ENDOPHYTIC FUNGI OF *TECTONA GRANDIS* L. (TEAK)

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DECLARATION

I declare that while registered as a candidate for the degree of Doctor of Philosophy I have not, in the duration of this research programme, been registered as a candidate for another award from any other academic or professional institution.

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Related studies undertaken in connection

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ABSTRACT

Taxonomic diversity, biology and ecological aspects of fungal endophytes of *Tectona grandis* (teak) from Chiang Mai Province, Thailand were investigated. It was found that the endophyte assemblages of mature leaves sampled from natural forest and plantation teak were not significantly different. Members of the Xylariaceae, especially *Daldinia eschscholzii, Nemania subannulata, Hypoxylon haematostroma* and *Xylaria cubensis* were frequent isolates. Widely reported endophytic fungi such as *Phomopsis, Colletotrichum, Cladosporium* and *Fusarium* were also isolated. There is little evidence to support host specificity for the majority of the isolates.

Differences in endophyte assemblages between young and mature leaves were shown to occur with a much lower infection percentage in the young leaves. Species of *Phomopsis* and *Colletotrichum* were dominant in the young leaves but members of the Xylariaceae dominated in mature leaves. This pattern was the same for both natural forest and plantation samples.

However comparison of taxa isolated from leaf lamina, midrib and veins gave conflicting results. Samples from mature leaves from natural forest trees exhibited little variation with greater variation in taxa recovered being found to occur between sampling years than between position of isolation from the leaf. In plantation leaves, although the results were similar to those from natural forest tree leaves for two of the years sampled, in 1997 the overall recovery rate was highest for the lamina, followed by veins and then the midrib. There was no evidence obtained to link individual taxa with specific regions of the leaf. It is now possible to devise a sampling strategy to obtain suitable diversity of endophytic isolates from teak leaves for industrial screening of these fungi.

Techniques were developed to overcome current problems of identification of xylariaceous endophytes in the absence of their teleomorph. Inoculation of suitable woody substrata combined with selective incubation was used to induce teleomorph formation in many of the isolates and this together with chemical profiling enabled identification to species of many of these isolates. Rates of development of specific species were obtained and differences in environmental conditions necessary for development of teleomorphs to maturity were noted for members of different genera. Thus species of *Daldinia* and

Hypoxylon required drier conditions than species of *Xylaria* and *Nemania* which only developed under wet shaded conditions.

Xylariaceae from the natural forest, plantation, and forest surrounding the plantation were surveyed and a number of the Xylariaceae recovered as endophytes were found to be new to science, new records for Thailand or were recorded as endophytes for the first time.

Abbreviations

SEM	=	Scanning Electron Microscopy
DIC	=	Differential Interference Contrast
µm	=	micrometre
mm	=	milimetre
cm	=	centimetre
diam	=	diametre
°C	=	degree celcius
km	=	kilometre
min	=	minute
NMR	=	Nuclear mass resonance

Contents

Chapter 5: Taxonomy of endophytic species and relevant Xylariaceae

Chapter 1

Introduction

" Tropical regions are undoubtedly a black box with respect to our knowledge of endophyte distribution and ecology. Thus, they represent a new and exciting avenue for all interested fungal ecologists and taxonomists, at the same time opening the possibility of discovering novel ways of exploiting, both scientifically and economically, fungal endophytes" (Rodrigues & Petrini, 1997).

1. 1. General considerations

Since De Bary (1866) made the first reference to fungal endophytes, describing them as any fungus whose hyphae invade tissues or cells of living autotrophic organisms, a variety of definitions have been reported. However, the definition given by Petrini has been most widely used. He stated that "all organisms that live inside plant tissues for at least part of their life cycle without causing any disease symptoms in the host are endophytes" (Petrini, 1991). Endophytes have been isolated from a wide range of plant groups including mosses, ferns, lichens, orchids, grasses and trees, encompassing a broad geographical range (Marchisio *et al.,* 1985; Hawksworth,1988; Clay,1989; Fisher & Petrini, 1990; Petrini & Fisher, 1990; Warcup, 1991; Fisher, Petrini & Sutton, 1993; Swatzell, Powell, & Kiss, 1996). Studies over the past 20 years have shown endophytic fungi to occur universally and to exhibit impressive diversity in tropical plants (Dreyfuss & Petrini, 1984; Rodrigues & Samuels, 1990). Their activities in nature, and significance to the host are little known but some endophytes have been reported to be prolific producers of interesting antibiotics and metabolites (Noble *et al.,* 1991; Calhoun *et al.,* 1992; Polishook *et al.,* 1993; Jensen & Frisvad, 1996; Hallmann & Sikora, 1996). In certain cases endophytic fungi are likely to be a latent inoculum for future infection in woody host species, especially under stress conditions (Chapela, 1989; Carroll, 1990; Whalley, 1996). Moreover, the endophytes in some grasses are known to be biocontrol agents e.g. clavicipitaceous endophytes reduce pest damage either by directly killing or damaging the pest, reducing its population growth, or by deterring the pest before it can do any damage (Clay, 1989). Furthermore, in many

cases mutualism has been suspected in endophytic symbiosis. Carroll (1988) discussed such symbiosis in well documented case histories.

