Temporal variation in endophyte assemblages of *Plumeria rubra* leaves

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The temporal pattern of endophyte infection in the leaves of *Plumeria rubra*, a tropical deciduous tree, was studied by sampling the leaves of an individual tree for a period of one year. Endophytes could be isolated from the leaves throughout the study period. Older leaves were more densely colonised than the younger leaves. Hyphomycetes dominated the endophyte assemblage of the younger leaves, while the older leaves harboured more coelomycetes. This study indicates that there is temporal variation of endophyte assemblages in leaves of some tropical plant hosts.

Key words: Fungal endophytes, *Plumeria rubra*, endophyte community

Introduction

Endophytic fungi, which cause asymptomatic infections in plants, are important organisms as many of them produce novel secondary metabolites of industrial potential (Schulz *et al*., 2002; Worapong *et al*., 2002) and some of them enhance the fitness of their host plants (Redman *et al*., 2002). The occurrence and distribution of endophytic fungi in the leaves of tropical plants (Stone *et al*., 2000; Romero *et al*., 2001; Suryanarayanan *et al*., 2001; Toofanee and Dulymamode, 2002) and plant communities (Kumaresan and Suryanarayanan, 2001, 2002; Cannon and Simmons, 2002; Suryanarayanan *et al*., 2002) have been relatively well-studied. Besdies the brief study of Tomita (2003), there are, few studies on seasonal infection patterns, especially for deciduous trees as most of the studies are based on samples taken at one time in one year (Wilson, 2000). Temporal information is essential since this cryptic guild of fungi could serve as a benchmark for assessing global fungal diversity (Hawksworth, 1991; Arnold *et al*., 2000, 2001). We have therefore studied the foliar endophytes of *Plumeria rubra*, a deciduous, tropical tree over a period of

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one year in order to assess whether there are temporal changes in endophyte communities.

Methods and materials

Plumeria rubra is a native of Central America and it has been planted throughout India as an ornamental tree. It sheds its leaves during the month of March and new foliage is produced in April. Healthy leaves were sampled from a single medium-sized tree growing in Chennai (13º04' N lat, 80º16' E long). Monthly collections were made for a period of 12 months from October 2001 to September 2002. For each sampling, ten leaves were processed within two hours of collection. The leaves were thoroughly washed in running water and 22 segments of 0.5cm^2 size were cut from the midrib region of each leaf. The segments were surface sterilized in 70% ethanol for 5 seconds followed by 4% NaOCl for 90 seconds and were then washed in sterile water for 10 seconds (Suryanarayanan *et al*., 1998). Two hundred surface sterilized segments were then plated on Petri-dishes containing Potato Dextrose Agar (PDA) amended with Chloramphenicol (150 mgl^{-1}). Ten segments were plated in each Petri-dish and were kept in a light chamber with a light regimen of 12 hour: 12 hour light-dark cycle (Bills and Polishook, 1992; Suryanarayanan *et al*., 1998) for 21 days at 26 ± 1°C. The fungi that grew out from the segments were periodically isolated and identified. In studies on fungal endophyte communities, mycelia sterilia are invariably isolated and grouped into morphotypes based on cultural characteristics. The study of Lacap *et al*. (2002) based on rDNA sequences substantiates the validity of such morphotypes as taxonomic groups. In the present study also, a few sterile forms were isolated and were categorized as morphotypes based on cultural characteristics such as colony surface, texture and hyphal pigmentation (Suryanarayanan *et al*., 1998).

Colonization frequency of an endophyte species was calculated as the number of segments colonized by a single endophyte divided by the total number of segments observed × 100. Diversity index (H') was calculated using the method of Ludwig and Reynolds (1988). Relative percentage of occurrence of different groups of fungi (viz. coelomycetes and hyphomycetes) was calculated by dividing the number of segments colonized by a group of fungi by the total number of segments colonized by all the groups of fungi.

Results and Discussion

The leaves of *P. rubra* harboured endophytes throughout the year (Table 1). The endophyte assemblage was made up of ascomycetes and their

anamorphs. A few mycelia sterilia were also isolated. *Colletotrichum* spp. and *Phyllosticta* sp. 1 were the dominant endophytes during the wet period (Table 1). These two genera are ubiquitous endophytes and have been reported from several plant hosts (Brown *et al*., 1998; Suryanarayanan *et al*., 2002).

