Fungal diversity and succession on pods of *Delonix regia* (*Leguminosae*) exposed in a tropical forest in Thailand

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The succession of fungi on pods of *Delonix regia* has been investigated at Khao Yai National Park, Thailand. The fungi colonising *Delonix regia* pods were dominated by anamorphic fungi. Succession of fungi begins with colonisation by classical seed fungi, e.g. *Aspergillus, Chaetomium, Penicillium* and *Rhizopus* species, when pods were dry and attached to the tree. As soon as the pods fall on the forest, the classical seed fungi are replaced rapidly by litter fungi, e.g. *Dictyochaeta*, *Helicosporium, Phaeoisaria*, *Phoma* and *Sporoschisma* species. The moisture content of the pods may be an important factor in determining the mycota they support.

Key words: fungal diversity, legume fruits, succession, tropical forests.

Introduction

While many fungal succession studies have investigated various plant substrata, few have focused on fruits and seeds, with the exception of those on grain in storage e.g. rice in Malaysia (Kuthubutheen, 1984). We therefore embarked on a study of fungal succession on fruits and seeds exposed on the natural forest floor to better understand nutrient cycling processes in tropical ecosystems. The objective of this study was to investigate the fungal diversity of *Delonix regia* pods exposed in a natural forest and to follow the sequential occurrence of fungi involved in the decay process.

Materials and methods

Mature dry pods of *Delonix regia* (*ca.* length: width: thickness was 60: 4.5: 0.7 cm) were collected from boundary areas of Khao Yai National Park, Thailand, while they were still attached to the trees. Pods were cut into 15 cm long samples (130 samples). Five of these samples were randomly selected, surface sterilized with 10% sodium hypochloride and incubated in moist

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Table 1. Frequency of occurrence of fungi colonising *Delonix regia* pods collected from the trees.

Taxon	(%)	Taxon	(%)
Aspergillus flavus	100	*Cladosporium sphaerospermum*	40
Chaetomium globosum	100	*Eurotium* sp.	40
Rhizopus sp.	100	Sterile mycelium taxon 2	40
Aspergillus niger	80	*Aspergillus candidus*	20
Aspergillus sydowii	80	*Memnoniella echinata*	20
Penicillium sp.	80	*Myrothecium* sp.	20
Sterile mycelium taxon 1	80		

chambers to identify the fungi present, while the other 120 samples were placed separately in a litterbag (10 cm × 20 cm and 2 mm mesh size) and exposed in an evergreen forest of Khao Yai National Park, Thailand. Litterbags were exposed in May 2001 at two terrestrial sites: an area near road marker km 29.2 (km 29.2) and an area near Pha Kra Jai Waterfall (Pha Kra Jai). Five samples from each site were removed randomly and returned to the laboratory in June 2001 and then monthly until all samples had been retrieved. The results for the first five months of exposure are presented in this paper.

On return to the laboratory, samples were washed with water to remove attached soil and any plant particles before incubation in moist chambers. Samples were examined under a dissecting microscope after incubation for one week and up to 3 months. Colonising fungi were identified using standard texts (e.g. Sutton, 1973; von Arx and Müller, 1975; Carmichael *et al.*, 1980) and recorded in terms of percentage frequency of occurrence. Fungi were isolated and deposited in the BIOTEC Culture Collection (BCC) and dried samples placed in the BIOTEC Bangkok Herbarium (BBH).

Results

Seventy fungi (50 anamorphic fungi, 12 ascomycetes, 5 sterile species, 2 basidiomycetes and 1 zygomycete) were recorded on *Delonix regia* pods: eight on incubated pods collected from the trees; 57 on the exposed pods on the forest floor, while *Chaetomium globosum*, *Memnoniella echinata*, *Myrothecium* sp. and 2 sterile species were common to both sets of pods (Tables 1-3). The 62 species on exposed pods comprised 44 anamorphic fungi, 11 ascomycetes, 5 sterile species, and 2 basidiomycetes.

Pods collected and incubated in the laboratory without being exposed on the forest floor were dominated by *Aspergillus* spp., *Chaetomium globosum*, *Penicillium* spp. and *Rhizopus* sp., with a frequency of occurrence ranging from 80-100% (Table 1). The average number of fungi occurring on each pod sample was 8, and the ratio of anamorphic fungi to ascomycetes was 4:1.

Table 2. Colonisation and frequency of fungal occurrence of *Delonix regia* pods exposed at km 29.2 site, Khao Yai National Park, Thailand.

