Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia

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Brown, K.B., Hyde, K.D. and Guest, D.I. (1998). Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia. Fungal Diversity 1: 27-51.

An ecological investigation of foliar endophytic fungal communities on a *Musa acuminata* (Banana) species complex was undertaken in Hong Kong and Queensland, Australia. The study yielded twenty-four taxa. *Colletotrichum gloeosporioides*, *Pestalotiopsis palmarum* and *Nigrospora oryzae* were the dominant endophytes isolated from banana in Hong Kong. Isolates of the family Xylariaceae and a *Phoma* species were most frequently isolated from indigenous banana in the wet tropics of north Queensland. Endophyte fungal communities from hosts from banana plantations in north Queensland and south eastern Queensland were found to differ from each other and to those of the other communities.

Introduction

The Musaceae is a monocotyledonous family that contains two genera; *Musa* with 37 species and *Ensete* with 7 species. The family has its highest species diversity in South East Asia with representatives occurring across the Old World Tropics (Price, 1995). Due to the commercial and subsistence value of *Musa* spp. they have been introduced throughout the tropics and subtropics, as well as in more marginal climates such as those in Florida, Israel and Greece. Most fungal disease research has focussed on the commercial cultivars (Jeger *et al.*, 1995).

Recent investigations of endophytic fungi in perennial plants have followed a similar approach where healthy plant tissues are systematically sampled for internal fungi (Petrini, 1986; Carroll, 1988; Wilson, 1995). This approach differs from conventional research in plant pathology where disease tissue is selectively sampled for single pathogenic taxa. A considerable amount of research has been undertaken on endophytes in temperate regions (summarized in Petrini, 1991), however, very few hosts or regions have been investigated for endophytes in the

tropics. The extent of tropical endophyte research is represented in the publications of Petrini and Dreyfuss (1981), Dreyfuss and Petrini (1984), Rodrigues and Samuels (1990), Pereira, Azevedo and Petrini (1993), Fisher *et al.* (1994a), Rodrigues (1994), Fisher *et al.* (1995), and Wright et al. (1996). This research has been primarily motivated by the search for novel bioactive compounds and for a knowledge of the taxonomy and ecological roles of the endophytes encountered (Petrini, 1991). The potential of asymptomatic endophyte colonizations for protection against disease has also provided impetus for research (Clay, 1991; Petrini, 1993; Freeman and Rodriguez, 1994; Dorworth and Callan, 1996). Endophytic fungi may also become problematical under environmental stress. Understanding their endophytic life stage may help design management regimes that could reduce postharvest losses (Wright, 1998). This report contains information relevant to each of these motives, placed in the context of the endophytic fungal community of *Musa* spp.

The definition of an endophyte is far from clear and Hawksworth *et al.* (1995) suggest that the term should be clearly defined when used. In this study, the widely used definition of Petrini (1991) is followed: "...all organisms inhabiting plant organs that at some time in their life, can colonise internal plant tissues without causing apparent harm to the host". This includes symptomless latent pathogens and those fungi which also have an epiphytic phase of their life cycle.

Ecological factors including leaf age (Petrini, 1991), tissue type (e.g. Bissegger and Sieber, 1994; Fisher *et al.*, 1995) and anthropogenic modifications (e.g. Riesen and Close, 1987) can significantly affect endophyte communities. There can also be significant differences between endophyte assemblages on a host plant in its endemic range, compared to the assemblages found on an introduced population. Introduced plants support different endophytic taxa and less diverse assemblages, than plants in their endemic range (Carroll, Müller and Sutton, 1977; Espinosa-Garcia and Langenheim, 1990; Fisher, Petrini and Sutton, 1993; Fisher *et al.*, 1994b). This study is one of the first to compare endophytic assemblages of indigenous host plants with that of introduced hosts in tropical regions. The ecological effects of leaf age, tissue (midrib/lamina) and fungicide use on endophyte abundance and community composition are also assessed.

This is also one of the first study of symptomless endophytes from *Musa acuminata* species complex and it represents the first investigation of endophytes from wild strains of any crop plant in the tropics. There are no previous accounts of endophyte research on other members of the genus *Musa* or any other members from the family Musaceae. The only information on

internal fungi from banana addresses specific latent pathogens such as *Colletotrichum gloeosporioides* or *Pyricularia grisea* on banana fruit in commercial hybrids (Meredith, 1963; Binyamini and Schiffmann-Nadal, 1972; Verhoeff, 1974). This study is also the first to sample the sub-species *Musa acuminata* subsp. *banksii* for fungi, either endophytes or epiphytes. Shivas and Alcorn (1996) produce a checklist of the fungi from hosts in the wet tropics in north Queensland and a number of plant pathogens have been recorded from *Musa acuminata* subsp. *banksii*. They include *Cordana musae*, *Glomerella cingulata*, *Mycosphaerella musae*, *Nodulisporium* sp., *Periconiella musae*, *Phyllachora musicola* and *Pseudocercospora musae* (Herbarium records, BRIP)

Materials and methods

Sampling regime and site descriptions

The aims of the sampling regime were to collect samples from as many plants and from as broad a range of sites as possible; within the constraints of statistical validity and time available for processing. Five of the seven previous accounts of endophyte assemblages from the tropics are primarily qualitative taxonomic accounts and have not had strict sampling constraints. Rodrigues (1994) and Fisher *et al.* (1995) tested ecological hypotheses and used more robust sampling regimes for their quantitative analyses.

To test whether host plants outside their natural range supported different endophytic assemblages to host plants within the natural range, two locations were sampled chosen on the basis of accessibility. Hong Kong represents a location outside the natural range of *Musa acuminata*, while north Queensland is part of the endemic range. The five sites in Hong Kong and the five sites in north Queensland covered as wide a variety of environmental conditions as possible. All of the sites included have annual rainfalls of over 1500 mm, although site specific rainfall data is not available. In the present study all collections were made in the corresponding "dry" season to eliminate the possible effect of seasonality on the endophyte community composition or species abundance. Seasonality has previously been shown to have an effect by Rodrigues (1994) in a study of the palm *Euterpe oleracea* Mart. Rodrigues (1994) found that the same fungal species were isolated at four seasonal intervals with the highest endophyte diversity and occurrence in the wet season.

Healthy leaves were collected in Hong Kong in early May 1996. A total of five sites were sampled with two plants per site. Two leaves were removed from each plant, one old leaf, defined as either the third or fourth most recently opened leaf, and a young leaf which was always the youngest opened leaf. Within a site the plants were always less than five meters apart and thus probably

represented propagules of the same rhizome. This is an important factor as community analysis treats the plants from the same site as a single genetic individual. No strict sampling method of plant age could be used due to the very low densities of the host plant, therefore a relatively broad range of different aged plants were collected from each location to approximate a normal distribution. Collections were made in secondary forest as there is no primary moist forest in Hong Kong that contains *Musa acuminata*. No deliberate attempts were made to sample plants along environmental gradients such as rainfall or altitude; the density and predictability of occurrence precluded this approach. Sites 2-4 were sampled from well developed secondary forest while sites 1 and 5 were from drier, more open sites. Sites 2 and 3 were over 800 meters in altitude, while sites 1, 4 and 5 were all under 200 meters.

