Fungal endophytes associated with *Cordemoya integrifolia*

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Fungal endophytes associated with leaves of the endemic plant *Cordemoya integrifolia* have been studied. The diversity and frequency of endophytic fungi in young and old leaves of *Cordemoya integrifiola* occurring inside and outside the Maccabhé Conservation Management Area (CMA) were investigated. Endophyte assemblages examined were quite diverse, consisting of 26 fertile fungal taxa and one sterile morphospecies. *Pestalotiopsis* sp. and *Penicillium* sp. were the dominant taxa. Differences were observed between the endophytic communities isolated from different tissues and tissues of different ages. Old leaves supported more endophytes than relatively younger ones. Likewise, more endophytic fungi were recorded in the veins and petioles than in the intervein tissues.

Key words: *Cordemoya*, endophytes, Mauritius.

Introduction

The term 'endophyte' was introduced by De Bary (1866) and was initially applied to any organism found within a plant. The word's meaning has since evolved to include any microorganism that inhabits for a period of its life cycle, the interior of plants, especially leaves, branches and stems, without causing apparent damage to its host (Wilson, 1995). The definition is used in this study.

Research on fungal endophytes of trees over the past two decades has shown that virtually every leaf and stem tree is infected with fungal endophytes. Several reports have been published on endophytes, most from studies in temperate regions. Reviews have dealt with taxonomy, the biology, evolution and occurrence of endophytes. A number of factors such as age of foliage and plant, location and wetness of the site, and season have been shown to affect the distribution and species diversity of fungal endophytes (Petrini, 1991; Fisher *et al.*, 1993, 1995; Fröhlich *et al.*, 1999). Furthermore, within the leaves there can be variation in endophytes between the lamina, midrib and petiole (Johnston, 1994; Wilson and Carroll, 1994), and even cell type (Petrini, 1991).

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Fungal studies in Mauritius have so far dealt only with parasitic and saprobic fungi associated with sugarcane and the native forest. Peerally (1973) described the agaric *Tricholoma mauritianum* collected from a planted forest. Lutchmeeah (1993) reported *Phomopsis folliculicola* as a pathogen of *Trochetia boutoniana*, an endemic plant. More recently, fungi associated with mangroves (Poonyth *et al*., 1999a,b) and those associated with some endemic plants of Mauritius (Dulymamode, 2000) have been described.

Until now, no scientific study solely devoted to endophytic fungi has been carried out in Mauritius. *Cordemoya integrifolia* was selected for study because it is endemic to Mauritius and has not been investigated for the presence of endophytes. Moreover, diversity and succession of saprobic mycota have been studied in this plant (Leung, 2000).

Materials and methods

Leaves of *Cordemoya integrifolia* were collected from plants inside and outside the Conservation Management Area plot at Maccabhé, Black River Gorges National Park, Mauritius. At each site, three young and three mature leaves with their petioles were randomly picked from five parent plants and placed in labelled individual plastic bags.

The material was processed in the laboratory on the same day within the shortest time possible. The leaves were washed with running water. The leaves were surfaced sterilised by immersing them in 75% ethanol for one minute, then in 5% sodium hypochlorite for three minutes followed by immersion in 75% ethanol for 30 seconds. From each surface sterilised leaf, the petiole, lamina and the midrib were separated from each other. From each of the three parts, five square disks of approximately 0.5 cm × 0.5 cm were randomly cut.

The five disks were then placed at more or less equal distances on 2% oxoid malt extract agar (MEA) plates, supplemented with 250 mgL^{-1} streptomycin sulphate to suppress bacterial growth. All the plates were incubated at 25 C for 15 days and regularly checked for fungal growth. When two or more types of fungal mycelia were obtained on the same plate, they were separated by carefully sub-culturing onto different plates. Subsequent isolation was carried out by transfer of mycelium, conidia or ascospores to 2% MEA plates.

The fungi isolated were incubated at 25 C and allowed to grow and sporulate and the fungi were identified using relevant keys and taxonomic notes from Barnett and Hunter (1988), Ellis (1971, 1976) and Hawksworth *et al* (1995).

Table 1. Fungal taxa isolated from the lamina (L), midrib (M) and petiole (P) of mature and immature leaves of *C.integrifolia* at the 2 sites.