Endophytic fungi obtained from plants are diverse and include members of the Ascomycotina, Deuteromycotina and Basidiomycotina (Petrini & Fisher, 1986; Fisher, Petrini & Scott, 1992; Bettucci & Saravay, 1993). The role of endophytic fungi in host-plant-endophyte-pathogen or host-plant-endophyte-insect interactions, that lead to antagonistic symbiosis or mutualism and secondary metabolite production for pharmaceutical and agricultural benefits, have been widely studied (Bills *et al.*, 1992; Calhoun *et al*,. 1992; Whalley & Edwards, 1995). Thus, accurate identification of such fungi is crucial following the application of suitable sampling and isolation strategies.

1. 2. Grass endophytes

1. 2. 1. Biology

The presence of clavicipitaceous endophytic fungi within the tissues of several grasses has been known for some time (Clay, 1986,1988,1989; White, 1987). Species of fungi in the family Clavicipitaceae, tribe Balansiae systemically infect plants of three families, the Poaceae, Cyperaceae and Juncaceae, with the overwhelming majority of hosts being grasses (Clay, 1989). Of the five genera in the tribe Balansiae (*Atkintonella, Balansia, Balansiopsis, Epichloe* and *Myriogenospora*), *Balansia* is the largest, with 15-20 species (Diehl, 1950). The Balansiae are systemic and perennial (Diehl, 1950; Clay 1986) and most of them are endophytes whose vegetative hyphae are intercellular and run parallel to the long axis of host cells in leaf and stem tissues (Clay, 1988,1989). Fungal infection in most hosts causes sterility by inhibition of flowering or mechanical abortion of the developing inflorescence (Bradshaw, 1959; Clay 1986). Fruiting bodies of the Balansiae can be seen on the leaves and aborted inflorescences of host species (Clay, 1986,1988,1989). The anamorphs, generally placed in the genus *Acremonium* (Sect.) *albo-lanosa*, are very closely related to the Balansiae, which are not known to exhibit sexual reproduction. *Acremonium* resembles the asexual stage of *Epichloe typhina* (Fr.) Tul. (Clay, 1986; White, 1987) and is placed with the asexual form of *E. typhina* in the Deuteromycotina, based on growth characteristics in culture (White & Cole, 1985; White & Morgan-Jones, 1987).

Unlike the Balansiae, they do not sporulate or produce fruiting bodies or produce any visible signs of infection, but their hyphae occur intercellularly in leaf and stem tissue (Clay, 1988,1989). They are transmitted maternally by growth of hyphae into developing ovules and seeds of infected maternal plants (Neill, 1941; White, 1987). For example, in perennial ryegrass seed (Clay, 1989), endophytic hyphae of *Acremonium lolii* can invade the aleurone layer. They do not produce symptoms on their hosts or suppress flowering and can be vertically transmitted from generation to generation. These anamorphic fungi with affinities to *E. typhina* offer the greatest potential for exploitation as biocontrol agents (Clay, 1989; Siegel, Latch & Johnson, 1987).

Recently, a number of publications have indicated that associations between grasses and fungal endophytes may be mutualistic in nature (Clay, 1986). Many grasses are infected by systemic fungal endophytes (family Clavicipitaceae, Order Hypocreales) that produce a number of biologically active compounds both *in vivo* and *in vitro* which are toxic to various grass herbivores. Mechanisms by which fungal symbionts of plants can influence herbivory of the host are described by Clay (1987). Most evidence, however, suggests that resistance results primarily from fungal secondary compounds e.g. ergot alkaloids, produced by a seed-borne endophyte species in the tribe Balansiae, *Acremonium coenophialum* Morgan-Jones & Gams, in the case of tall fescue (Hinton & Bacon, 1985; Bacon *et al.*, 1986; Lyons, Plattner & Bacon, 1986). Feeding trials have shown that these alkaloids can be extremely toxic to insects (Clay & Cheplick, 1989; Johnson *et al.*, 1985). Clay (1989) reviewed those alkaloids produced by endophyte-infected ryegrass and tall fescue such as peramine, lolitrems and loline (Table 1).