Taxa	Frequency of occurrence at each month (%) [1]					
	1[2]	2	3	4	5	1-5
Dominant taxa						
Dictyochaeta sp.	80	80	100	80	40	76
Phaeoisaria clematidis	20	100	100	40	40	60
Sterile mycelium taxon 3	80	60	40	100	20	60
Sterile mycelium taxon 2	100	100	40	-	-	56
Cylindrocladium sp.	60	100	60	40	-	52
Chaetomium globosum	60	60	20	-	-	48
Clonostachys sp.	40	100	20	20	20	40
Phoma sp.	60	40	40	40	-	36
Sesquicillium sp.	20	-	40	100	20	36
Melanochaeta hemipsila	40	-	60	-	40	28
Physalidium elegans	-	60	40	20	20	28
Thozetella nivea	20	20	40	40	20	28
Other taxa						
Ascomycete sp. 1	-	-	20	60	-	16
Ascomycete sp. 2	-	-	20	60	-	16
Helicosporium sp.	-	20	60	-	-	16
Sporoschisma saccardoi	40	-	60	-	-	16
Chaetopsina fulva	-	-	60	-	-	12
Helicomyces roseus	40	-	20	-	-	12
Memnoniella echinata	40	-	20	-	-	12
Sterile mycelium taxon 4	-	20	40	-	-	12
Ascomycete sp. 3	-	-	20	-	20	8
Cirrenalia sp.	-	-	20	20	-	8
Hyphomycete sp. 1	-	-	40	-	-	8
Hyphomycete sp. 2	-	20	-	-	20	8
Lanceispora sp.	-	20	-	-	20	8
Sporoschisma nigroseptatum	-	-	40	-	-	8
Acremonium sp.	-	20	-	-	-	4
Ascomycete sp. 4	-	-	-	-	20	4
Ascomycete sp. 5	-	-	-	-	20	4
Basidiomycete sp. 1 (teeth fungi)	-	20	-	-	-	4
Basidiomycete sp. 2 (agaric)	-	-	20	-	-	4
Chloridium sp.	-	-	-	-	20	4
Coelomycete sp. 1	20	-	-	-	-	4
Coelomycete sp. 2	-	-	-	-	20	4
Dactylaria sp.	-	-	-	20	-	4
Dictyosporium tetraseriale	-	-	20	-	-	4
Gonytrichum macrocladium	-	-	-	-	20	4
Hyphomycete sp. 3	-	20	-	-	-	4

[1] Frequency of occurrence (%) = number of samples on which a given taxon occurred / number of samples examined) × 100; [2] Period of exposure (months).

Table 2 continued.

Taxa	Frequency of occurrence at each month (%)					
	1	2	3	4	5	1-5
Myrothecium sp.	20	-	-	-	-	4
Nectria sp.	-	20	-	-	-	4
Pithomyces alabamensis	-	20	-	-	-	4
Pseudorobotrytis terrestris	-	-	-	-	20	4
Trichoderma sp. 1	-	-	-	-	20	4
Trichoderma sp. 2	-	-	-	-	20	4
Verticillium sp.	-	-	-	-	20	4
Volutella sp.	-	-	20	-	-	4
Total species	16	19	27	13	20	46

Exposed pods on the forest floor at km 29.2 yielded 46 fungi, including 32 anamorphic taxa, 9 ascomycetes, 2 basidiomycetes and 3 sterile species (Table 2). The ratio of anamorphic fungi to ascomycete taxa was 3.6:1. Species diversity increased during the first three months of exposure then declined during the last two months. Parallel with number of species, frequency of occurrence of most fungi was also higher during this early phase of seed decay. *Dictyochaeta* sp., *Phaeoisaria clematidis* and one sterile species were recorded every month with high frequencies of occurrence (60-76%) (Table 2). There were 16 pioneer species, with sterile mycelium taxon sp. 2, 3 and *Dictyochaeta* sp. present at a high percentage frequency. Some taxa occurred at low frequencies of occurrence (e.g. Coelomycete sp. 1, *Myrothecium* sp.) and may play no significant role in the decay process. Of the 12 most dominant species, *Dictyochaeta* sp., *Phaeoisaria clematidis*, *Sesquicillium* sp. and sterile mycelium taxon sp. 3 increased in their frequency of occurrence over the 5-month experiment period, while most declined in their frequency (e.g. *Cylindrocladium* sp., sterile mycelium taxon sp. 2). Species diversity was greatest at month 3 when 27 taxa were recorded, but some of these taxa only occurred once during the exposure period (e.g. *Chaetopsina fulva*, Hyphomycete sp. 1, *Sporoschisma nigroseptatum*). At 5 months, 20 species were still sporulating on the pods, but at a low frequency of occurrence (20%). Only *Dictyochaeta* sp., *Phaeoisaria clematidis*, and *Melanochaeta hemipsila* occurred at a higher frequency of 40%.