Site	Inside CMA						Outside CMA						Total	%
Age	Mature leaves			Immature Leaves			Mature leaves			Immature leaves				Colonisation Rate
Parts/Fungi	L	M	P	L	M	P	L	M	P	L	M	P		
Acremoniella	-	-	-	-	-	-	-	2	1	-	-	1	4	0.7797
Acremonium	-	-	-	4	4	2	-	-	-	-	-	-	10	1.9493
Aspergillus	-	-	-	-	-	-	6	11	9	4	2	4	36	7.0175
Cephalosporium	-	-	-	3	-	-	-	-	-	-	-	-	3	0.5848
Chlamydomyces	-	-	-	-	-	-	1	1	2	-	1	1	6	1.1696
Cladosporium	5	3	3	2	1	2	10	7	3	1	9	3	49	9.5517
Colletotrichum	-	-	-	-	-	-	-	2	1	-	-	-	3	0.5848
Fusarium	-	-	-	2	7	9	1	2	1	1	3	4	30	5.8480
Helminthosporium	-	1	1	-	-	-	-	-	-	-	-	-	2	0.3899
Humicola	-	-	-	-	2	3	-	-	1	-	-	-	6	1.1696
Hyalodendron	-	1	-	-	-	-	-	-	-	-	-	-	1	0.1949
Meliola	-	-	-	1	-	1	-	-	-	-	-	-	2	0.3899
Moniliella	1	7	10	-	-	-	-	-	-	-	-	-	18	3.5088
Myrothecium	-	-	-	-	-	-	1	1	1	-	-	1	4	0.7797
Nigrospora	4	7	5	-	4	1	1	1	3	1	4	1	32	6.2378
Papulospora	-	-	-	1	3	3	-	2	1	-	1	3	14	2.7290
Penicillium	12	13	14	8	13	7	6	2	5	4	6	2	92	17.934
Periconia	-	-	-	-	-	-	2	2	1	1	5	9	20	3.8986
Pestalotiopsis	10	17	14	9	15	13	2	6	13	2	7	8	116	22.612
Phialiophora	-	-	-	-	-	-	-	1	1	1	-	1	4	0.7797
Phoma	-	-	-	1	2	2	1	2	3	1	1	4	17	3.3138
Rhinocladiella	-	2	-	-	-	-	-	-	-	-	-	-	2	0.3899
Rhizoctonia	1	3	2	-	-	-	-	1	3	-	-	-	10	1.9493
Sclerotina	-	-	-	-	1	2	-	-	-	-	-	-	3	0.5848
Scolecobasidium	3	1	1	-	-	-	-	-	-	-	-	-	5	0.9747
Thermomyces	-	1	1	-	-	-	-	-	-	-	-	-	2	0.3899
Sterile Mycelia	6	1	5	1	1	1	1	1	2	-	2	1	22	4.2885
Sub-total	**42**	**57**	**56**	**32**	**53**	**46**	**32**	**44**	**51**	**16**	**41**	**43**	513	

Results and discussion

Isolation frequency

Nine hundred samples of *Cordemoya integrifolia* from the two sites were examined and 513 isolates were recorded. The results are summarised in Table 1. In this study, a total of 27 genera of endophytic microfungi were isolated from *C. integrifolia*. However, only a few were present in significant amount (Table 1). The dominant taxa belong to genera which have often been recorded as endophytes. They are usually very widespread within the same host and can be encountered after sampling of very few host individuals (Petrini *et al.*,

1992). In the present study, *Pestalotiopsis* and *Penicillium* were predominant at both sites. Most taxa, however, were recovered sporadically. suggesting It is possible that the environmental factors are not conducive to their growth or that more competitive endophytes have already achieved a significant colonization of the host tissue.

Site analysis.

Overall isolation rate for the assemblages of endophytes recovered and the diversity indices at each site are shown in Table 2. There was a significant difference in endophyte assemblages between the two sites (Chi-square, $p < 0.005$). Greater overall isolation rates were obtained inside the Conservation Management Area than outside it. The two sites are different in thief floral composition and distribution. *Cordemoya integrifolia* plants within the Conservation Management Area are clustered and surrounded by other native plants. Outside the Conservation Management Area, the host sampled is sparsely distributed and is surrounded by exotic plants. The low isolation rates observed outside the Conservation Management Area can be explained in terms of the limited source of endophytic inoculum owing to the sparse distribution of the host. On the other hand, a greater diversity of endophytes was observed outside the Conservation Management Area (Table 2), suggesting that endophytes from the surrounding vegetation can also grow in *C. integrifolia* through natural openings such as stomata. It can therefore be concluded that the surrounding exotic vegetation outside the plot provide a greater diversity of endophytic inoculum than the native vegetation within the Conservation Management Area.