1. 2. 2. Effects of endophyte infection

Effects of endophyte infected grass on insects have been the subject of several interesting investigations (Clay, 1989). Field experiments have demonstrated correlation between endophyte infection level with diversity and abundance of insects present (Prestidge, Pottinger & Barker, 1982; Funk *et al.*,1983; Clay, 1989; Ahmad *et al.*, 1986; Lopez, Faeth & Miller, 1995). Laboratory choice and no-choice experiments were used to study host-endophyte-insect relationships (Barker *et al.*, 1984; Clay, Hardy & Hammond, 1985; Hardy, Clay & Hammond, 1985; Johnson *et al.*, 1985; Latch, Christensen & Gaymor, 1985; Siegel, Latch & Johnson, 1985;

Ahmad *et al.*, 1987). The results showed negative effects of endophyte-infected grasses on insects. Clay (1989) listed 15 insects on 23 host species that have been reported to be negatively affected by endophyte-infected grasses. The infected grasses are more toxic and result in reduced insect survival, growth and developmental rate compared to uninfected conspecifics. In laboratory and greenhouse choice experiments, many insects actively discriminated between endophyte-infected and uninfected perennial ryegrass and tall fescue (Barker *et al.*, 1984; Hardy *et. al.*, 1985; Latch *et al.*, 1985; Johnson *et al.*, 1985; Siegel *et al.*, 1985). Survival and population growth rates of insects on seeds of infected grasses were significantly lower than on uninfected seed. On infected tall fescue seed, beetle populations rapidly went extinct (Cheplick & Clay, 1988). In the study of an endophytic fungus, *Acremonium starrii* White & Morgan-Jones and redlegged grasshoppers, *Melanoplus femurrubrum* (De Geer) Lopez *et al.* (1995) found however, that the endophyte did not deter herbivory by adult grasshoppers in the laboratory and the presence of *A. starri* did not affect grasshoppers under field conditions.

Table 1. Alkaloids which are produced by endophyte-infected ryegrass and tall fescue

Alkaloids	Authentication
Ergonovine	Bacon *et al.*, 1986; Lyons *et al.*, 1986.
Lolitrems	Gallagher *et al.*, 1981.
Loline	Jones *et al.*, 1983; Siegel *et al.*, 1987.
Peramine	Rowan & Gaymor, 1986; Rowan *et al.*, 1986.
Pyrrolizidine	Jones *et al.*, 1983.

The anti-herbivore properties of endophyte-infected grasses require careful consideration because they may limit the exploitation of endophytes as biocontrol agents. For example, Bacon *et al.* (1977) noted problems with livestock on endophyte-infected pastures and Clay (1988) listed several antiherbivore effects of fungal endophytes that infect grasses.

There is evidence that endophyte-infected grasses may possess properties that are of applied value as well. For example, rats fed infected tall fescue seed showed a number of significant physiological abnormalities compared to rats fed uninfected seed (Neal & Schmidt, 1985).

Nematode counts were significantly higher in plots of endophyte-free tall fescue than in plots of infected tall fescue. Resistance to nematodes may be present in a wide variety of endophyte-infected grasses (West *et al.*, 1987). There is also antagonism between endophytic fungi and common grass pathogens according to cultural studies by White & Cole (1986) and there are many examples of plants infected by one fungus being more resistant to their common pathogens (Clay, 1987). Infection by endophytic fungi also may benefit grasses in other ways. Several researchers have suggested that endophyte-infected plants are more drought-tolerant than uninfected plants. Infected plants are able to persist and recover more rapidly than uninfected plants following severe water stress, providing a considerable advantage in environmentally stressful habitats (Belesley *et. al.*, 1987; Siegel *et al.*, 1987; West *et al.*, 1987).

1. 3. Non-grass endophytes

1. 3. 1. Distribution and Diversity

Unlike grass endophytes, non-grass endophytes usually colonize non-grass hosts and are members of a broad range of the Ascomycotina or Deuteromycotina (Petrini, Petrini & Rodrigues 1995). Representatives of the endophytes can disseminate independently by air and water and can be horizontally transmitted. Endophytes have been isolated from a wide range of evergreen, shrubs, herbaceous, coniferous and deciduous plants (Spurr & Welty, 1975; Petrini & Carroll, 1981; Sherwood-Pike, Stone & Carroll, 1986; Sridhar & Barlocher, 1992; Schulz *et al.*, 1993; Sieber & Dorworth, 1994) and since the latter half of the 1970s, their ubiquitous occurrences have been demonstrated repeatedly. Endophytes have been found in twigs, leaves, bark, xylem and roots of almost all plants examined (Petrini & Fisher, 1990; Sridhar & Barlocher, 1992; Fisher *et al.*, 1993). Even under poor conditions, such as heavy metal pollution or acid rain, endophytic fungi have been found to occur in plant tissue. The effect of poor environmental condition on endophytes has been discussed (Helander *et al.*, 1993 a,b; Ranta *et al.*, 1994; Helander, 1995). Endophytic fungi in aerial parts of vascular plants have also been extensively studied over the past ten years (Rodrigues & Samuels, 1990). Endophyte distribution has been the most intensively studied, and its assemblage in woody plants is both widespread and diverse