Leaves of *Musa acuminata* subsp. *banksii* from north Queensland were sampled from five sites in late August 1996, a dry period. Sites were of varying altitude and rainfall in accordance with the protocol used in Hong Kong. The first site (site 6) was in an area of well developed lowland rainforest, that is defined as "complex mesophyll vine forest" (Winter *et al.*, 1987) in the Licuala State Forest near Mission Beach with very high rainfall of over 2,500 mm annually and a low altitude. The second site (site 7) was in tall monsoon forest, or "semi-deciduous mesophyll vine forest" (Winter *et al.*, 1987) on the floodplain of the Gilles river in the Bellenden Ker National Park near the Gilles Highway. Sites 8 and 9 were also in monsoon forest along the Gilles Highway. The final site was in upland rainforest, or "complex notophyll rainforest", in the Gilles State forest at an altitude of approximately 900 meters with a higher rainfall than sites 7, 8 and 9. At each site two plants were sampled using one old leaf each. Young leaves were not collected in Queensland due to the limited time of the present study.

Further collections of the commercial *Musa acuminata* species complex were made from plantations in north Queensland and south eastern Queensland. Only one site was chosen in north Queensland; this site (site 12) was a banana plantation at Mission Beach and the fungicide mancozeb/benomyl had been applied periodically to the crop. The plantation was adjacent to Licuala State Forest and the lowland rainforest that included site 6 and allowed a direct comparison between fungicide treated and untreated sites in the same locality. Only one site was selected in south eastern Queensland. These leaves were collected by Natalie Moore (QDPI) and mailed to the University of Melbourne for fungal isolation. The site was also a banana plantation and was not sprayed with fungicides. Ten old leaves were collected from each site to replicate at least the same number of the Hong Kong and wild north Queensland protocols.

All leaves from Hong Kong and north Queensland were processed within 72 h or refrigerated if stored for longer. The south eastern Queensland sample was in transit for over a week and arrived when the researcher was in north Queensland and therefore isolations were not made until two weeks after collection. Most previous tropical endophyte research has involved the plant material being posted without significant loss of community resolution (Petrini and Dreyfuss, 1981; Dreyfuss and Petrini, 1984; Rodrigues and Samuels, 1990; Fisher *et al.*, 1994a; Rodrigues, 1994; Fisher *et al.*, 1995).

Isolation of endophytes

Sixteen leaf discs and 16 midrib discs (3 mm diam.) were taken from every leaf sample collected. The standard triple ethanol-sodium hypochlorite-ethanol surface sterilization technique was used to allow comparison with other studies (Perreira *et al.*, 1993). To determine the most appropriate sodium hypochlorite immersion time for *Musa* leaves a series of trials were performed in which 0.5, 1, 2 and 4 min immersion times were tested. In Hong Kong sixty-four leaf discs with 2 and 4 min sodium hypochlorite immersion times yielded one isolate. The same trial performed on both the lamina and midrib of *M. acuminata* subsp. *banksii* leaves from north Queensland yielded one isolate from each of sixty-four discs. This was a far lower rate of isolation than previous research on all plants surveyed, and thus it was concluded that the treatment was too penetrative, especially as the leaf discs were visibly "bleached". The longest time of immersion that yielded a similar number of endophytes to previous studies (Petrini, 1991) was 1 min sodium hypochlorite immersion.

The most appropriate surface sterilization protocol, as determined by sodium hypochlorite immersion trials was 1 min in ethanol (75 %), 1 min in sodium hypochlorite (3.25 %), and 1 min in ethanol (75 %) without rinsing with sterile-water. This protocol is shorter than in most previous studies. Many previous studies have rinsed the plant material with sterile water after immersion and have sampled woody material which may be less permeable to sterilizing chemicals. Banana leaves are thinner and may be more permeable, than other leaves, such as the fan palm *Licuala ramsayi* (Rodrigues, 1994).

The surface sterilized leaf discs and midrib segments were tranferred to potato-dextrose-agar (PDA: 39 mg/L) amended with Rose of Bengal (20 mg/l). As the aim of this study was to isolate the most representative range of fungi for ecological community analysis this "non-selective" medium was used. Bills (1996) suggests so-called "non-selective" media are highly selective as they favour taxa with robust and rapid radial growth and high numbers of propagules. PDA was used in this study as it permits the growth of the largest number of

predicted taxa of the most readily available agar media. Rose of Bengal was used to inhibit bacterial growth and to slow fungal growth (Bertoni and Cabral, 1988).

Plates were incubated at 25 C and when hyphae appeared they were subcultured onto malt extract agar (MEA: 34 gm/L, Difco). MEA was used as it has been found to encourage higher sporulation (Petrini, 1986). The axenic cultures and the original isolation cultures were both subjected to UV light and cold storage to induce sporulation so accurate identifications could be made, following the procedures of Leach (1971) and Petrini (1986).

Identification

Pure cultures were sorted into morphotypes on the basis of colony surface textures, hyphal pigments, exudates, growth rates, and any sporulating structures. Reproductive structures including asci, ascospores, conidia and conidiophores were examined by preparing squash mounts. Permanent slides were prepared using lactophenol or lactophenol-cotton blue (Nag Raj, 1993).

Statistical analysis

Species abundance were calculated as the percentage of discs that were colonized by fungi, as described in previous studies (Rodrigues, 1994). To assess the significance of the factor defined as location, site, leaf age and tissue type (midrib or lamina) on the endophyte community, a "nested" log linear regression was applied. A multiple linear regression was used in preference to ANOVA (analysis of variance) because the data consisted of presence/absence values of endophyte abundance in each of the 16 replicated discs (Sokal and Rohlf, 1995).

The regression analysis was partially successful, however, it could not be manipulated to include all interactions in one analysis. A chi-square goodness of fit test was subsequently used to analyse simple non-interactional models. This test was used as it also can be used for data in the form of frequencies in a non-normal distributed sample, and also, it is appropriate for a small numbers of factors used in each of the subsequent comparisons (Sokal and Rohlf, 1995). To statistically test the significance of locations (i.e. Hong Kong) against tissue type on species abundance, the data was transformed to percentages so it represented continuous data and could be applied to a nested ANOVA (Wilkinson, 1990). This was done as sites and locations represented the same data and the regression analysis would not run these factors simultaneously.

For the community analysis a non-parametric multidimensional scaling (NMDS) ordination was performed. The NMDS ordination creates a

dissimilarity matrix with one figure for dissimilarity based on species abundance in ecological community analyses. This is followed by a ranking of the dissimilarity values of distances between points, in this case sites, in a "monotonic" relationship. The final stage involves developing a proportional relationship between the factors (Faith, Minchin and Belbin, 1987). The result can then be graphically displayed for ease of observation and for clarifying trends and relationships.

The NMDS ordination method was used as it has been found to be a more robust measure of community compositional dissimilarity with less quantitative and hence, graphical distortion than other ordination methods such as Principal Component analysis or Correspondence analysis (Faith *et al.*, 1987). Other endophyte research involving community analysis have all used these other methods with the exception of Rodrigues (1994) who also used an NMDS ordination.

Percentage data for fungal species occurrence frequencies and species abundance were used. All identified taxa at the time of analysis were included in the NMDS ordination. The NMDS ordination was performed on the program DECODA. The data was standardized to unit maxim and the Bray-Curtis index was used. The Bray-Curtis measure of distance was used as it has been found to have a robust monotonic and linear relationship with respect to ecological distance (Faith *et al.*, 1987).