Table 1. CF% of endophytes isolated from the leaves of *Plumeria rubra* sampled during various months (Only those endophytes with CF% more than or equal to 5 in at least one month of sampling)

Endophyte	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Aspergillus niger	2.5	1		0.5			1	1.5	6	9	7	1.5
Aureobasidium sp. 2	1						1				27.5	
Cladosporium sp. 1	2		0.5				6.5	2				1
Colletotrichum sp. 1	3.5	36.5	36.5	32	24.5	29						11.5
Colletotrichum sp. 2	1			12.5							0.5	5.5
Colletotrichum sp. 3	1.5	8.5	32	30	17	41						6
Colletotrichum sp. 4			1.5	7.5	22.5	18						
Colletotrichum sp. 5	1	1.5		11	5.5	1.5						
Glomerella sp. 1	1	0.5	5	7	6.5	5						
Glomerella sp. 2					6.5							
Penicillium sp. 1							5	2.5	2.5	5.5	1	
Phoma sp. 1	1.5	7.5	1.5	0.5					0.5	1	2	1
Phomopsis sp. 1	9	12.5	20	3	16	8.5				4	2.5	3.5
Phyllosticta sp. 1	14	40.5	35.5	27	30.5	49					0.5	7.5
Phyllosticta sp. 2					8							
Scytalidium sp. 1								5				0.5
Sporormiella sp. 1	7.5	4.5	0.5			1.5						5
Sterile form 1	4	8.5	2		3	3						1.5
Sterile form 2	0.5							2.5	5			
Trichoderma sp. 1		3.5	2		1.5		5.5	2			1.5	1.5
Xylariaceous form 1	0.5	3.5	3	5		6.5					1	1.5
Total CF%	62.5	140	148	146.5	144.5	169	24	26	26.5	29	49.5	67.5
Species	24	19	20	18	13	14	9	13	13	10	14	19

Endophytes with CF% < 5

Acremonium sp. 1, *Alternaria* sp. 1, *Aspergillus flavus*, *Aspergillus* sp. 1, *Aspergillus* sp. 2, *Aureobasidium pululans*, *Colletotrichum* sp. 6, *Corynespora* sp. 1, *Curvularia* sp. 1, *Drechslera* sp. 1, *Fusarium* sp. 1, *Fusarium* sp. 2, *Geotrichum* sp. 1, *Guignardia* sp. 1, *Lasiodiplodia theobromae*, *Lasiodiplodia* sp. 1, *Memnoniella* sp. 1, *Pestalotiopsis* sp. 1, *Nigrospora* sp. 1, *Phialophora* sp. 1. , *Piptochephalis* sp. 1, *Sporormiella* sp. 2, Sterile form 3, Sterile form 4, *Verticillium* sp. 1

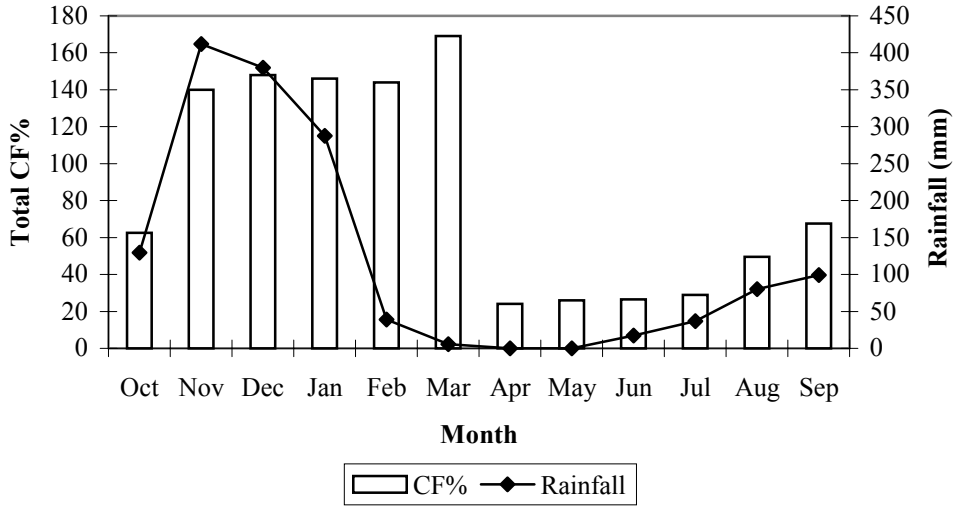
Although spatial patterns of endophyte infections have been studied for several tropical plants (Suryanarayanan *et al*., 2002), there are no studies on the temporal variation of endophytes in tropical hosts. The young leaves had low endophyte colonization frequencies, a trend that has been observed for many tropical plants (Rodrigues, 1994; Rajagopal and Suryanarayanan, 2000; Kumaresan and Suryanarayanan, 2002). The colonization frequency of the endophytes increased with the age of the leaf and reached a maximum during the month of March when the leaves were senescent and about to be shed (Table 2). The number of endophytes that can be recovered from the leaf tissue increases with the age of the leaf in several plant hosts including Douglas-fir (Stone, 1987), *Trachycarpus fortunei* (Taylor *et al*., 1999), *Azadirachta indica* (Rajagopal and Suryanarayanan, 2000). In ericaceous plants (Okane *et al*., 1998) and *Rhizophora apiculata* (Kumaresan and Suryanarayanan, 2002), the senescent leaves have more endophytes in them than the mature or young leaves. This increased colonization of old leaves is due to superinfection of the leaves over time by air-borne inoculum (Carroll *et al*., 1977; Rodrigues *et al*., 1993; Suryanarayanan and Vijaykrishna, 2001). Kumaresan and Suryanarayanan (2002) showed that the endophyte assemblage of senescent leaves of *R. apiculata* is more diverse than that of the young leaves indicating that the susceptibility to endophytes and saprobic fungi increases with leaf age. However, in *P. rubra*, the diversity of endophyte assemblage of mature leaves (during the month of March) was one of the lowest of all months, although such leaves had the maximum colonization frequency (Table 2). This indicates that although the susceptibility of the leaves to endophytes increases with age, the leaves continue to recruit only some species of fungi as endophytes.