(Carroll, 1995) but there is variation among plant species in both recovered frequency and number of fungal species recorded. This may be related to biochemical, cytological and morphological differences, and to the ecological conditions under which the plant is growing (Clay, 1986,1988; Carroll, 1986; Petrini, 1986,1991; Fisher & Petrini, 1990). Such fungi have been isolated from virtually every host and every plant tissue investigated. These hosts include a wide variety of conifers, a number of *Quercus* species, members of the Fagaceae, species of *Eucalyptus*, the tropical palm *Euterpe oleracea* Mart. and many other woody species (Bertoni & Cabral, 1988; Petrini & Fisher, 1988; Petrini & Fisher, 1990; Rodrigues & Samuels, 1990; Bettucci & Saravay, 1993; Rodrigues, 1994; Wu, 1997). Most of these investigations have been carried out on plants from temperate (Petrini, 1986) and subtropical regions (Cabral, 1985; Bertoni & Cabral, 1988), although a few are from tropical regions. e.g. Dreyfuss & Petrini, (1984); Rodrigues & Samuels (1990); Rodrigues, (1994). Patterns of distribution have been described in several different situations i.e among diverse stands, between geographically close but ecologically distinct stands, among individual trees within a stand, among needles on a single branch, among microscopic samples of needles or leaves and many small patches of bark (Carroll, 1995).

A number of studies have examined the variation in endophytic mycoflora in samples. Among stands, Carroll (1995) reviewed several studies concerning factors that appear to be important determinants of endophyte infection frequency. Those factors are geographically disjunct sites of collections, liquid precipitation, ecological diversity and environmental and climatic differences (Carroll, 1979; Legualt, Dessureault & Laflamme, 1989; Rollinger & Lansenheim, 1993; Fisher *et al.*, 1994; Rodrigues, 1994). There are several reports from studies of endophyte assemblages. For the same plant species, endophyte assemblages can differ between localities, particularly those which are far apart and such fungi are therefore regarded as site-specific endophytes (Fisher & Petrini, 1990; Fisher *et al.*, 1994). Bernstein and Carroll (1977) found no correlation between height in the crown and endophyte infection frequencies. However, Johnson & Whitney (1989a) showed the opposite. No correlation between compass direction and pattern of infection was noted by Johnson & Whitney (1989a). In an investigation of how endophytes vary within and between individual plants, Bayman *et al.* (1997) found that there was considerable variation in endophytes within individual plants and within individual roots and

leaves of 7 *Lepanthes* Swartz species. Differences among orchid species in number and types of endophytes were significant, however the heterogeneity of endophytes in single plants and plant organs overshadowed differences between species (Bayman *et al.*, 1997). The incidence of colonization with different endophytes showed a significant tendency to increase with advancing age of the host tissue (Bernstein & Carroll, 1977; Fisher, Anson & Petrini, 1986; Hata & Futai, 1993). In her study of the foliar fungal endophytes of Amazonian palm (*Euterpe oleracea*)., 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. Furthermore, the distinct endophytic community is determined by the type or morphology of the plant structures examined e.g. vein, intervein or xylem (Bertoni & Cabral, 1988; Petrini & Fisher, 1988). Information on the distribution of endophytes within the leaf comes from culture work experiments in which the physical size of individual sampling units is arbitrarily determined (Carroll, 1995). The investigations found that endophytes occupy extremely limited domains within plant tissues and may often be confined to the lumen of single cells (Stone, 1987; Suske & Acker, 1987,1989; Bissegger & Sieber, 1994). In a study of endophytes of pine needles Hata & Futai (1996) found *Leptostroma* and *Cenangium ferruginosum* Fr. ex Fr. dominated the middle segment of the needles whilst a species of *Phialocephala* was most frequent in the basal segments.

1. 3. 2. Taxonomy

The identification of endophytic fungi has proved to be extremely difficult, largely because of the lack of information on the cultural characters of species already described, but also because very little is known about the peculiar microhabitat represented by plant tissues (Petrini, 1986). Ascomycetes, basidiomycetes, deuteromycetes and members of the Oomycota have so far been isolated as endophytes (Petrini, 1985; Fisher *et al.*, 1986; Fisher & Petrini, 1992; Fisher *et al.*, 1994). Representatives of these fungal groups are shown in Table 2. Within the deuteromycetes, the situation is complicated by the lack of reliable descriptions of known hyphomycetous or coelomycetous species in culture, as well as the poor knowledge of the pleomorphic states which

Table 2. Fungi isolated as endophytes from some host plants.