Results

Species abundance

Leaves of *Musa* spp. showed a highly variable incidence of endophytic fungal abundance. Overall species abundance varied between locations (Fig. 1); south eastern Queensland plantation (67 %) had the highest species abundance, followed by wild north Queensland (57.5 %), Hong Kong (53.7 %) and north Queensland plantations (36.4 %). The young leaf sample from Hong Kong had the lowest rate of colonization with 29.6 % of leaf discs infected. Old Hong Kong leaves without surface sterilization (the control) had a 100 % recovery of fungi (Fig. 1). A nested ANOVA analysis comparing the species abundance at the four locations found that species abundance in leaves from the north Queensland plantation were lower ($p = 0.033$) than at the other three sites. A Chi-squared analysis comparing species abundance in young and old leaves from Hong Kong (Fig. 2) showed that species abundance increased with leaf age ($p = 0.002$).

In young leaves in Hong Kong the midrib had a higher species abundance than the lamina in all but one site. The largest difference was at site 5, where 22

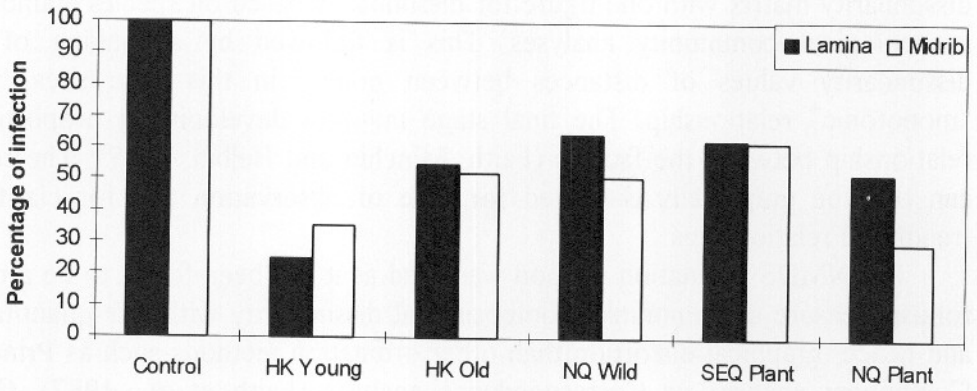


Fig. 1. Species abundance in the lamina and the midrib from Hong Kong and Queesland, including all experimental groups.

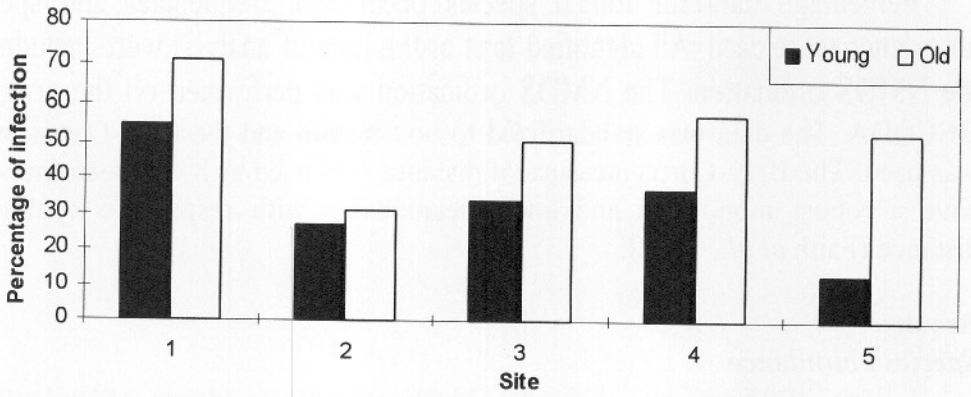


Fig. 2. Species abundance for young and old leaves in Hong Kong.

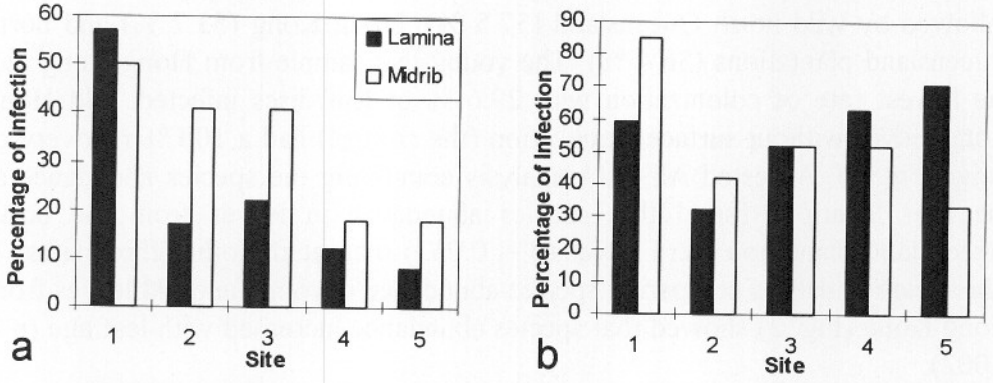


Fig. 3. Species abundance in the lamina and midrib from (a) young and (b) old leaves in Hong Kong.

% of midrib leaf discs and 6 % the lamina discs were colonized (Figs. 3a, b). In the old leaves sampled in all locations 22 plants had higher species abundance in the lamina than the midrib; 3 plants were equal, and 5 plants had higher species abundance in the midrib (Figs. 3b, 4a, b, c). A regression analysis comparing colonization of the lamina and midrib at each site revealed no significant differences at the 5 % level of confidence in species abundance at any site, or overall.