Table 2. Number of species, isolates and species diversity of endophytes recovered from *P. rubra* sampled during various months

Month	Species	Isolates	Diversity (H')
October	24	125	2.66
November	19	280	2.16
December	20	296	2
January	18	293	2.22
February	13	289	2.26
March	14	338	1.94
April	9	48	1.86
May	13	52	2.32
June	13	53	2.19
July	10	58	1.94
August	14	99	1.67
September	19	135	2.6

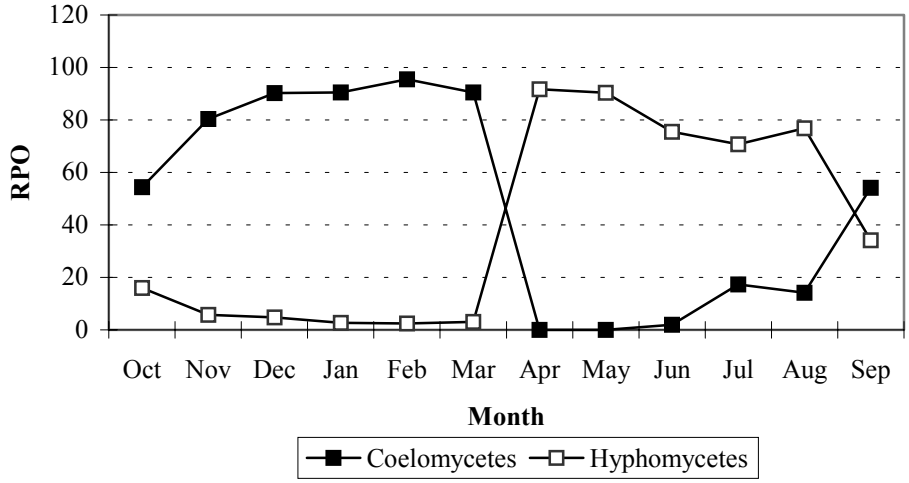
Precipitation is one of the major factors that influences infection by foliar endophytes. A strong correlation has been observed between endophyte infection levels and cumulative precipitation (Wilson, 2000). In many instances, leaves sampled during the wet season harbour more endophytes than those screened during the dry season (Rodrigues, 1994; Wilson and Carroll, 1994; Suryanarayanan *et al*., 1998). Such a trend was apparent with the leaves of *P. rubra* also (Fig. 1). However, high colonization frequencies were observed in the months of February and March during which the leaves were fully mature and there was very little precipitation (Fig. 1). Thus, leaf age was an overriding factor influencing the colonization density of foliar endophytes in *P. rubra*.

Fig. 1. Total Colonization Frequency of foliar endophytes of *Plumeria rubra* sampled during various months



Ascomycetes and their anamorphic states invariably constitute the endophyte populations of leaves (Petrini, 1986; Wilson, 2000). In the case of *P. rubra*, the relative percentage of occurrence of different groups of fungi revealed that hyphomycetes dominated the endophyte assemblage of young leaves, while coelomycetes dominated older leaves (Fig. 2). Thus, there appears to be a temporal relationship with reference to endophyte colonization

Fig. 2. Relative Percentage of occurrence of coelomycetes and hyphomycetes in *Plumeria rubra* leaves



in this host. Such a qualitative difference in the recruitment of endophytic fungi in young or old leaf tissue might depend on, apart from other factors, the physical and chemical status of the leaves. Leaves of tropical plants are densely colonized by endophytes (Suryanarayanan *et al*., 2002) and endophytes are known to produce several different types of secondary metabolites (Schulz *et al*., 2002). Thus, metabolically active endophytes in the leaf tissue can influence its physiological status. It is pertinent to mention here that endophytes can regulate leaf abscission (Wilson, 2000). More detailed studies are needed to elucidate the role of endophytes in leaf senescence and abscission.

Kumaresan and Suryanarayanan (2002) have shown that the endophyte assemblages of leaves are not static, but undergo changes after leaf fall. The present study shows, for the first time, that the endophyte assemblage is dynamic even in intact leaves. Such changes in the assemblages are likely to be more apparent in short-lived (deciduous) leaves than in persistent leaves.

Our study indicates that, at least in some tropical deciduous trees, one can witness temporal changes among endophytic fungi. Studies on other deciduous tree hosts would provide more data on this phenomenon and the regulation of leaf metabolism by such endophytic fungi if any.

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