Fungi	References
Ascomycetes	
Guignardia sp.	1
Phaeosphaeria eustoma (Fuck.) Holm	1
Phomatospora barkeleyi Sacc.	1
Chaetomium sp.	11
Gelasinospora reticulispora (Greis) C. & M. Moreau	11
Pezicula spp.	5,9
Pleospora herbarum Rabenh.	11
Sporormiella spp.	11
Deuteromycetes	
Hyphomycetes	
Acremonium spp.	1,6,12,13
Alternaria spp.	6,7,11,12
Aureobasidium spp.	6,7,9,10,12,14,16
Cladosporium spp.	1,7,9,10,11
Curvularia pallescens	12
Fusarium spp.	1,4,6,11,14
Hormonema spp.	7,8,9,13,16
Nigrospora spp.	7,12
Phialophora spp.	1,6,11,13,16
Penicillium spp.	10,12
Rhizoctonia spp.	8,11,13,17
Trichoderma spp.	7,12
Coelomycetes	
Colletotrichum spp.	1,4,9
Coniothyrium spp.	1,2,6,8
Cryptosporiopsis sp.	5
Diplodina acerina	15
Phomopsis spp.	3,4,5,7,8,13,14,15
Phyllosticta sp.	10
Pestalotiopsis spp.	2,4,7,12
Basidiomycetes	
unidentified basidiomycetes	12

References: 1, Dreyfuss and Petrini, 1984; 2, Bertoni and Cabral, 1988; 3, Rodrigues, 1994; 4, Rodrigues and Samuels, 1990; 5, Sieber, Sieber-Canavesi & Dorworth, 1991; 6, Schulz *et al.*, 1993; 7, Fisher *et al.*, 1993; 8, Petrini and Fisher, 1988; 9, Fisher and Petrini, 1990; 10, Johnson and Whitney, 1989; 11, Fisher and Anson, 1986; 12, Bettucci and Saravay, 1993; 13, Sieber, 1989; 14, Petrini and Fisher, 1990; 15, Sieber and Dorworth, 1994; 16, Helander *et al.*, 1994; 17, Warcup, 1991.

can occur in a single species during its life cycle. Some strains of coelomycetes and hyphomycetes, for instance, show a broad variation in shape and size of their conidiomata, conidiophores and conidia in culture (Petrini, 1986). The task of identification of some ascomycetous fungi, especially Xylariaceae, to species level from their culture characteristics is very difficult as they rarely produce morphologically diagnostic structures and teleomorphs are seldom formed (Brunner & Petrini, 1992; Rodrigues, Leuchtmann & Petrini, 1993). Accurate identification to species level can be accomplished only when colonies are compared with descriptions or cultures derived from ascospores of reliably identified teleomorphs. The more frequently isolated anamorphs could be named mostly only to genus level; no further identification was possible because the cultural characters of only a limited number of xylariaceous species have been described (Petrini & Petrini, 1985; Rogers, 1985; Callan & Rogers, 1990; Rodrigues *et al.*, 1993). In the last few years cultural, biochemical and numerical techniques have been repeatedly applied to solve taxonomic problems of endophytic fungi (Suske & Acker, 1987,1989; Sieber-Canavesi, Petrini & Sieber, 1991). Electrophoresis techniques, for example, have been widely used to solve taxonomic problems within fungal taxa (Leuchtmann & Clay, 1990; Bonde, Peterson & Mass, 1991; Oudemans & Coffey, 1991; Leuchtmann *et al.*, 1992; Leuchtmann, 1994) and to determine taxonomic relationships of endophytic isolates of *Xylaria* Hill ex Schrank species (Brunner & Petrini, 1992; Rodrigues *et al.*, 1993).

1. 4. Secondary metabolites

Some endophytic fungi are known to produce secondary metabolites which serve many purposes. In xylariaceous fungi, many of which live endophytically, secondary metabolites produced by representatives of at least one third of these genera have been isolated and identified. The major compounds, which are produced in static culture, can be grouped as dihydroisocoumarins, punctaporonins, cytochalasins, butyrolactones and succinic acid derivatives (Whalley & Edwards, 1995) as shown in Tables 3, 4 and 5. Compounds which are produced in static culture can be used in conjunction with traditional taxonomic characters for systematic purposes in the Xylariaceae (Whalley & Edwards, 1987,1995). A total of 115 *Cryptosporiopsis* Bubak & Kabat isolates from different hosts can be grouped into 3 species (*C. abietina* Petrak, *C. fasciculata* (Tode ex Ttul.) Petrak and *C. quercina* Petrak) by classifying them according to the