Fungal taxonomic composition

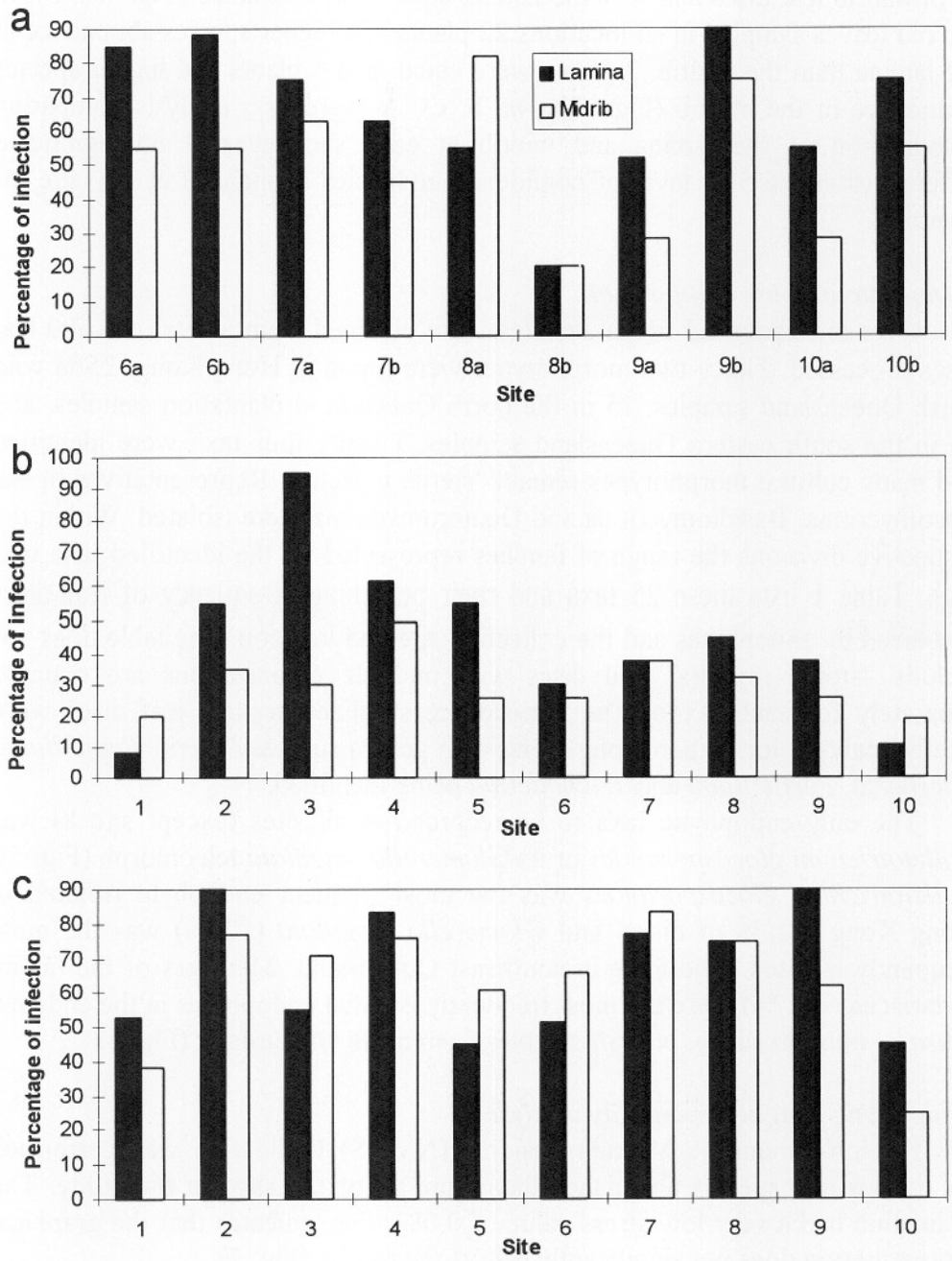
Over one thousand fungal isolates were obtained from a total of 1600 leaf discs processed. Thirty-two morphotypes were found in Hong Kong; 25 in wild north Queensland samples; 15 in the north Queensland plantation samples; and 18 in the south eastern Queensland samples. Twenty four taxa were identified and many cultural morphotypes remain "sterile mycelia". Representatives of the Ascomycotina, Basidiomycotina and Deuteromycotina were isolated. Within the respective divisions the range of families represented by the identified taxa was high. Table 1 lists these 25 taxa and their percentage frequency of leaf discs colonized by endophytes and the collection site and location. The table does not include "sterile mycelia" and discs with multiple colonizations are counted separately for each taxon. The non-surface sterilized control leaf discs were briefly analysed for taxa in Hong Kong with genera such as *Mucor*, *Penicillium*, *Alternaria*, *Verticillium* and *Trichoderma* being identified.

The only endophytic taxa to be recorded at all sites (except site 8) was *Colletotrichum gloeosporioides* or its *Glomerella cingulata* teleomorph (Fig. 5). *Colletotrichum gloeosporioides* was the most frequent endophyte isolated in Hong Kong (22 % of discs) and *Glomerella cingulata* (12 %) was the most frequently isolated endophtye in southeast Queensland. Members of the family Xylariaceae (11 %) were the most frequently isolated endophytes in the endemic *Musa acuminata* subsp. *banksii* samples from north Queensland (Fig. 6).

Endophytic fungal community analysis

A Non-Parametric Multidimensional (NMDS) Ordination was performed on species and species abundance data from all of the sites in the study. The ordination had a very low stress value of 0.083; this indicates that the graphical representation does not significantly distort the results (Fig. 7).

The ordination creates distinct clusters of sites thus demonstrating that clear differences between experimental groups exist. Sites one to five, from Hong Kong, cluster closely together and represent the same community type; as do the wild *M. acuminata* subsp. *banksii* samples from north Queensland. Only one site



Figs. 4. Species abundance in the lamina and midrib of replicate leaves from (a) the north Queensland wild (location 2) sample, (b) north Queensland plantation (location 4) and (c) southeast Queensland plantation (location 3).

Table 1. Taxa isolated as endophytes from *Musa* spp.; as a percentage of total leaf pieces colonized in each location. Letters in columns indicate experimental sample group HK, Hong Kong (10 plants analysed); NQw, north Queensland native *M. accuminata* ssp. *banksii* (10 plants); NQp, north Queensland plantation (2 plants); SEQp; south east Queensland plantation (2 plants).

Taxon	HK	NQw	NQp	SEQp
Alternaria alternata	<1			
Cladosporium cladosporioides	<1	<1		<1
Colletotrichum gloeosporioides	17	3.5	15	
Colletotrichum musae	2			
Curvularia sp.				<1
Cryptosporiopsis sp.	<1			
Epicoccum nigrum				3
Fusarium lateritium	1			
Fusarium solani				<1
Glomerella cingulata				11
Lasiodiplodia theobromae	<1			
Microsphaeriopsis sp.		<1		
Nigrospora musae		2		
Nigrospora oryzae	9.5			
Pestalotiopsis palmarum	12	3	1	
Phoma sp.		8.5		
Phomopsis sp. 1	1			
Phomopsis sp. 2	<1			
Phomopsis sp. 3		<1		
Phyllasticta musicola	2.5			
Xylaria sp. 1		9	5	
Xylaria sp. 2		3		
Xylariaceae sp. 1		<1		
Xylariaceae sp. 2		<1		
Basidiomycetes		2	2	

was collected and analysed from each of the Queensland plantations (sites 11 and 12). Both of the plantation sites were found outside the related community clusters; this indicates that these samples were significantly different to each other and to the more extensively sampled groups.

The Hong Kong endophyte communities are characterized by cosmopolitan and potentially pathogenic taxa including *Colletotrichum gloeosporioides* and *Pestalotiopsis palmarum*. Many fungal taxa separate the clusters in the ordination; a high proportion of species were only isolated from one experimental group such as *Epicoccum nigrum* and *Fusarium solani* in south east Queensland and *Xylaria* spp. and *Microsphaeriopsis* sp. In wild north Queensland samples. The lists of identified taxa (Tables 1, 2 and 3) and the

