Cryptogamie, Mycologie, 2004, 25 (4): 315-321

Fungal saprobes on dead leaves of *Magnolia liliifera* (Magnoliaceae) in Thailand

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Abstract – An investigation of saprobic fungi associated with dead leaves of *Magnolia liliifera* at Doi Suthep-Pui National Park, Chiang Mai, Thailand was carried out from June to September 2001. Ninety dead leaves fallen on the forest floor were collected and examined for fungi. Thirty-seven taxa of saprobic fungi comprising 20 ascomycetes and 17 anamorphic fungi (4 coelomycetes and 13 hyphomycetes) were discovered. Dominant species were *Bionectria ochroleuca*, *Cylindrocladium floridanum*, *Dokmaia monthadangii*, *Gliocladium* sp. 1, *Hyponectria manglietiae*, *H. manglietiagarrettii*, *Hypoxylon* sp., *Lasiosphaeria* sp., *Pseudohalonectria suthepensis* and *Sporidesmium crassisporum* which comprised 12.2%, 17.8%, 11.1%, 21.1%, 41.1%, 14.4%, 14.4%, 17.8%, 15.6%, 52.2% of the total collections, respectively. The diversity of fungi on this host is compared with that found in other studies.

Biodiversity / fungal diversity / fungal estimates

INTRODUCTION

There have been several estimates of global fungal numbers ranging from 100,000 to 9.9 million and this has been discussed extensively by Fröhlich & Hyde (1999), Hawksworth (2001) and Hyde (2001). The generally accepted 1.5 million fungi is dependent, in part, on there being, on average, 5-6 unique species to every host plant (Hawksworth, 1991, 2001). Studies on bamboo, grasses, palms, sedges, Pandanaceae and Musaceae in the tropics have shown that many of the saprobic fungi occurring on these hosts are unique to the host family, genera and species (Photita *et al.*, 2001; Wong & Hyde, 2001; Yanna *et al.*, 2002; Hyde *et al.*, 2002ab;

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McKenzie *et al.*, 2002; Whitton *et al.*, 2002, 2003). Estimates of fungal diversity are important in order to place value on threatened natural habitats (Hawksworth, 2003). This is important to protect unsustainable exploitation of these habitats (Cannon, 1997). The tropical region comprises a great variety of tree species that produce high amounts of leaf litter (Cooke & Rayner, 1984), but few mycologists have investigated the diversity of microfungi inhabiting tropical leaf litter (Bills & Polishook, 1994). The present study aims to investigate the diversity of saprobic fungi occurring on dead leaves of *Magnolia liliifera* (Magnoliaceae) in its natural habitat. We chose this host as it has not been previously examined, and its leaves are relatively large (18-30 × 8-12 cm) and thick and thus likely to support a diverse range of fungi. *Magnolia liliifera* is also a prominent tree on Doi Suthep and Doi Inthanon forests in Chiang Mai.

MATERIALS AND METHODS

Ninety fallen brown leaves from *Magnolia liliifera* (montha-doi) were haphazardly collected from Doi Suthep-Pui National Park, Chiang Mai, Thailand during the rainy season, between June and September 2001. Leaves were returned to the laboratory in individual plastic bags and incubated with an addition of a piece of moistened sterile tissue. Samples were examined following 2 days of incubation and then for up to 2 weeks for the presence of microfungi. The fungi were recorded, identified and isolated by single spore isolation (Choi *et al.*, 1999). It was also noted whether the fungi were sporulating on either the upper or lower surface of the leaves, and if they were on the leaf lamina, veins or petioles. Small pieces of the leaves containing the fungi were excised and dried and prepared as herbarium specimens.

