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### Fungal colonization of *Rhizophora apiculata* and *Xylocarpus granatum* poles in Kampong Kapok mangrove, Brunei

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Test poles of *Rhizophora apiculata* and *Xylocarpus granatum* were exposed in the intertidal region of Kampong Kapok mangrove, Brunei. Samples were recovered bi-monthly over the course of one year and the fungal colonization noted. *Halosarpheia minuta* was an early colonizer on *R. apiculata* and *Lulworthia* sp. of *Xylocarpus* granatum. The relationship of fungal colonization with depth is also investigated. Keywords: mangrove fungi, colonization, succession.

Several recent studies have provided insight into the ecology of intertidal mangrove fungi (Hyde, 1988; 1989; 1990a; 1990b; 1990c; 1991; TAN & al., 1989a; 1989b). Frequency of occurrence has been examined by several authors and although the picture is far from complete, a core of common fungi is now recognised (Hyde, 1989; TAN & LEONG, 1991). These, however, vary from one mangrove community to the next (TAN & LEONG, 1991) and reasons for this include vertical distribution, age of samples, sample size, salinity, type of samples collected and presence or absence of bark (Hyde, 1990a; 1990b; 1990c; 1991; TAN & LEONG, 1991). Intertidal fungi have been shown to be vertically distributed (Hyde, 1988; 1989; 1990c), while evidence of fungal succession has been reported by LEONG & al. (1991). The structure of the fungal communities is also dependent on the mangrove host (Hyde, 1990a).

#### **Materials and methods**

The study was carried out at Kampong Kapok in Brunei, a mangrove stand composed mostly of *Rhizophora apiculata* and *Xylocarpus granatum*, with a salinity of 16–25% and water temperature of 27 C – 32 C. Poles (300 x 2.5 – 3.5 cm) were cut from living prop roots of *R. apiculata* and trunks of young *X. granatum*, the leaves and small branches being removed. Poles of both species were placed in the intertidal region near a steep overhanging bank (Fig. 1). The poles were sunk into the mud at the bottom and tied to prop roots at

the top, placed in such a way that the poles stretched across the entire tidal range (Fig. 2). Poles were placed in the mangrove in early January 1987 and recovered bi-monthly until January 1988. Six poles of *R. apiculata* and four of *X. granatum* were randomly removed from the mangrove at each sampling period. The samples were first sawn into manageable pieces with their vertical position noted, washed clean of mud, then returned to the laboratory to be incubated in moist chambers for 1 week before examination for the presence of fungal fruiting bodies. Tidal details are as in Tab. 1.

#### Results

#### Decay of poles

The process and rate of decay of poles of *X. granatum* and *R. apiculata* were different. Over 12 months, samples of *R. apiculata* underwent negligible decay, particularly those in the mud, while those above were colonised by a limited number of bark inhabiting fungi. The bark remained intact, only occasionally splitting and revealing the core wood (Fig. 3). Removal of the bark from samples at 12 months revealed sound wood beneath. A few marine borer holes were present, while marine crustaceans colonised the bark surface, particularly below mean tide.

In contrast *X. granatum* poles were severely decayed within 12 months. The part of the poles in the mud lost their bark within 4 months, but the wood beneath was sound. The part in the intertidal region also lost its bark within four months, the exposed wood being quickly decayed, evidently by fungi and marine borers, and several of the samples completely disintegrated (Fig. 4).

#### Colonization by fungi

Each substratum was colonized by markedly different fungal communities (Tab. 1, 2), but it should be noted that fungi on *Rhizophora* were on bark, while those on *Xylocarpus* were mainly recorded from xylem. One of the earliest and most common fungus to occur on *R. apiculata* was *Halosarpheia minuta*, which produced ascomata within 2 months. *H. minuta* was common on bark throughout the period of this study and occurred in the lower tidal regions (1 - 3). *Lulworthia grandispora* was next to produce ascomata followed by *Manglicola guatemalensis* and *Anthostomella* sp. (6 months), and *Capillatispora* corticola and *Hypophloeda rhizospora* (8 months). *Capillatispora* was common in the later stages in the lower tidal region, while *Anthostomella* sp. and *Hypophloeda rhizospora* were common in the upper zone. Other fungi including *Splanchnonema* sp. (Figs. 6, 7) were noted on few occasions.