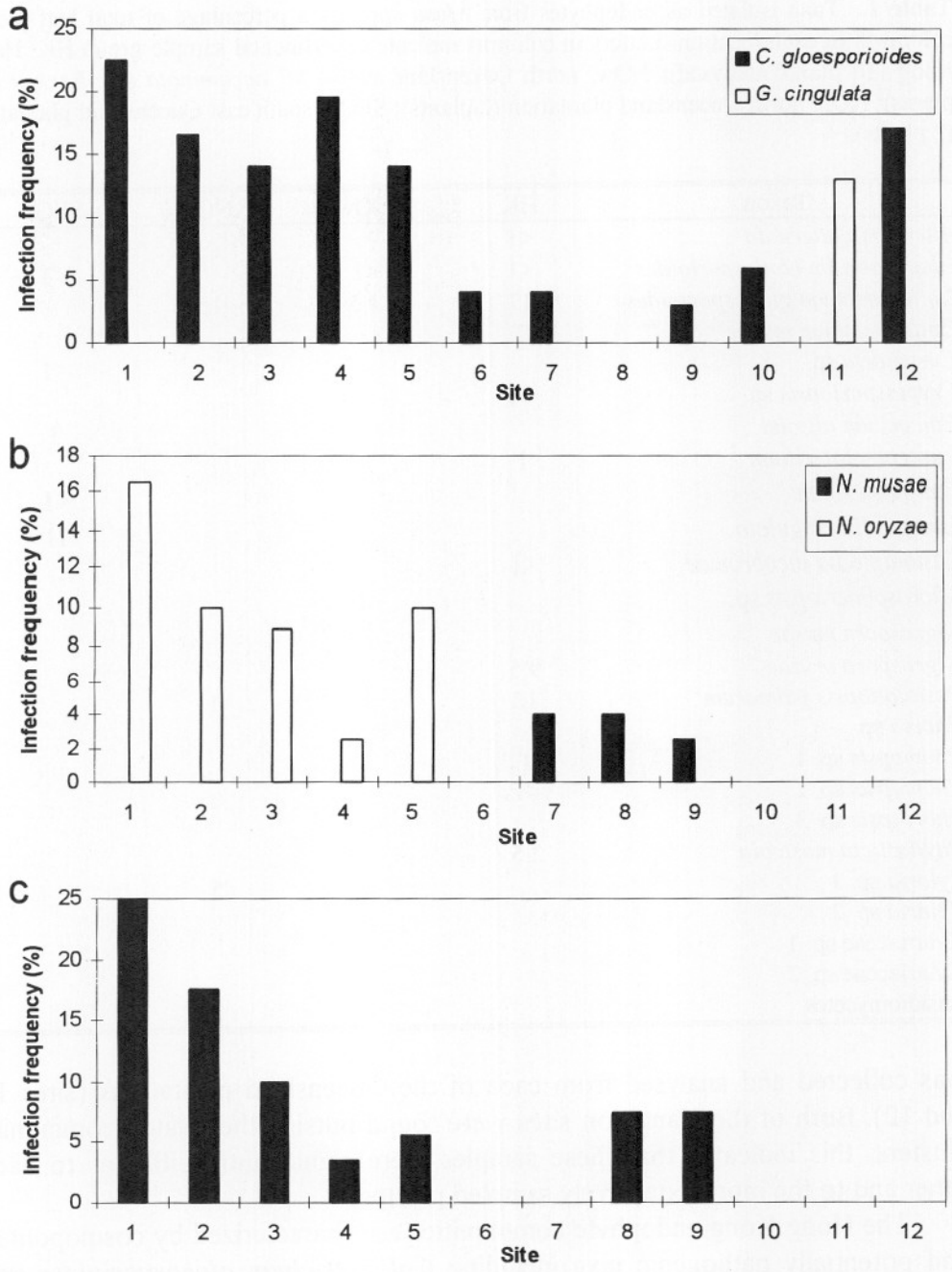


Fig. 5. Frequency of occurrence of abundant endophytic taxa at each site: 1-5 represent Hong Kong sites; 6-10 north Queensland wild sites; 11 southeast Queensland plantation; and 12 north Queensland plantation site. (a) *Colletotrichum gloesporioides* and *Glomerella cingulata*; (b) *Nigrospora musae* and *N. oryzae*, and (c) *Pestalotiopsis palmarum*.

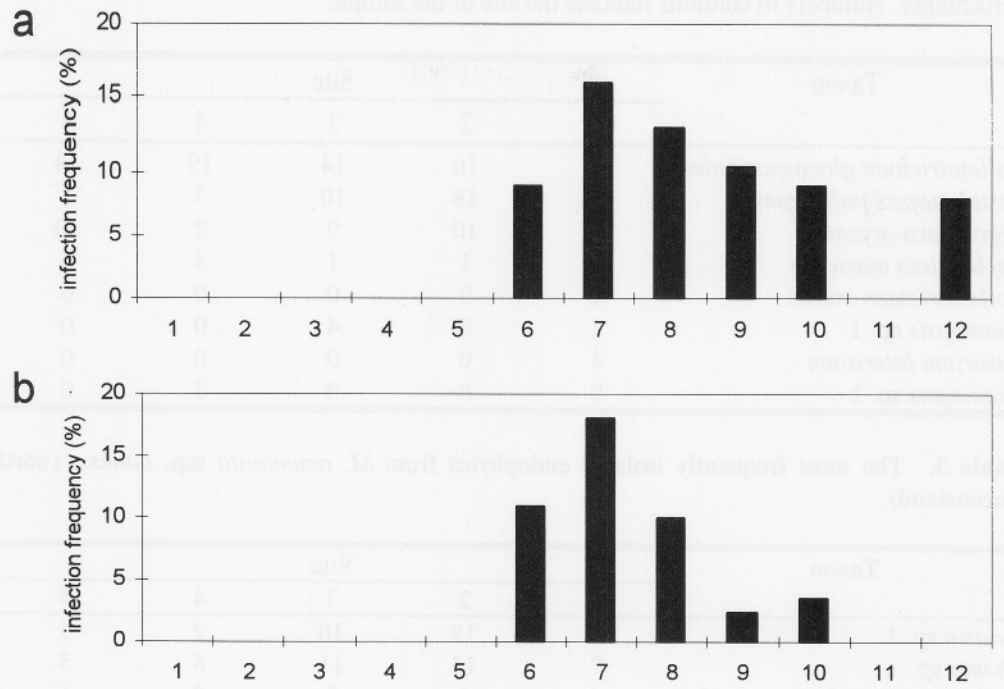


Fig. 6. Frequency of occurrence of the most abundant endophytic taxa at each north Queensland site. 1-5 represent Hong Kong sites; 6-10 north Queensland wild sites; 11 southeast Queensland plantation site; and 12 north Queensland plantation site. (a) Family Xylariaceae; (b) *Phoma* spp.

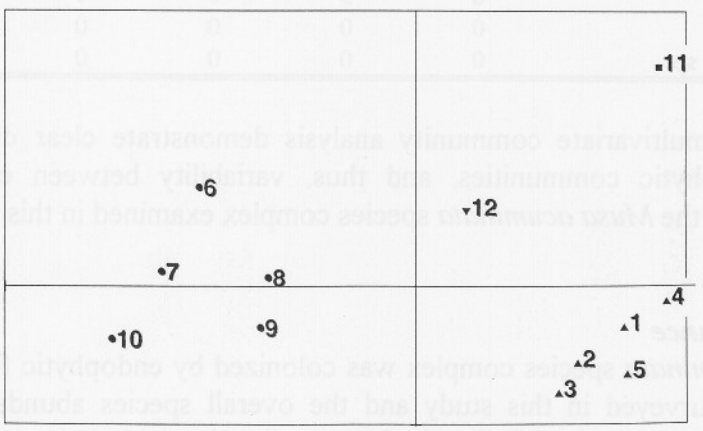


Fig. 7. Two dimensional ordination of endophyte communities (▲ Hong Kong; ● north Queensland endemic (*M. acuminata* subsp. *banksii*); ■ south-east Queensland plantation; ▼ north Queensland plantation; stress = 0.083).

Table 2. The most frequently isolated fungal endophytes from *Musa* sp. (Hong Kong) as percentages. Numbers in columns indicate the site of the sample.