Statistical analysis and sample calculation

The fungi found in this study are presented in terms of percentage occurrence. Fungal taxa with an overall percentage occurrence equal to or higher than 10 are regarded as dominant species. Percentage similarity is the numerical method for comparing the species diversity between two different samples or communities. In this study percentage similarity was used to measure similarity between species diversity on the upper leaf surface and species diversity on the lower leaf surface.

$$\text{Percentage occurrence} = \frac{\text{Number of leaves on which fungus was detected} \times 100}{\text{Total number of leaf samples examined at each sampling time}}$$

Percentage similarity = 2c/a+b
a: the number of species on upper leaf surface
b: the number of species on lower leaf surface
c: the number of species in common between upper and lower leaf surface

RESULTS AND DISCUSSION

The taxa recorded on dead leaves of *Magnolia liliifera* are listed in Table 1 with their percentage occurrence. Thirty-seven fungal taxa were identified, comprising 20 ascomycetes and 17 anamorphic fungi (4 coelomycetes and 13 hyphomycetes). Dominant species, found on more than 10% of samples were *Bionectria ochroleuca*, *Cylindrocladium floridanum*, *Dokmaia monthadangii*, *Gliocladium* sp. 1, *Hyponectria manglietiae*, *H. manglietiagarrettii*, *Hypoxylon* sp., *Lasiosphaeria* sp., *Pseudohalonectria suthepensis* and *Sporidesmium crassisporum.*

The fungi recorded on dead leaves of *Magnolia liliifera* can be compared with those recorded during a successional study of fungi on senescent leaf baits of this host at the same site (Promputtha *et al.*, 2002). In this paper and others papers (Promputtha *et al.*, 2002, 2003, 2004abc) we gave the host as *Manglietia garrettii.* With careful examination of these similar hosts and with the help of botanists we have now determined that the host is *Magnolia liliifera.*

During the successional study on *Magnolia liliifera*, 22 fungal taxa were identified, compared to 37 taxa identified on randomly collected decaying leaves in the present study. All of the fungi recorded by Promputtha *et al.* (2002) were recorded in the present study, but 15 extra taxa were recorded on the randomly collected decaying leaves. The numbers of leaves examined in the successional study (70) was less than in this study (90), which may account for fewer fungi being discovered. In some other studies, where baits have been used to investigate fungi on a substrate, there have been fewer fungi recorded than on randomly collected samples of the same substrate (Tan *et al.,* 1989; Hyde, 1991; Poonyth *et al.*, 2001). However, successional studies using baits have also resulted in more fungi being identified as substrates at different stages of decay are examined (e.g. Zhou & Hyde, 2001; Ho *et al.*, 2002; Yanna *et al.*, 2002). Examination of fungi on naturally occurring samples is likely to result in finding fungi that only occur during certain stages of decay (Yanna *et al.*, 2002). When decaying leaves are collected there may be a bias towards collecting leaves that are at the middle stage of decay and this is thought to be reflected in the lower numbers of fungi identified (Yanna *et al.*, 2002). The times for fungal communities increase to the peak of species diversity are varied among different hosts (Yanna *et al.*, 2002). For example, complete decomposition of sugarcane bagasse required 20 weeks and the highest diversity were recorded between week 6-13 (middle stage of decay) (Sandhu & Sidhu, 1980). During one year of complete decomposition process of fronds of *Phoenix hanceeana*, species diversity also peaked at the middle stage of decomposition, 120 days for leaves, 150 days for rachis tips 200 days for mid-rachides and rachis-bases (Yanna *et al.*, 2002). In the two months decomposition of senescent leaves of *Magnolia liliifera*, the number of species was maximum during the middle phase (day 24-40) of decomposition (Promputtha *et al.*, 2002).

A species with high percentage occurrence is found on many leaf samples, but occurrence does not indicate how dominant a particular species may be. For example, *Gliocladium* sp. 1 occurred on 21.1% of the leaves examined but was found on only a small area on each of the leaves. On the other hand, *Hyponectria manglietiae* and *Lasiosphaeria* sp. were found on 41.1% and 17.8% of the leaves respectively. Entire leaves were covered with ascomata of these two species indicating high activity. It is important in future studies to devise a system that records the area of leaf occupied or provides a dominance index as well as frequency of occurrence, as this will provide more data on the importance of a taxon.

