CHAPTER 8

GENERAL DISCUSSION AND CONCLUSION

This study was initiated in order to obtain data on the fungal endophytes, saprobes and parasites developing on six zingiberaceous species, a host family that has been little studied for these organisms. The relationships between endophytic, saprobic and pathogenic fungi from Zingiberaceae were observed.

8.1 Diversity of endophytic and saprobic fungi in Zingiberaceae

Numerous studies have reported that infections of terrestrial and aquatic plants by endophytes are ubiquitous, having been found throughout a broad range of host orders, families, and genera worldwide. In the present study, low numbers of fungi were found common among zingiberaceous species from different sites, and fungal compositions on the plants from different sites was less closely coherent than those from same sites. This indicates that fungal communities also vary between sites. There were no seasonal patterns of fungal occurrence on the Zingiberaceae collected in this study. Although the study areas have a tropical climate there are seasonal differences in rainfall, with distinct wet and dry seasons. No seasonal differences were observed between the endophytic fungi isolated from *Licuala* species (palm) in Brunei, which has a tropical climate (Fröhlich and Hyde, 1999; Fröhlich *et al.*, 2000). This may have been due to the small seasonal differences in rainfall. Seasonal patterns of phylloplane fungal communities have, however, been recorded on

Eucalyptus viminalis, which were directly correlated with humidity and invesely with temperature (Cabral, 1985). Isolation of endophytic fungi from *Euterpe oleracea* during the wet and dry seasons in Brazil resulted in more isolates being found during the wet seasons (Rodrigues, 1994). The effect of seasonality may be more acute in temperate regions where greater fluctuation in temperature, humidity and rainfall usually occur. Further studies are, however, required to elucidate the effects of seasonality.

Bayman *et al.* (1997) found that there was considerable variation in endophytes within individual plants and within individual roots and leaves of seven *Lepanthes* species. Differences among these orchid species in number and types of endophytes were also significant, however the heterogeneity of endophytes in single plants and plant organs overshadowed differences between species. The incidence of colonization with different endophytes showed a significant tendency to increase with advancing age of the host tissue (Bernstein and Carroll, 1977; Fisher *et al*., 1986, Hata and Futai, 1993). Rodrigues (1994) found that overall fungal colonization was positively correlated with leaf age, plant growth stage, site and the interactive effects of growth stage versus season, and growth stage versus site. Moreover, the distinct endophytic community is determined by the type or morphology of the plant structures examined e.g., vein, intervien or xylem (Bertoni and Cabral, 1988; Petrini and Fisher, 1988).

A high number of fungal taxa was found in the present study, indicating that the fungi on zingiberaceous plants are diverse and could be a source of undescribed species. Although the zingiberaceous species contained similar endophytic fungal communities as those of other monocotyledons and dicotyledons (Rodrigues, 1994;

Menendez *et al.*, 1995; Taylor *et al.*, 1999; Kumaresan and Suryanarayanan, 2001; Photita *et al*., 2001b; Baayen *et al*., 2002; Okane *et al*., 2003; Rodrigues *et al*., 2004), several species new to science were described (Bussaban *et al.*, 2001b, c, 2002, 2003a, b). Endophytes and saprobes isolated from Zingiberaceae are believed to be excellent potential sources of bioactive compounds.

The recurrence of certain fungi on different tissue types may be due to nutritional requirements, or the ability of the fungi to utilise different substrates (Adaskaveg *et al.*, 1991; Ingold and Hudson, 1993). Alternatively, it may be related to the distribution of endophytes, a theory, which requires testing. Unfortunately, most endophytes identified from Zingiberaceae are common anamorphic fungi such as *Colletotrichum gloeosporioides*, or sterile mycelia and therefore the saprobes found on zingiberaceous plants are not similar to the endophytes reported by Bussaban *et al.* (2001). Further studies are needed to identify the sterile isolates to establish if they are the same species as the saprobes. The enzymatic activities of these fungi also warrants further investigation in order to understand whether their abundance on specific tissue types is due to differing enzymatic capabilities (Yanna, 2001). The comparison of fungal diversity on zingiberaceous species with other hosts must be done carefully. There are several methods employed in the study of fungal diversity and many indices to interpret the diversity. Determination of the adequacy of sample size is also important.

8.2 The relationships among endophytic, saprobic and pathogenic fungi from Zingiberaceae

Some endophytes are thought to benefit the host plants by enhancing absorption of soil nutrients such as phosphorus, conferring drought tolerance, providing protection from insect attack, and/or inhibiting the development of plant pathogens (Thomson *et al.* 1986; Breen 1993, 1994; Latch 1993; Stone *et al.*, 2000; Liu *et al.*, 2001). Endophytes are, however, also thought to become pathogenic when the host plant is stressed (Millör, 1980; Andrews *et al.*, 1985). Endophytes have been often isolated by plant pathologists and described as latent or weak pathogens (Kulik, 1984; Brown *et al.*, 1998; Photita *et al.*, 2004). This study collected and identified the diseases that occur on the selected zingiberaceous plants. The relationships between endophytic, saprobic and pathogenic fungi were observed by testing the pathogenicity of these fungi against tissue cultured ginger, and by testing endophytic and saprobic fungi for their antagonism against ginger pathogens.