Figs. 1-4. – Experimental details. – 1. Vertical mangrove bank at Kampong Kapok. – 2. Exposed poles. – 3. Poles of *Rhizophora apiculata* (exposed for 8 months). – 4. Cut poles of *Xylocarpus granatum* (exposed for 8 months).

Tab. 1. – Colonization of prop roots of *Rhizophora apiculata* by intertidal fungi. Six samples were examined after each exposure. Highest tide during 1987 = 2.7 m; lowest = 0.1 m.

Tidal level		0 - 1	(0.2	- 0	.8 m	ı)	1-2 (0.8 - 1.4  m)							2-3 (1.4 - 2.0  m)							3-4 (2.0 - 2.6  m)					
Period of exposure (months)	2	4	6	8	10	12	2	4	6	8	10	12	2	4	6	8	10	12	2	4	6	8	10	12		
Taxon																										
Halosarpheia minuta Leong	5	6	6	6	3	6	5	6	5	6	3	6	1	_	2	2	4	6	_	_	_	_	_	_		
Lulworthia grandispora Meyers	_	_	1	1	_	3	_	_	_	_	_	3		_	_	_	_	_	_	_	_	_	_	_		
Manglicola guatemalensis Kohlm.	_	_	_		_	4	-	_	1	_	1	1		_	_	_	_	_	_	_	_	_	_			
Anthostomella sp.	_	_	_	_	_	_	_	_	_	_	_	_		_	2	_	_	-	_	_	2	3	2	4		
Capillatispora corticola Hyde	-	_	_	_	6	5	_	_	_	3	6	6		-	_		1	1			_		_	_		
Hypophloeda rhizophorae Hype & Jones	_	_	_	-	_	_	_	_	_	_	_	_		_	_	-	_	_	_	_	_	1	3	2		
Cytospora sp.		_			_	-		_	-	_	_	-		_	_	_	_	_	_	_	2	_	_	_		
Splanchnonema sp.	_	_	1	_	_	_	_	_	_	1	_	_		_	_		-		_	_	_	_	_	_		
Cirrenalia pygmea Kohlm.	_	_	_	_	_	_	_	_	_	1	1	_		_	-	_	_	-	_	-	-	_	_	_		
C. tropicalis KOHLM.	_	_	_	_	_	_	_	_	_	1	_	_		_	_	_	_	_	_	_	_	_	_	_		
C. pseudomacrocephala Kohlm.	_	_	_	_	-	_	_	_	_	1	_	-		_	_	_	_	_	_	_	_	_	_	_		
Phoma sp.	_	_	_	_	_	_	_	_	_	_	_	_		_	_	_	_	_	_	_	_	_	1	_		
Xylomyces sp.					_	_		_	_	1	_	_		_	_	_	_	_	_	_	_	_	_	1		
Immature ascocarps	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-		



Figs. 5-7. – Interference light micrographs of succession fungi. – 5. unidentified Hyphomycete sp. – 6. Splanchnonema sp. Asci. – 7. Splanchnonema sp. Ascospores. Scale bars = 10 μm.

In Xylocarpus, Lulworthia sp. (ascospores 200-335  $\mu$ m) were the first to occur on bark or wood below mean tide. A *Diplodia* sp. was an early colonizer above mean tide along with several unidentified coelomycetes. In the later stages several fungi including *Xylomyces* sp., *Cirrenalia* spp., *Periconia prolifica* and an unidentified species in the Hyphomycetes (Fig. 5) were identified below mean tide. Ascomycetes included *Leptosphaeria australiensis*, *Lulworthia* spp. and *Halosarpheia marina*. In the higher tidal regions *Calathella* sp. and *Hypoxylon oceanicum* were very common.