Tab. 1. Percentage occurrence of fungal taxa on dead leaves of *Magnolia liliifera* in Doi Suthep-Pui National Park, northern Thailand.

Fungal taxa	*Upper surface*			*Lower surface*			*Overall percentage occurrence*
	L	*V*	*P*	*L*	*V*	*P*	
Albonectria rigidiuscula	1						1.1
*Anthostomella monthadoia**				4			4.4
Anthostomella tenacis	2						2.2
Bionectria ochroleuca	11			11			12.2
Bionectria palmicola				1			1.1
Bionectria sp.	1			1			1.1
Colletotrichum gloeosporioides				1			1.1
Cylindrocladium floridanum	16			16			17.8
Dactylaria dimorphospora				2			2.2
Dactylaria longidentata	4			4			4.4
Dokmaia monthadangii	10			10			11.1
Fusarium sp. 1	3						3.3
Fusarium sp. 2	1						1.1
Gliocladium sp. 1	19			19			21.1
Gliocladium sp. 2	2			2			2.2
Gliocladium sp. 3	1			1			1.1
Guignardia sp. 1					1		1.1
Giugnardia sp. 2					1		1.1
Haematonectria haematococca	1						1.1
*Hyponectria manglietiae**	37			37			41.1
*Hyponectria manglietiagarrettii**	13			13			14.4
*Hyponectria suthepensis**	6			6			6.7
Hypoxylon sp.	13			13			14.4
Ijuhya parilis				1			1.1
Lasiosphaeria sp.	16			16			17.8
Munkovalsaria magnoliae					1		1.1
Periconia jabalpurensis				5			5.6
Phaeosphaeria sp.	6			6			6.7
Phoma sp. 1	1			1			1.1
Phoma sp. 2	1			1			1.1
Phomopsis sp.	1			1			1.1
Physalospora sp.				1			1.1
*Pseudohalonectria suthepensis**					14	14	15.6
Rhytisma sp.	1						1.1
Sporidesmium crassisporum	47	47	47	47	47	47	52.2
Stachybotrys parvispora				3			3.3
Volutella sp.	8			8			8.9
Total no taxa 37	**25**	**1**	**1**	**27**	**5**	**2**	

* New species known only from *Magnolia liliifera* with descriptions presently in press or in preparation.
L: Leaves V: Veins P: Petioles.

The diversity of fungi on dead leaves of *M. liliifera* can be compared with diversity recorded on other hosts in tropical regions, especially Thailand. Photita *et al.* (2001) identified 46 taxa from *Musa acuminata* in Hong Kong, only one of which, *Colletotrichum gloeosporioides*, was found on *M. liliifera* in this study. Somrithipol *et al.* (2002) examined 130 samples of *Dolenix ragia* pod in Khao Yai

National Park, Thailand and found 70 fungi, but no fungi overlap with this study. Photita *et al.* (2003) recorded 80 fungi on dead tissues of *Musa acuminata* from five sites at Doi Suthep-Pui National Park, Thailand, and only one of these taxa (*Dactylaria dimorphospora*) was recorded in the present study. Yanna *et al.* (2001) identified 81 fungal taxa from fronds of *Phoenix hanceana* in Hong Kong. Yanna *et al.* (2001) identified 91 studies diversity was higher, probably because collections included several plant parts. The hosts were also monocotyledons. The overlap of fungi on *M. liliifera* with those fungi found on other hosts in Thailand (Doi Suthep-Pui National park and Khao Yai National Park) is low. This may be result of host-specificity (Zhou & Hyde, 2001). The overlap of fungi occurring on *M. liliifera,* with that on other unrelated hosts in the same region (Doi Suthep-Pui National Park) are low (Photita *et al.*, 2002). This may be a result of host-specificity or recurrence (*sensu* Zhou & Hyde, 2001). Saprobic fungi are thought unlikely to be host-specific (Zhou & Hyde, 2001). However, the results presented here indicate that the fungi on a host are quite different to others hosts in the same area (Photita *et al.*, 2001). An important factor that influences the biodiversity of saprobic fungi on various hosts is therefore host-recurrence. The basis for host-recurrence is, however, unknown and requires further research, but may be related to host recurrence of endophytes.