Pods exposed on the forest floor at Pha Kra Jai yielded 40 fungi, including 30 anamorphic taxa, 4 ascomycetes, 1 basidiomycete and 5 sterile species (Table 3). The ratio of anamorphic taxa to ascomycete taxa was 7.5:1. Species diversity was highest in the first month of exposure, declining in the next three months and finally increasing in the last month. *Dictyochaeta* sp., *Phaeoisaria clematidis*, *Phoma* sp. and *Sporoschisma nigroseptatum* were

Table 3. Colonisation and frequency of fungal occurrence of *Delonix regia* pods exposed at Pha Kra Jai site, Khao Yai National Park, Thailand.

Taxa	Frequency of occurrence at each month (%)					
	1	2	3	4	5	1-5
Dominant taxa						
Phaeoisaria clematidis	100	100	100	100	80	96
Dictyochaeta sp.	100	100	100	80	60	88
Sporoschisma nigroseptatum	40	20	100	80	60	60
Phoma sp.	80	80	40	40	40	56
Helicosporium sp.	20	-	80	60	100	52
Cylindrocladium sp.	20	20	20	60	80	40
Sterile mycelium taxon 2	20	20	60	20	80	40
Chaetomium globosum	80	80	-	-	-	32
Helicomyces roseus	20	80	-	-	20	24
Nectria sp.	40	80	-	-	-	24
Pseudorobillarda sojae	80	40	-	-	-	24
Thozetella nivea	-	-	40	60	20	24
Other taxa						
Chaetopsina fulva	40	-	-	60	-	16
Memnoniella echinata	60	40	-	-	-	16
Clonostachys sp.	20	40	-	-	-	12
Physalidium elegans	-	-	-	60	-	12
Volutella sp.	40	-	-	20	-	12
Basidiomycete sp. 2 (agaric)	-	-	20	20	-	8
Selenosporella sp.	-	-	40	-	-	8
Sesquicillium sp.	-	-	40	-	-	8
Sporoschisma saccardoi	40	-	-	-	-	8
Sporoschismopsis sp.	-	40	-	-	-	8
Sterile mycelium taxon 4	20	-	-	-	20	8
Sterile mycelium taxon 3	20	20	-	-	-	8
Trichoderma sp. 3	-	-	20	-	20	8
Ascomycete sp. 6	-	-	-	-	20	4
Ascomycete sp. 7	-	-	-	-	20	4
Beltrania rhombica	-	-	-	20	-	4
Catenularia sp.	-	-	-	-	20	4
Cirrenalia sp.	-	-	-	-	20	4
Coelomycete sp. 3	-	-	20	-	-	4
Dictyosporium tetraseriale	-	-	-	-	20	4
Hyphomycete sp. 4	-	-	20	-	-	4
Hyphomycete sp. 5	-	20	-	-	-	4
Monotosporella sp.	-	-	-	-	20	4
Myrothecium sp.	20	-	-	-	-	4
Nawawia filiformis	-	-	-	-	20	4
Sterile mycelium taxon 1	20	-	-	-	-	4
Sterile mycelium taxon 5	-	20	-	-	-	4
Trichoderma sp. 4	-	-	-	-	20	4
Total species	20	16	14	13	19	40

recorded every month with a high frequency of occurrence (60-96%) (Table 3) and were present throughout the exposure period

Pioneer species, such as *Dictyochaeta* sp., *Phaeoisaria clematidis*, *Phoma* sp. and *Pseudorobillarda sojae* occurred at a high frequency of occurrence (80-100%). *Phaeoisaria clematidis* was present throughout the exposure period, while others declined over the 5 months (e.g. *Dictyochaeta* sp., *Chaetomium globosum*, *Nectria* sp.). However, *Cylindrocladium* sp. and *Helicosporium* sp. increased in frequency of occurrence over the experimental period (20-100%, 20-80%, respectively). Apart from the dominant species, other fungi were sporadic in their occurrence or present with a low frequency of occurrence.

Twenty-two taxa, including 17 anamorphic taxa, 3 sterile species, 1 ascomycete and 1 basidiomycete, occurred on seeds at the two sites. The overall similarity index to the two sites was 51.2% (Table 4), with lowest similarity for the ascomycetes (15.4%) when compared with the basidiomycetes (66.7%) and anamorphic taxa (54.8%). Similarity between fungi colonising pods collected directly from the tree and exposed pods were low (20%) and this further decreased following the period of pod exposure (Table 6).