#### Discussion

TAN & al. (1989a) and LEONG & al. (1991) have shown that a series of fungi colonize submerged mangrove wood. Early colonizers of *Avicennia alba, A. lanata, Bruguiera cylindrica and Rhizophora apiculata* in Singapore were *Lignincola laevis, Lulworthia* sp. and *Verruculina enalia, while Dactylospora haliotrepha* appeared only later. The succession shown in this study, however, cannot be compared with that shown by TAN & al. (1989a) and LEONG & al. (1991)

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Tab. 2. – Colonization of prop roots of Xy locarpus granatum by intertidal fungi. Four samples were examined after each exposure. Highest tide during 1987 = 2.7 m; lowest = 0.1 m.

Tidal level			0-1 (0.2 - 0.8 m)							1-2 (0.8 - 1.4  m)						2-3 (1.4 - 2.0  m)							3-4 (2.0 - 2.6 m)					
Period of exposure (months)	2	4	6	8	10	12	2	4	6	8	10	12	2	4	6	8	10	12	2	4	6	8	10	12				
Taxon																												
Lulworthia sp. $(200 - 335 \ \mu m)$			3	2	1	3	1	4	2	_	_	_	3	2	_	_	_	_	_	_	_	_	_	_				
Diplodia sp.	_		_	_	_	_	_	_	_	_	_		2	2	2	_	_	_	3	-	-			_				
Xylomyces sp.	_		2	1	1	_	_	-	_	_	3	_	2	2	2	_	_	-	_		_	-	_	_				
Unidentified Coelomycetes			_	_	_	_	_	_	_	_	_	_	2	1	1	_	_	-	_	_	2	3	1	-				
Calathella sp.	_		_	_	-	_		_	_	_	-		_	1	2	1	1	3	_	_	1	_	4	4				
Hypoxylon oceanicum SCHATZ	_		_	_	_	_	_	_	2	_	_	-	_	_	2	2	4	3	_	_	_			_				
Periconia prolifica ANAST.			3	3	_	_	_		_	1	_	_	_	_	_	_	_	-	_	_	_	_	_	_				
Cirrenalia pygmea Kohlm.	_		4	_	-	_	-		2	_		_	_	_	_	_	_	-	-	-	_	_	-	-				
C. tropicalis KOHLM.	-		2	_	1	_	_	-	_	_	_	_	_	_	_	_			_	_	_	_	_	_				
C. pseudomacrocephala Kohlm.	_	_	_	4	2	_	_		1	1	_	_	_	_	_	_	_	_	-	_	_	_	_	_				
Lulworthia sp. (spores $100 - 200 \ \mu m$ )	_	-	_	_	_	_			2		_	_	_	_		_	_	_	_	_	_	_	_	_				
L. grandispora MEYERS	_	_	_	_	_	_	_		1	1	_	_	_	_	_	1	1	-	_	_	_	_	_	_				
Hyphomycete sp.	_	_	_	_	_	-	_		_	_	2	2	_	_	_	_	_	_	-	_	_		_	_				
Leptosphaeria australiensis (CRIBB & CRIBB) HUGHES	_		_	_	_	1	_		-	_	1	3	_	_	_	-	1	2	_	_	_	_		_				
Halosarpheia marina (CRIBE & CRIBB) KOHLM.			_	_		_	_		_	_	1	_	_	_	_	_	_	-	-	_	_	_	_	_				
H. ratnagiriensis PATIL & BORSE	_	-	_	_	_	_	_		_	_	_	_	_	_	_	-	1	-	-	_	_	_	-	_				
Phialophorophoma cf. litoralis LINDER	-		_	_	_	_	-		_	_	1	_	_	_	_	_	_	_	1	_	_	_		1				
A. chesapeakensis SHEARER & MILLER	_	_	_	_	_	1	_		_	-	_	_	_	_	_	_	_	_	_	_	_	_	_	_				
Splanchnonema sp.	-	-	_	-	_	-	-		-	-	_	_	-	-	-	-	-	-	1	-	-	-	-	-				