Taxon	Site				
	1	2	3	4	5
Colletotrichum gloeosporioides	22	16	14	19	14
Pestalotiopsis palmarum	25	18	10	3	5
Nigrospora oryzae	16	10	9	2	10
Phyllosticta musicola	0	1	1	4	6
Colletotrichum musae	4	0	0	0	6
Phomopsis sp. 1	2	0	4	0	0
Fusarium lateritium	4	0	0	0	0
Phomopsis sp. 2	0	0	0	2	0

Table 3. The most frequently isolated endophytes from *M. acuminata* ssp. *bankssi* (north Queensland).

Taxon	Site				
	1	2	3	4	5
Xylaria sp. 1	11	19	10	2	3
Phoma sp.	7	14	11	6	5
Colletotrichum gloeosporioides	4	4	0	3	6
Pestalotiopsis palmarum	0	0	7	7	0
Xylaria sp. 2	0	5	2	6	0
Nigrospora musae	0	4	4	2	0
Basidiomycete	0	3	0	3	4
Phomopsis sp. 3	0	5	0	0	0
Xylariaceae sp.	0	0	0	0	3
Microsphaeriopsis sp.	0	0	0	0	3

results of the multivariate community analysis demonstrate clear distinctions between endophytic communities, and thus, variability between endophytic communities of the *Musa acuminata* species complex examined in this study.

Discussion

Species abundance

Musa acuminata species complex was colonized by endophytic fungi in all of the plants surveyed in this study and the overall species abundance were within the range of other tropical endophyte studies that included mature leaves (e.g. Fisher *et al.*, 1995; Rodrigues, 1994). The species abundance of unsterilized leaf discs (the control), were much greater than any of the experimental groups suggesting the isolation methods were effective. Variation of species abundance existed at all levels of sampling in this study; variation

occurred between: different locations (i.e. Hong Kong and south eastern Queensland); different sites within the same location; plants within the same site (or "clump"); different tissue types (lamina or midrib); and between young and mature leaves.

At the largest scale of sampling, location was found to influence fungal endophyte species abundance. The plantation leaves from north Queensland had significantly lower species abundance than leaves from other locations; this was not unexpected as the plantation was treated with the protectant fungicide mancozeb/benomyl. Riesen and Close (1987) have shown that endophytic colonization of healthy barley leaves is significantly reduced after application of the fungicide propiconazole; and have postulated that the elimination of latent pathogens growing symptomlessly as endophytes may increase yields from apparently healthy crops. The effect of fungicides on endophyte species abundance has not been independently assessed although, in the case of the north Queensland banana plantation, it appears to reduce endophyte species abundance.

There was a high species abundance in the south eastern Queensland plantation. A possible explanation for the high species abundance may be the differences in sampling techniques. Leaves from south east Queensland were processed two weeks after collection, therefore, epiphytic taxa may have penetrated further into the leaf tissue than in other samples. Recently penetrated hyphae or haustoria could have survived surface sterilization procedures (Verhoeff, 1974). Bertoni and Cabral (1988) found that epiphytic fungi including *Alternaria alternata* and *Cladosporium cladosporioides* may form limited colonizations through substomatal chambers and also avoid the effects of surface sterilization. The leaves collected from south east Queensland appeared to be older than all other leaves sampled.

Endophyte species abundance also varied according to the site within each location. This variation is probably a reflection of the range of environmental conditions sampled in Hong Kong and north Queensland. Sites differed primarily by rainfall, altitude and forest types; these are all factors that may influence fungal species abundance as they affect humidity, temperature and potential inoculum sources.

Species abundance also varied between the lamina and the midrib tissue. Many studies have found differences in species abundance between plant tissues, such as bark, stems and leaves (Bissegger and Sieber, 1994; Fisher *et al.*, 1994b). Differences between endophytes occurring in the midrib (vein) and lamina (intervein) tissue have also been recorded (Rodrigues, 1994; Fisher *et al.*, 1995). In the present study, the lamina had a higher endophyte species

abundance than the midrib in mature leaves. This was found to be significant only with a 10 % confidence interval (0.086) in all sites sampled. When the Hong Kong results were analysed independently, the lamina was found to have a significantly higher species abundance than the midrib with a 1 % confidence interval. Inversely, the midrib in young leaves from Hong Kong samples had significantly higher species abundance than the lamina.

The old leaves also had a significantly higher species abundance than young leaves. This comparison was only made in Hong Kong. In previous research young leaves were also colonized by significantly fewer endophytes than older leaves (Rodrigues, 1994; Fisher *et al.*, 1995). Rodrigues and Samuels (1990) suggest taxa isolated from tightly rolled palm leaves are less likely to be colonized by air spora. The species abundance in both old and young leaves; and the lamina and midrib, from both age classes, suggest that colonization occurs primarily from air spora, as with epiphytes. In mature leaves and in recently opened leaves the few endophytes present are more likely to be involved in intimate infections growing throughout the plant, such as *Fusarium* spp. in the vasculature of the palm *Licuala ramsayi* (Rodrigues, 1994).

Fungal taxonomic composition

The endophytes recovered in this study are from genera previously recorded as endophytes in other tropical plants. No novel species were identified, although the high level of sterile mycelia indicates the identified taxa do not represent the entire endophyte mycota. Other tropical endophyte studies have found fewer novel species than earlier predictions (e.g. Hawksworth, 1991). Four new *Idriella* species were described by Rodrigues and Samuels (1992) and *Letendraeopsis palmarum* was described by Rodrigues and Samuels (1994). Petrini and Dreyfuss (1981) described two novel species, *Anthostomella aracearum* and *Chaetosphaeria endophytica*, from a study of three host families.

Twenty-four fungal taxa were identified in this study of *Musa acuminata* species complex. This is similar to the other tropical endophyte surveys from perennial monocotyledons; Rodrigues (1990), 12 taxa; Pereira *et al.* (1993), 13 taxa; Fisher *et al.* (1994a), 23 taxa; Rodrigues (1994), 62 taxa. These results are not directly comparable as different sized samples were taken in each study and different periods of time were spent identifying taxa and awaiting sporulation. Of these studies, only Rodrigues (1994) was able to identify most of the *Xylaria* species and anamorphs from mature reproductive structures; *Xylaria* cultures are known to take up to six months to sporulate with optimum conditions in culture (Petrini and Petrini, 1985). The range of plant tissue types and the

environmental conditions also affect the number of endophytic taxa potentially isolated from a host plant.

The ecological roles of isolated taxa can be grouped into two categories; one that contains potential pathogens of *Musa acuminata* species complex and ones containing fungi known to be primarily saprotrophs. Many of the fungi recovered are common, cosmopolitan taxa and many of these species are recorded banana pathogens. Significant proportions of non-pathogenic fungi were also isolated, particularly from the sites from wild bananas in north Queensland.

Can endophytes of* Musa *spp. become pathogens?

No data of asymptomatic endophytes are documented for the family Musaceae and the only previous endophyte knowledge of internal fungal infection has been gleaned from research into latent pathogens such as *Colletotrichum musae* or *Colletotrichum gloeosporioides* (Jeger *et al.*, 1995). Many pathogens of the Musaceae, have however, been found to cause disease in Strelitziaceae in the tropics (Jeger *et al.*, 1995). *Fusarium oxysporum* f.sp. *cubense* is known to cause vascular wilt on members of the Strelitziaceae. Moko disease is the most serious bacterial pathogen of *Musa* species and the pathogen *Pseudomonas solanacearum* causes disease in the Musaceae and Strelitziaceae, but no other family (Hyde *et al.*, 1992; Jeger *et al.*, 1995).