Colonisation of the host tissues by endophytic fungi is also dependent on successful penetration of the protective external plant layers, which can be achieved only by either mechanical fracture of the protective tissues or by enzymatic digestion of cuticular and epidermal layers. Experiments have demonstrated that endophytes produce enzymes that are able to degrade most substrates present on the surfaces or in the cell wall of the host (Petrini *et al.*, 1992). Spore deposition, germination, and/or production of infection structures depend on ecological parameters such as wind speed, light, temperature and, and relative humidity which vary at different heights above the ground. Plants of *C. integrifolia* inside the Conservation Management Area occur as shrubs and thus have their leaves closer to the soil. Comparatively, plants outside the plot are much taller (up to 15 m) with leaves well above ground level. The most heavily colonised leaves are found closer to the soil where conditions of higher humidity facilitate the penetration and colonisation by endophytic fungi. The proximity of *Cordemoya* leaves with litter can also contribute to the occurrence of a higher number of endophytic fungi.

Table 2. Overall isolation rates and Diversity Index of endophytic fungi at each site.

Site	Inside CMA	Outside CMA
No. of samples	450	450
No. of isolates recovered	286	227
Overall isolation rate (No. of isolates per sample)	0.64	0.51
Diversity Index	2.31	2.46

Table 3. A summary of isolation rates and Diversity Index for old and young leaves of *C. integrifolia.*

Site/Age	Total number of samples	Total No. of isolates	Isolation rates	Diversity Index
Inside CMA (Mature)	225	155	0.68	2.007
Inside CMA (Immature)	225	131	0.58	2.134
Outside CMA (Mature)	225	127	0.56	2.439
Outside CMA (Immature)	225	100	0.44	2.358

Table 4. A summary of isolation rates and Diversity indices for old and young lamina, midrib and petioles of *C. integrifolia.*

Site/Tissue	Total number of samples	Total number of isolates	Isolation rates	Diversity Index
Inside CMA (Mature)				
Lamina	75	42	0.56	1.99
Midrib	75	57	0.76	2.46
Petiole	75	56	0.75	2.37
Inside CMA (Immature)				
Lamina	75	29	0.39	1.99
Midrib	75	53	0.71	2.15
Petiole	75	49	0.65	2.34
Outside CMA (Mature)				
Lamina	75	32	0.43	1.82
Midrib	75	45	0.60	2.00
Petiole	75	50	0.67	1.92
Outside CMA (Immature)				
Lamina	75	16	0.21	1.82
Midrib	75	41	0.55	1.99
Petiole	75	43	0.57	2.21

Age specificity

The isolation rates in Table 3 indicate that there are consistently more isolates recovered from old leaves than young ones. However, there was no significant difference between the numbers of isolates (Mann-Whitney test, $p > 0.005$). The results correspond to findings of Rodrigues and Samuels (1990, 1994) who concluded that frequency of colonization increases with the age of

the organs or tissue based on similar studies. Mature leaves of *C. integrifolia* were exposed for a longer time to fungal propagules and their subsequent development as endophytes. Mature leaves also offer larger quantities of nutrients to fungal endophytes as they undergo higher photosynthetic rate. However, it is interesting to note that some endophytes recorded in young leaves are absent or occurred less frequently in mature leaves (Table 1). These include *Acremonium*, *Cephalosporium*, *Fusarium*, *Humicola*, *Meliola*, *Papulospora*, *Phoma*, *Sclerotina* inside the Conservation Management Area and *Periconia* outside the Conservation Management Area. One explanation could be that the intrinsic environment of the leaves undergoes constant changes as they mature and thus support different endophytes. Interestingly, young leaves showed slightly greater diversity index inside the plot and a lower diversity index outside the plot.

Vein and intervein analysis

There was no significant difference between the numbers of isolates recovered in the vein and inter-vein tissues [Mann-Whitney test ($p > 0.005$)]. However, more isolates are recovered from the veins than from the laminas irrespective of leaf age (Table 4).

These results are consistent with the findings of Rodrigues and Samuels (1990) who reported that endophytes from the leaf blade of *Licula ramsayi* tended to be concentrated in the leaf vein, and also occurred in the veins of unfurled leaves, thereby confirming transmission of endophytes via seeds. Here, no experiment was performed on unfurled leaves. The lamina is found to be less susceptible to endophytic infection than the midrib and/or petiole. The physical properties of the leaf affect spore retention and spore deposition. These include the behaviour of water reaching the leaf and the pattern of runoff and evaporation, all which favour the petiole and the midrib. Also, the number of stomata occurring at specific parts of the leaf play a role in the colonization of endophytes by air-borne source. Midrib and petioles tend to have more vascular bundles than intervein region and therefore support more endophytic fungi in term of nutrition. The diversity of fungal endophytes in the lamina was relatively lower than the other tissues examined.

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