freshwater habitats of the estuaries of Cheapeake Bay (Shearer & Crane, 1980). Since then, it has been reported from other woody mangrove species (Jones *et al.*, 1988; Jones & Kuthubutheen, 1989). *Lignincola laevis* Höhnk is cosmopolitan and is known to occur mostly on lignicolous substrata (Kohlmeyer & Kohlmeyer, 1979; Kohlmeyer, 1984). Recently, this was reported on *Pemphis acidula* Forst. a dead shoreline tree in India (Chinnaraj, 1993). *Tirispora unicaudata* is a new species to be described by Jones, Vrijmoed, Read and Moss (in press).

Table 3 also lists the vertical distribution of fungi observed on prop roots of *Rhizophora* spp. by Hyde (1988) and a comparison is made between his data and those of the present investigation.

In conclusion, forty-four species of higher fungi were recorded from 120 samples of standing senescent *Acanthus* stems collected from April to December 1992. The Deuteromycotina was dominant with 32 species, followed by 11 Ascomycotina and 1 Basidiomycotina. The very frequent species include: *Acremonium* sp., *Colletotrichum gloeosporioides* cf., *Phoma* sp., *Fusarium* sp., *Tubercularia* sp. and *Phialophora* sp. cf. These very frequent species collected in this study are different from other mangrove fungi reported on lignocellulose substrata. A vertical zonation of fungi on the standing senescent *Acanthus* stems was also observed. This investigation demonstrates that both the tissue type (lignicolous versus herbaceous) and varying degrees of exposure due to tidal inundation are important factors governing species distribution colonizing a vertically-orientated substratum in the mangroves.

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