since experimental techniques differed. In this study poles surrounded by a bark layer have been used, while in the work in Singapore the samples were split in two, revealing the xylem. The fungi developing on *R. apiculata* in this study are bark-inhabiting species. Nutrition is probably gained only from the bark since the internal wood tissue has remained undecaved. The fungi mainly differ from those recorded by TAN & al. (1989) since these are unlikely to be barkinhabiting species but probably recorded from the exposed xylem. In nature the dving prop roots may first be colonized by bark-inhabiting fungi, which eventually cause the bark to drop off. After 1-3years the inner woody tissue is exposed and further decayed by fungi, probably similar to those recorded by TAN & al. (1989a). Obviously a simplified model like this only serves to illustrate succession; in nature the situation is more complex as up to 60 fungi are known from Rhizophora apiculata (Hyde, 1990a). Many factors affect the mycota colonising the wood. HYDE (1991) has shown that dead young "fleshy" prop roots of Rhizophora apiculata are colonised by Phomopsis sp., although this may be an antagonistic or neutral endophyte, while Hype (1989; 1990c) has demonstrated vertical distribution to be an important factor.

Some of the fungi recorded on *X. granatum* are similar to those recorded by TAN & al. (1989a) since wood was exposed early on in the experiment due to loss of bark. *Lulworthia* sp. (spore range 200-335  $\mu$ m) and *Xylomyces* sp. were early colonizers of bark and then wood. *Lulworthia* sp. was also an early colonizer of submerged wood in Singapore (TAN & al., 1989a). *Xylomyces* sp. was not recorded in studies in Singapore, but this species is common on *Xylocarpus* and rare on other hosts (HYDE , 1990a). Late colonizers on *Xylocarpus* were *Calathella* sp. and *Hypoxylon oceanicum*, the latter also recorded as a later colonizer in Singapore.

The resistance of *R. apiculata* prop roots to decay when compared to poles of *Xylocarpus granatum* is notable. The prop roots of *R. apiculata* contain tannin cells (TOMLINSON, 1986), are also very thick and few fungi are apparently able to develop within this substratum. In Borneo 25-30% tannin was recovered from bark (CHAPMAN, 1976). Poles of *R. apiculata* examined after one year of submersion still supported a protective layer of bark with sound wood beneath. Marine borers also seemed to be excluded by the bark. In marked contrast, *Xylocarpus* poles decayed quickly, losing bark within 4 months and many disintegrating within 12 months. Bark of *X. granatum* contained 20.4% tannin in Indo-Malaysia (CHAPMAN, 1976) and it is difficult to explain why bark was lost so quickly. Younger trees, however, contain lower tannin levels in the bark than older trees (CHAPMAN, 1976) and this may account for the losses. Marine borers played a major role in the breakdown of material in the lower intertidal zone. *Hypoxylon oceanicum* was also abundant, particularly at level 3. The wood in the upper zone (level 4) was decayed more slowly, maintained its bark and was colonised invariably by *Calathella* sp.

The present study highlights some of the fungi involved in the early decay of *Rhizophora apiculata* and *Xylocarpus granatum* and shows a marked difference in the rate with which they are broken down in the intertidal region. In *Rhizophora* the fungi collected can be regarded as early colonizers of bark and are fungi infrequently collected in random sample studies (HYDE, 1990a; TAN & LEONG, 1991). This should be expected, since in the previous studies easily detached samples were collected and were already considerably decayed. The results on *Xylocarpus* also indicate that marine borers play a major role in the breakdown of wood in the lower intertidal region, but have less effect above mean tide, where fungi seem to play the dominant role in decomposition.

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