Studies with dead leaves of dicotyledonous trees include those of Parungao *et al.* (2002) and Polishook *et al.* (1996). Parungao *et al.* (2002) examined 10 leaves from different tree species in rainforests in northern Queensland, Australia and found between 0 and 14 fungal taxa on each leaf species. Polishook *et al.* (1996) identified between 8 and 15 species from leaves of *Manilkara bidentata* and 9 and 11 species from leaves of *Guarea guidonia* in Puerto Rico. Thirty-seven fungi for one host in a relatively small area thus appears to be a high number for dicotyledonous tree leaves.

Different fungi were recorded on leaf lamina, petioles and veins of *M. liliifera*. Most fungi were found on the lamina. Of the 37 species identified, 32 were found only on the lamina and 3 were only on the veins. The difference is due to the differential tissue type recurrences of species such as *Guignardia* sp. 1 and 2 and *Munkovalsaria magnoliae* on the veins, *Pseudohalonectria suthepensis* on the veins and petioles and *Sporidesmium crassisporum* on the lamina, petioles and veins, while other species (e.g. *Cylindrocladium floridanum*, *Hyponectria manglietiae*) were found only on the lamina tissues. These results indicate that some fungi occur more frequently on certain tissue types. This has been also shown with palms (Yanna *et al.*, 2001).

Fungi were recorded from both of leaf surfaces, upper and lower leaf surfaces. Of the 37 species found, 19 taxa occurred on both sides of leaves, and the percentage similarity of the fungi between the two sides of the leaves was 67.8%. *Albonectria rigidiuscula*, *Anthostomella tenacis*, *Fusarium* sp. 1, and 2, *Haematonectria haematococca*, and *Rhytisma* sp. were found only on the upper surface of leaves, while *Anthostomella monthadoia*, *Bionectria palmicola*, *Colletotrichum gloeosporioides, Dactylaria dimorphospora*, *Guidnardia* sp. 1 and 2, *Ijuhya parilis*, *Munkovalsaria bipolaris*, *Periconia jabalpurensis*, *Physalospora* sp., *Pseudohalonectria suthepensis* and *Stachybotrys parvispora* were found only on the lower side. The reasons for some fungal species being restricted to a particular leaf surface is unclear. When leaves fall from the tree they can land with either surface touching the ground and there does not appear to be any apparent advantage for any fungus to be restricted to developing on a particular leaf surface. The restrictions to a leaf surface exhibited by some fungi in this study may be the result of under sampling. The surface of leaves touching the ground had no

significant effect on fungal communities developing on baited senescent leaves of the same host (Promputtha *et al.*, 2002).

Of the 37 fungi recorded in this study, *Anthostomella monthadoia*, *Dokmaia monthadangii*, *Hyponectria manglietiae*, *H. manglietiagarrettii*, *H. suthepensis*, *Munkovalsaria magnoliae* and *Pseudohalonectria suthepensis* are newly discovered species and to date are known only from *M. liliifera*. Other species, not identified to species level, may also be specific to *M. liliifera*. Many of the fungi recorded in this study are rare species and it is uncertain whether they are specific to *M. liliifera*. From the point of view of fungal diversity numbers, it is important to establish if these rare species are specific to *M. liliifera*.

Acknowledgements : Funds for this research were provided by The Royal Golden Jubilee Ph.D. Program under The Thailand Research Fund. S. Thongkantha, B. Bussaban and W. Photita are thanked for help with technical, photographic and other suggestions. K.D. Hyde thanks The Institute of Science and Technology Development of Chiang Mai University for providing funds to visit Chiang Mai University. J.F. Maxwell is thanked for help in identifying specimens.

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