Discussion

Fungal diversity

Fungal diversity on the exposed pods was high at both sites: 46 species were recorded at km 29.2 and 40 species at Pha Kra Jai. Some of the fungi colonising pods at km 29.2 were similar to those reported by Sivichai *et al.* (2000) on submerged wood of *Dipterocarpus alatus* and *Xylia dolabriformis* at the same location. Sixty-two fungi were recorded on *Xylia dolabriformis* wood and 53 on *Dipterocarpus alatus* wood during a 12 month study period. The ratio of anamorphic to ascomycete taxa on the two timbers were 3.4:1 and 2.3:1 respectively (Sivichai *et al.*, 2000), while on pods in the present study it was 3.6:1. Fungi common to both studies, including their frequency of occurrence, are listed in Table 6. The frequency of occurrence of *Phaeoisaria clematidis*, *Sporoschisma saccardoi* and *Thozetella nivea* was higher on pods than on the submerged wood, while *Helicomyces roseus* had a higher frequency of occurrence on submerged wood as opposed to the pods.

Fungal succession

We categorized fungi colonising *Delonix regia* pods into 3 groups: classical seed fungi (dominant on pods collected from the trees but not placed

Table 4. Comparison of fungal similarity between km 29.2 and Pha Kra Jai sites.

	Number of species		Number of sharing species	Similarity (%)
	Km 29.2	Pha Kra Jai		
Ascomycetes	9	4	1	15.4
Basidiomycetes	2	1	1	66.7
Anamorphic taxa	32	30	17	54.8
Sterile mycelium	3	5	3	75
Total	46	40	22	51.2

Table 5. Comparison of fungal similarity between collected (incubated) pods and exposed pods at the sites: km 29.2 and Pha Kra Jai.

	Exposed pod (month)									
	Km 29.2					Pha Kra Jai				
	1	2	3	4	5	1	2	3	4	5
Collected	20	12.5	10	0	0	24.2	13.8	0	0	0
1 month		50	63.6	53.3	37.8		72.2	41.2	54.5	46.2
2 month			52.3	50	41			40	41.4	40
3 month				60	38.3				59.3	54.5
4 month					42.4					50

Table 6. Frequency of occurrence of fungi common to two studies at km 29.2, Khao Yai National Park, Thailand.

Species	Exposed pods of *D. regia* (this study)	Submerged wood of *D. alatus* (Sivichai *et al.*, 2000)	Submerged wood of *X. dolabriformis* (Sivichai *et al.*, 2000)
Phaeoisaria clematidis	60	3.3	3.3
Thozetella nivea	28	13.3	13.3
Sporoschisma saccardoi	16	1.7	-
Helicomyces roseus	12	81.7	58.3

on the forest floor); litter fungi (dominant on exposed pods throughout the exposure period, often at a high frequency of occurrence) and sporadic inhabitants (those occurring sporadically on exposed seed pods with a low frequency of occurrence).

The classical seed fungi encountered on the collected pods were taxa in the genera *Aspergillus*, *Chaetomium*, *Cladosporium*, *Eurotium*, *Penicillium* and *Rhizopus* (Sinha, 1992). This group disappeared after the pods were exposed on the forest floor and were replaced by litter fungi. *Chaetomium globosum*, however, was also collected on the exposed pods. The dominance of the classical seed fungi on the pods collected directly from the tree and the decline and disappearance of these fungi during the exposure period on the forest floor reflects the preferred conditions of these seed species. It may be that the

classical seed fungi require a low humidity for development (Sinha, 1992). The moisture content of the pods exposed on the forest floor increases with time, which may account for the decline in classical seed fungi and the dominance of the litter fungi.

As indicated earlier, some of the fungi colonising the pods exposed on the forest floor have also been reported from test blocks submerged in streams (Goh and Hyde, 1999; Sivichai *et al.*, 2000; Tsui *et al.*, 2001) and also on submerged litter in tropical freshwater streams (Kuthubutheen, 1993). Fungi common to these substrata and niches include species in the genera *Chaetopsina, Cryptophiale, Dactylaria, Dictyochaeta, Kionochaeta, Helicomyces, Monotosporella, Phaeoisaria* and *Sporoschisma.* Goh (1997) regarded these as facultative aquatic fungi and most are dematiaceous. However, the fact remains that these fungi colonise substrata submerged in river and streams and often at a high frequency of occurrence (Sivichai *et al.* 2000). Goh (1997) points out that many of these hyphomycetes have "long mononematous stipitite conidiophores, which stand erect from the submerged substrata and bear masses of conidia at their apices". Clearly, there is a continuum of hyphomycetes from terrestrial leaf litter, to submerged leaf litter to immersed trapped wood. The lignicolous and leaf litter species have to withstand periodic drying and re-hydration in an aquatic freshwater habitat, and therefore have developed different strategies for the dispersal of their spores. The terrestrial origin of these fungi is supported by the data presented in this study, but nevertheless they also are very successful in the colonisation substrata under amphibious and submerged conditions (Kuthubutheen, 1993; Goh, 1997; Sivichai *et al.*, 2000). Thomas (1996) has broadened the definition of what is an aquatic freshwater mycota, many of which, as shown here are also common in terrestrial habitats.

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