secondary metabolites produced (Jensen & Frisvad, 1998). It has also been suggested that a fungitoxic compound, cryptosporiopsin, produced by *Cryptosporiopsis*, could be used as a biocontrol agent against a number of wood rot fungi and bacteria (Stillwell, Wood & Strunz, 1969). Another toxic compound from endophytic *Cryptosporiopsis* strains isolated from *Vaccinium myrtillus* L. showed antimicrobial properties against the ascomycetous yeast *Candida albicans* (Robin) Berkhout (Fisher, Anson & Petrini, 1984). The endophyte *Hormonema dematioides* Lagerb & Melin, a slow growing, black yeast usually associated with conifer wood, produced preussomerin D, which is an effective natural antibiotic compound. In standard disc assays, this compound exhibited broad spectrum, intermediate potency, antifungal activity when evaluated against selected filamentous fungi, clinically important yeasts and Gram positive bacteria (Polishook *et al.*, 1993). There is an evident toxicity of fungal endophyte secondary metabolites to the plant parasitic nematode, *Meloidogyne incognita*. In this case the toxic compound was produced by the non-pathogenic *Fusarium oxysporum* Schlecht emend. Sny. & Hans. isolated from the cortical tissue of surface sterilized tomato roots. The compound is not only effective for the in biological control of *M. incognita* in pot experiments but can also reduce significantly the growth of soil-borne plant pathogenic fungi such as *Phytophthora cactorum* (Leb. & Cohn) Schrot, *Pythium ultimum* Trow and *Rhizoctonia solani* Kuhn *in vitro* (Hallmann & Sikora, 1996). Endophyte toxins have been considered as a probable cause of herbivore antagonism (Clay, 1989; Carroll, 1995). In balsam fir needle, Calhoun *et al.* (1992) identified toxins from 3 strains of balsam fir endophytic fungi that were reported to cause reduced growth rate and mortality of spruce budworm larvae. Moreover, Carroll (1995) suggested that the effectiveness of toxigenic endophytes could be greatly enhanced under field conditions by the synergistic interactions of toxins produced by different species of endophytes inhabiting the same small portion of leaf or stem tissue. The anticancer compound taxol, from *Taxus brevifolia* Nutt., has recently been found as a product of the endophyte *Taxomyces andreanae* Strobel, Stierie & Hess opening up the possibility that other valuable natural products found in small quantities in plants might in future be obtained via fermentation of host endemic endophytic fungi (Stierie & Strobel, 1995).

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Table 3. Distribution of dihydroisocoumarins (Whalley & Edwards, 1995).

Note: Numbers and symbols represent the following: 1, mellein; 2, iso-ochracein; 3, ramulosin; 4, 4-hydroxyisoochracein; 5, 5-methylmellein; 6, 5-formylmellein; 7, 5-methoxycarbonylmellein; 8, 6-methoxy-5-methylmellein; 9, 5-hydroxymethylmellein; 10, 5-carboxymellein. +, present as major metabolite; *, present in trace amount only.

[a] At present a herbarium name awaiting formal description (Pouzar, pers. comm).

[b] Transferred to *Nemania* by Laessøe and Spooner (1994).

[c] Transferred to *Nemania* by Pouzar (1985a, 1985b).

[d] Transferred to *Euepixylon* Fuisting by Laessøe and Spooner (1994).

[e] Note yet formally transferred from *Hypoxylon* (= *H. microplacum* (Berk. & Curt.) Mill.

Table 4. Distribution of succinic acid derivatives (Whalley & Edwards, 1995).

Note : Diacid 1, 2-butyl-3-methylsuccinic acid; Diacid 2, 2-hexylidene-3-methylsuccinic acid.

[a] *Nemania illita* (Schwein.) Pouzar.

Table 5. Distribution of cytochalasins (Whalley & Edwards, 1995).

1. 5. Endophytes as plant pathogens

The fungal endophytes isolated from several plants are primarily common genera found to be either necrotrophic or saprotrophic e.g. *Phomopsis* (Sacc.) Bubak, *Colletotrichum* Corda, *Fusarium* Link, *Coniothyrium* Corda, *Alternaria* Nees, *Septoria* Sacc., *Hypoxylon* Bull. (Bassett & Fenn, 1984; Petrini, 1984; Petrini & Fisher, 1988; Rodrigues & Samuels, 1990; Sieber *et al.*, 1991; Fisher and Petrini, 1992; Fisher *et al.*, 1992; Fisher *et al.*, 1994; Whalley, 1996). Finding such a large number of potential plant pathogenic genera as endophytes supports the theory that endophytes can act as latent pathogens (Carroll, 1988; Stone, 1990; Petrini, 1991). Previous studies (Bernstein & Carroll, 1977; Canavasi, 1983; Sieber *et al.*, 1988, 1989) indicated that some pathogenic fungi have a latency period during which they live endophytically in apparently healthy plant tissues. Latency can last several days to many years depending on fungus virulence, host, climate and ecological condition. Thus, these pathogenic fungi could potentially be detected as endophytes in healthy tissues long before symptoms develop (Sieber *et al.*, 1981).