In the current study, isolates of endophytic, saprobic, or pathogenic fungi (genera which have previously been reported as zingiberaceous pathogens *Alternaria*, *Colletotrichum*, *Fusarium*, *Phomopsis*, *Phyllosticta* and *Pyricularia*), were tested for their pathogenicity against tissue cultured ginger. *Colletotrichum gloeosporioides*, *Fusarium* spp. and *Pyricularia costina* (each fungal taxa isolated as endophytes, saprobes or pathogens) caused disease symptoms or death of tissue cultured ginger. Some isolates of *Phomopsis* sp. and *Phyllosticta capitalensis*, isolated as endophytes, infected leaves of the test plant. After transplanting these gingers into plastic pots and incubation in greenhouse, the plants infected by some endophytes grew better than the control. However, studies on this aspect should be continued. The present study also showed the potential of zingiberaceous fungi to be antagonistic to plant pathogens in dual culture. Further research is needed to assess any inhibition of pathogens in the host.

8.3 Potential of zingiberaceous fungi to produce bioactive compounds

Strobel *et al.* (2004) noted plant selection strategy in searching for endophytes displaying bioactivity as follows: i) plants from unique environmental setting, especially those with an unusual biology and possessing novel strategies for survival; ii) plants that have an ethnobotanical history (use by indigenous peoples) that is related to the specific or applications of interest; iii) plants that are endemic, having an unusual longevity, or that have occupied a certain ancient land mass; and iv) plants growing in areas of great biodiversity which, perhaps, also have the prospect of housing endophytes with great diversity. The results of the present study demonstrate the potential value of investigating secondary metabolites (antimicrobial agents and enzymes) produced by fungi isolated from Zingiberaceae (medicinal plants) in a tropical region.

It has been reported that endophytes can produce various bioactive compounds e.g. *Acremonium* sp., the endophyte of European yew produces leucinostatin (Strobel *et al.*, 1997), *Xylaria* sp., an endophyte of *Spondias mombin* L. can produce secondary metabolites with potential use for biological control (Rodrigues *et al.*, 1993), and *Pestalotiopsis* spp. and *Monochaetia* sp. isolated from rain forest plants can produced ambuic acid (Li *et al.*, 2001). It has also been reported that endophytes isolated from some medicinal plants can produce the same bioactive compounds as the plants e.g., *Taxomyces andreanae*, *Pestalotiopsis* sp., *Pestalotiopsis microspora* and *Periconia* sp. endophytic fungi isolated from *Taxus brevifolia*, *T. wallachiama* and *Torreya grandifolia*, are also taxol producers (Stierle *et al.*, 1993; Strobel *et al.*, 1996a, b; Pulici *et al.*, 1997; Li *et al.*, 1998).

In the present study, selected strains of endophytic and saprobic fungi were screened for their ability to produce antifungal/antibiotic compounds. *Papulospora* sp. (CMUZE0118), *Colletotrichum gloeosporioides* (CUMZE0552) and *Phyllosticta capitalensis* (CMUZE0172) showed the highest activity against microbial test organisms, *Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. The results show that a wide range of endophytic and saprobic fungi can produce bioactive compounds that may have potential biotechnological applications. Determining the optimum conditions for these fungi to produce antimicrobial compounds, and determination of the chemical structures of such compounds requires further investigation.

8.4 Molecular and morphological characterization of fungi from Zingiberaceae

Molecular tools, because of their sensitivity, are now increasingly being used for the identification of fungi (Egger, 1995; Sreenivasaprasad and Mills, 1998; Berbee and Taylor, 2001; Pandey *et al*., 2003; Menkis *et al*., 2004; Rodrigues *et al*., 2004; Callac and Guinberteau, 2005; Didukh *et al*., 2005). A range of methods are in use and the method adopted in this thesis proved to be useful for confirming the identify of *Chaetomium globosum* (Chapter 7), for revealing differences between the morphologically similar fungal genera, *Dactylaria* and *Pyricularia*, and in providing evidence for synonymising the morphologically similar fungi *Myrothecium* and *Solhiemia* (Chapter 6). Molecular techniques have a great use to assist in the identification of taxa in species complexes and to define boundaries and relationships of taxa. In the present study, such techniques proved identification and determined the phylogenetic relationship of endophytic *Pyricularia* spp. The results from these techniques confirmed that endophytic and pathogeneic isolates of *Pyricularia costina* are close strains.