Diverse assemblages of pathogenic fungi have been found on *Musa* spp. (Stover, 1972; Wardlaw, 1972; Farr *et al.*, 1989). When an analysis of pathogenic taxa of *Musa* sp. (Table 5) and frequently isolated tropical endophyte genera is undertaken (Table 4) it is apparent that many potential pathogenic species, or genera, have also been encountered as endophytes.

The degree of correlation between the potential fungal pathogens of the *Musa* (Table 5) and the frequently isolated tropical endophyte genera (Table 4) is high. Genera that were found to be in common include: *Cladosporium*, *Colletotrichum*, *Curvularia*, *Drechslera*, *Fusarium*, *Guignardia*, *Lasiodiplodia*, *Nigrospora*, *Phoma*, *Phyllosticta* and *Verticillium*. This list contains many of the most serious pathogens of *Musa* species including *Fusarium oxysporum* and *Colletotrichum gloesporioides* (Jones et al., 1993). These result suggests that a endophytic stage may be important in the life cycles of some banana pathogens.

Some of the genera found as endophytes and associated with banana disease have been previously studied to establish if latent infection occurs before disease symptoms. *Colletotrichum* diseases have been extensively researched (Verhoeff, 1974; Cerkauskas, 1988). *Colletotrichum* species have been found to penetrate the fruit tissue either by hyphal growth or an appresorium (Simmonds,

Table 4. Frequently isolated endophyte genera from tropical hosts.

Genera	Hosts	Genera	Hosts
Ascomycetes		**Hyphomycetes**	
Anthostomella	1a, 1c, 2a, 2b, 2c	*Acremonium*	1a, 1b, 1c, 2a, 3
Chaetosphaeria	1a, 1b	*Cladosporium*	2a, 5
Glomerella	1a, 1b, 1c	*Curvularia*	1a, 3, 4
Guignardia	2a, 2c, 4	*Daldinia*	2a, 3
Phomatospora	1a, 1c, 2a	*Drechslera*	4
		Fusarium	1a, 1b, 1c, 2c, 3, 5
Coelomycetes		*Gliocladium*	1a
Ascochyta	1a, 1b, 1c, 2b	*Hypoxylon* anamorph	1a, 1c, 3, 5
Colletotrichum	1a, 1b, 1c, 2a, 3, 4	*Nigrospora*	2c, 3, 4, 5
Coniothyrium	1a, 1c, 2a	*Nodulisporium*	1a, 1b, 1c, 2a, 3, 4
Cryptocline	1a, 1b, 1c, 2a	*Periconia*	4, 5
Cryptosporiopsis	1a, 1b, 1c, 2b, 2c, 5	*Phialophora*	1a, 2c
Epicoccum	2b, 5	*Trichoderma*	3, 5
Lasiodiplodia	1a, 1c, 2a	*Xylaria*	1a, 1b, 1c, 2a, 2b, 2c, 3, 4,
Pestalotiopsis	1a, 1c, 2a, 2b, 2c, 3, 5		5
Phoma	1a, 1b, 1c, 2a, 2b, 3, 5	*Verticillium*	1a, 2a
Phomopsis	1a, 1c, 2a, 2c, 3, 4		
Phyllosticta	1a, 1c		

1a = Araceae, 1b = Bromeliaceae, 1c = Orchidaceae (Petrini and Dreyfuss, 1981).
2a = Pteridophyta, 2b = Piperaceae, 2c = Crassulaceae (Dreyfuss and Petrini, 1984).
3 = Palmae (Rodrigues, 1994).
4 = Cactaceae (Fisher *et al.*, 1994a).
5 = Compositae (Fisher *et al.*, 1995).

1963; Muirhead, 1981). Species known to be pathogenic, that have been isolated as endophytes are not necessarily always pathogenic strains. It has been demonstrated that if the infection is not latent, then the alternative hypothesis is that a mutation of a virulent pathogen has occurred and the fungus has become a non-pathogenic strain of the pathogen (Freeman and Rodriguez, 1994).

Potential pathogens

In the context of this study, it is not possible to conclude if banana pathogens isolated as species of endophytes in healthy leaves are the same pathogenic strains. The fungi may be non-pathogenic strains and experiments following Koch's postulates are required to confirm any pathogenicity. No previous endophyte community investigations on perennial plants have included pathogenicity experiments; and it has been assumed that these taxa are latent or quiescent pathogens that may become symptomatic when the host is

Table 5. Fungal pathogens from the genus *Musa*. Sources include: Wardlaw (1972); Stover (1972): Holliday (1980); Farr *et al.* (1989); Jones, Pegg and Thomas (1993); Jeger *et al.* (1995).

Pathogen	Disease	Pathogen	Disease
Oomycetes		*Curvularia* sp.	leaf spot
Phytophthora sp.	leaf rot	*Deightoniella torulosa*	black leaf spot
Pythium aphanidermatum	basal stem rot	*Drechslera gigantea*	eye spot
Pythium sp.	root rot, rootlet necrosis	*Fusarium lateritium*	end rot of fruit
		Fusarium oxysporum f.sp. *cubense*	panama wilt
Zygomycotina		*Fusarium pallidoroseum*	crown rot
Rhizopus stolonifer	fruit rot	*Fusarium subglutinans*	heart rot, end rot of fruit
Ascomycotina			
Ceratocystis paradoxa	fruit rot	*Helminthosporium* sp.	fine speckle, leaf spot
Chaetothyrina musarum	sooty blotch		
Guignardia musae	leaf freckle		
Mycosphaerella fijiensis	black sigatoka, black leaf steak	*Nigrospora sphaerica*	squirter disease
		Pseudocercospora musae	anamorph: yellow sigotoka
Mycosphaerella musicola	yellow sigatoka, leaf streak	*Pyricularia grisea*	pitting
		Verticillium theobromae	cigar end
Mycosphaerella musae	leaf speckle		
Mycosphaerella minima	leaf speckle	**"Deuteromycotina" – Coelomycetes**	
Phyllochora musicola	black-cross leaf spot	*Colletotrichum gloeosporioides*	anthracnose
		Colletotrichum musae	anthracnose, fruit and stem rot, leaf spot
Basidiomycotina			
Armillaria tabascens	root rot		
Haplobasidion musae	Malayan or diamond leaf spot	*Hendersonula toruloidea*	tip rot, leaf spot
		Lasiodiplodia theobromae (*Botryodiplodia theobromae*)	fruit and stem rot
Marasmiellus semiustus	root, stem and trunk rot		
		Pestalotia leprogena	ring spot
Marasmius sp.	root rot	*Phoma jolyana*	black finger disease
Uredo musae	rust		
Uromyces musae	rust	*Phyllosticta musae*	leaf spot
		Phyllosticta musarum	freckle, black spot
"Deuteromycotina" – Hyphomycetes			
Cercospora havi	brown or diamond spot	*Phyllosticta musicola*	leaf spot
		Trachysphaera fuctigena	fruit rot
Chalara paradoxa	basal stem rot		
Cladosporium cladosporioides	sooty mould	**"Deuteromycotina" - Other**	
		Rhizoctonia solani	root and stem rot
Cladosporium musae	leaf speckle		
Cordana musae	leaf blotch		

physiologically stressed (Kulik, 1984). Potentially pathogenic species found in this study include *Colletotrichum gloeosporioides*, *C. musae*, *Fusarium lateritium*, *F. solani*, *Lasiodiplodia theobromae*, *Pestalotiopsis palmarum* and *Phyllosticta musicola*.