Taxonomically endophytes are largely ascomycetes or deuteromycetes, that may become pathogenic only when they encounter an appropriate environment and when their host plants are subject to physiological stress (Chapela & Boddy, 1988a,b). The genus *Fusarium* is one of the most widely distributed and important groups of plant pathogens. The existence of avirulent

strains of *Fusarium* which persist endophytically in crop plants has been repeatedly noted (Matta, 1971; Leslie *et al.* 1990; Hallmann & Sikora, 1996). Literature reviewed by Leslie *et al.* (1990) indicates that at least *F. moniliforme* Sheldon can be transmitted through the seed and it usually causes characteristic stalk-rot in corn only when the plants are stressed by drought. The association between water stress and tree diseases caused by xylariaceous fungi has been recognized for many years. Whalley (1996) reviewed evidence that water status in the host plant is of the utmost importance to the development and subsequent presentation of diseases caused by members of the Xylariaceae.

1. 6. Endophytes as biocontrol agents

A number of endophytes have now been shown to function as antagonists to plant pathogens and insect pests. The widespread association between various endophytic fungi and certain plants has been extensively studied and frequently reviewed (Butin, 1986,1992; Carroll, 1988, 1990, 1995; Clay, 1989; Hata & Futai, 1995). Association between members of the Clavicipitaceae fungal tribe Balansiae and certain grasses results in resistance to attack from insect pests. These endophytes are consequently regarded as a useful source for biocontrol of insects in grasses. Unfortunately they also contribute to livestock poisoning (Clay, 1988,1989). Endophytes also colonize a diverse range of woody plants both evergreen and deciduous (Chapela, 1989; Petrini, Stone & Carroll, 1982; Sieber, 1989). In several cases it has been shown that infection by such endophytes can serve to deter feeding or may kill insect larvae within the colonized tissues. Diamandis (1981) reported that infection of first year needles of *Pinus brutia* L. with *Elytroderma torres-juanii* Diamandis and Minter protected them from grazing by the pine processionary caterpillar. Elm bark beetles interact with the endophyte *Phomopsis oblonga* (Desm.)Trav. In elm trees attacked by the beetles, colonization of the inner bark by *P. oblonga* clearly disrupted beetle breeding and led to a decline in beetle populations (Webber, 1981). Later Webber & Gibbs (1984) suggested that the *Phomopsis* could act as a biological control agent of the beetle which acts as a vector for Dutch elm disease. Conidia of a *Meria* Vuill anamorph of the ascomycete *Rhabdocline* Syd. isolated from Douglas fir can colonize galls produced by any of three species of gall-midge (*Contarinia spp.*) on needles. Larval mortality in infected galls is much

higher than in uninfected galls (Stone, 1987; Carroll, 1988). Studies by Taper, Zimmermann & Case (1986) and Taper & Case (1987) showed that a fungus (possibly a species of *Cryptosporiopsis*) causes significant mortality among larvae of a gall-making wasp on *Quercus agrifolia* Nee. This fungus seems to be associated with a negative correlation between tannin content and larval survival. The tannins inhibit gall colonization by the fungus and larval survival is higher on leaves with high tannin content than on those with low tannin content. Observations in the field suggested that certain species of endophytic fungi which occur, but are normally dormant, in leaves of *Quercus robur* L. can be stimulated to pathogenic development by the feeding activity of certain gall insects. As a result of the death of leaf tissue the gall is also killed. This antagonistic relationship between endophytic fungi and gall insects was shown in the following three samples: *Kabatiella apocrypta* (El.&Ev.) Arx vs. *Trioza ramota* Forster; *Gloeosporium quercinum* West. vs. *Neuroterus numismalis* Oliv.; *Dichomera saubinetii* (Mont.) Cooke vs. *Polystepha panteli* Tavares (Butin, 1992). Cerkauskas (1988) reported that fungal endophytes within a crop plant may prove strongly pathogenic to weeds in the same fields, a discovery which has lead to the development of mycoherbicides. Stiles & Glawe (1989) have reported that a number of fungi which parasitize and kill the soybean cyst nematode can also form asymptomatic infection in roots of soybean seedlings in the absence of the nematode.