Saprobes

Many of the taxa isolated from *M. acuminata* are primarily saprotrophic rather than pathogenic. Saprotrophic taxa that were isolated in moderate to high frequencies include *Epicoccum nigrum*, *Nigrospora musae*, *N. oryzae*, *Phomopsis* spp. and *Xylaria* species. A representative example of this saprotrophic existence are the members of the Xylariaceae found as the most frequent endophytes in north Queensland. The Xylariaceae are known to biodegrade cellulose and lignin and their ecological role is primarily in decomposing senescing plant material (Petrini and Petrini, 1985). *Xylaria* species have been found to be common in all tropical hosts surveyed (Rodrigues and Samuels, 1990; Rodrigues, 1994; Perreira *et al.*, 1993); this family are particularly well adapted to an endophytic existence (Whalley, 1995). In Hong Kong no xylariaceous species were isolated, possibly indicative of a disturbed host.

Potential mutualists

Some of the saprobes isolated from *Musa acuminata* species complex are from genera of fungi that have been linked to a mutualistic existence with their host plants. Carroll (1988) has proposed criteria to identify likely mutualists which include: (i) they do not cause apparent symptoms of disease; (ii) they have a high frequency of infection; and (iii) evidence exists of antibiotic and toxic metabolite production. One such genus is *Phomopsis*, with some of the strongest evidence of mutualistic relationships between perennial plant hosts and endophytes. Bills *et al.* (1992) isolated the tremorgenic mycotoxins, paspalitrem A and C, from an undescribed *Phomopsis* species; paspalitrem and the related lolitrem compounds from grass endophytes represent one of the best known fungal-plant protective mutualisms (Clay, 1991). This same *Phomopsis* species also produces two novel cytochalasins which is a group of compounds known to be potentially toxic to herbivores (Bills, 1996). Another protective mutualism involves *Phomopsis oblonga* which produces a compound antagonistic to the scolytid beetles which are the vectors of Dutch elm disease. Carroll (1988) also suggests that *Phomopsis* is a dominant successional species in temperate plants. *Phomopsis* was a common endophyte, although not dominant within the *M. acuminata* communities sampled. These previous observations of mutualisms,

bioactive compounds and competitive ability suggest this taxon may be beneficial to the host and should be considered for further investigation for biological control potential.

Other taxa isolated that may be mutualistic and may have potential for biological control strategies include the genera *Microsphaeriopsis*, *Nigrospora*, *Phoma* and *Xylaria*. Like *Phomopsis*, the genus *Xylaria* is known to produce and array of bioactive compounds including novel cytochalasins; its abundance as an endophyte of *M. acuminata* subsp. *banksii* deserves further attention as it may play an important ecological role within the endophytic communities.

This is the first report of *Nigrospora musae* as an endophyte. *Nigrospora oryzae* has previously been found as a common component of endophytic assemblages in hosts from the Compositae (Fisher *et al.*, 1995) and Papilionoideae (Pereira *et al.*, 1993). The closely related *Nigrospora sphaerica* was isolated at lower frequencies from tropical hosts from the families Piperaceae (Dreyfuss and Petrini, 1984) and Palmae (Rodrigues, 1994). The genus *Nigrospora* appears to be well adapted to an endophtyic existence. This is noteworthy as this genus is known to produce diterpene derivative aphidicolin antiviral compounds and triglycerides which can act as attractants for beetles such as *Tribolium confusum* (Domsch and Gams, 1993).

A *Phoma* species was also recorded as a major component of the endophytic community in *M. acuminata* subsp. *banksii* in north Queensland. Until an accurate species identification of the *Phoma* species can be made the ecological role of this taxa remains enigmatic. If it is *Phoma jolyana* then it is a recorded pathogen of *Musa acuminata* species complex, however, this genus has been shown to also produce bioactive compounds that may be beneficial to the host plant. Yang *et al.* (1994) demonstrated that a *Phoma* species from Nepales Yew (*Taxus wallachiana*) produced two antibacterial compounds. One an organic solvent plant extract was experimentally found to be antibacterial only when inoculated with the *Phoma* species. Due to the high frequency of occurrence of *Phoma* sp., *Xylaria* spp. and *Nigrospora musae* in *Musa acuminata* subsp. *banksii* and their possible beneficial presence, these taxa should be given a high priority for future investigation.

Biological control potential

Fungal endophytes have been proposed as potential biological control agents for weed control by latent pathogens (Petrini, 1993; Dorworth and Callan, 1996); and also induced resistance against disease through their ability to alter the alleochemical defences of a plant (Clay, 1991; Freeman and Rodriguez, 1994). Endophytes have practical advantages over epiphytes; these advantages

may include: host specificity (Petrini, 1996), less seasonal variation than epiphytes (Rodrigues, 1994), and they presumably would require less frequent applications than epiphytic fungal or bacterial agents. The present study is directly relevant to foliar pathogens of *Musa acuminata* species complex, one of which is *Mycosphaerella fijiensis*, the most serious pathogen of banana. As the results of the present study indicate, many frequently isolated endophytic taxa of *Musa acuminata* species complex, including *Nigrospora*, *Phoma*, *Phomopsis* and *Xylaria*, are from genera previously found to produce relevant antibiotic compounds. The endophytes of most relevance were found in much higher frequencies in the native banana (*Musa acuminata* subsp. *banksii*) suggesting banana from introduced areas and plantations may be at a disadvantage with respect to beneficial endophytes. Freeman and Rodriguez (1994) demonstrated that *Colletotrichum gloeosporioides* could be transformed from a virulent pathogen to a hypovirulent endophyte by genetic manipulation; this suggests potential pathogens isolated from *M. acuminata* may also be potential biocontrol agents. The concept of biological control of banana diseases deserves further attention. However, the variability of species abundance and species composition of endophyte assemblages observed in this study suggests the results would also be highly variable between sites and conditions, which may compromise their effectiveness.

Due to the economic importance of Sigatoka diseases on banana caused by *Mycosphaerella* species they are an excellent candidate for biological control research. Little work on biological control measures has been attempted; limited success has been achieved in experiments with antagonistic epiphytic bacteria (Jeger *et al.*, 1995). The possibility of using antagonistic fungal endophytes has in banana not been previously addressed. Inoculating plants with an antagonistic endophyte or a hypovirulent endophyte genetically engineered for resistance could be a future direction in biological control research. In relation to banana (*Musa acuminata*) they have potential as protective antagonists against fungal, bacterial and viral pathogens as well as nematodes. Each of these groups significantly reduce banana production worldwide (Jeger *et al.*, 1995). This example highlights the need for endophyte research into the Musaceae as it could be the basis on which disease control measures are developed.

Acknowledgements

Dr. L. Moore and Ron Peterson are thanked for providing specimens of banana leaves. Sally Fryar and Jacqui Wright is thanked for her comments on the draft manuscript.

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