1. 7. Xylariaceae as endophytes

Since Petrini (1991) provided a modern definition of endophytes numerous reports on endophytic fungi from temperate plants have appeared but only a few have been concerned with tropical hosts (Rodrigues & Samuels, 1990). These investigations have demonstrated an almost ubiquitous presence of xylariaceous species as endophytes (Petrini & Petrini, 1985; Whalley, 1996). Although members of the Xylariaceae are commonly present in temperate plant species they assume a much greater presence in tropical hosts and can become the dominant endophytic species (Rodrigues, 1994; Rodrigues & Samuels, 1990; Petrini, *et al.*, 1995). A total of eight xylariaceous genera have been isolated as endophytes (Table 6) with others such as *Camillea* Fr. expected in the future (Petrini *et al.*, 1995; Whalley, 1996). A major problem when investigating xylariaceous endophytes concerns difficulties in their identification since they often fail to produce

reliable diagnostic characters and rarely form their teleomorph in culture. However studies by Petrini (1992), Petrini & Petrini (1985), Petrini, Petrini and Fisher (1987), Petrini and Rogers (1986) and Petrini *et al.*, (1995) have resulted in the development of keys for identification to generic level for temperate isolates. Tropical isolates are much more difficult to identify because of their large numbers and remarkable diversity (Whalley, 1993,1996).

Table 6. Genera of the Xylariaceae with known endophytic representatives

Genus	Authentication
Anthostromella	Petrini & Petrini, 1985; Petrini *et al.*, 1987.
Biscogniauxia	Petrini & Müller, 1986.
Daldinia	Petrini & Petrini, 1985; Petrini & Müller, 1986.
Hypoxylon	Petrini & Müller, 1986.
Kretzschmaria	Petrini & Petrini, 1985; Petrini & Müller, 1986.
Nemania	Petrini & Petrini, 1985; Petrini and Rogers, 1986.
Rosellinia	Petrini & Petrini, 1985; Petrini, 1992.
Xylaria	Petrini & Petrini, 1985.

It is probably unlikely that identification on the basis of cultural and anamorphic features alone will ever be possible for most tropical isolates. The genus *Xylaria* is especially difficult in the absence of a teleomorph although studies involving a combination of morphological and biochemical data appear to be promising (Brunner & Petrini, 1992; Rodrigues, 1992; Rodrigues *et al.*, 1993). Another approach has been to match secondary metabolite profiles of endophytic isolates with those produced by cultures obtained from known teleomorphic material (Whalley & Edwards, 1995; Mekkamol *et al.*, 1996). In a preliminary study of *X. cubensis* (Mont.)Fr., secondary metabolites such as cubensic acid (Adeboya *et al.*, 1995a) were used to check the identity of isolates of *Xylaria* obtained from leaves of *Euterpe oleracea* and it was possible to confirm that many of the proposed identifications made by Rodrigues (1992) were correct and that *X. cubensis* was the dominant endophytic species in that plant. Current studies on secondary

metabolites of endophytes from teak leaves have enabled *X. cubensis* and other species of *Xylaria* to be recognised (Pittayakhajonwut, pers. comm.). Ideally the development of teleomorphs in cultures of endophytic Xylariaceae is required for identification.

A number of studies have also shown that the methods used to isolate endophytes might also influence the isolation of endophytic Xylariaceae (Chapela & Boddy, 1988a,b,c). In an investigation of endophytes of beech (*Fagus grandifolia* Ehrh.) and poplar (*Populus tremuloides* Michx) it was found that the frequency of isolation of Xylariaceae increased using selective isolation methods. Thus the application of different drying regimes produced isolation frequencies of 32% and 41% in the beech and poplar respectively (Chapela, 1989). It is also interesting to note that *H. fragiforme* (Pers.: Fr.) Kickx and *Entoleuca mammata* (Wahlenburg: Fr) J. D. Rogers & Y.-M. Ju (=*H. mammatum* (Wahlenburg) J. H. Miller were also isolated from beech and poplar respectively and these are their usual host trees on which their teleomorphs develop (Miller, 1961). Chapela, Petrini & Hagmann, (1991) later demonstrated that there is a complex and involved recognition system operating between *H. fragiforme* and beech. Ascospore germination appears to be stimulated by the monolignol glucosides present in the host tissue whereby the ascospores are primed to germinate through a process termed eclosion (Chapela, Petrini & Petrini, 1990; Chapela, Petrini & Bielser, 1993). The monolignol glucoside is seen as being a messenger which enables the spores to germinate rapidly on the preferred host, in this case beech (Chapela *et al,* 1990, 1993). Similar mechanisms might operate in other xylariaceous taxa such as *Daldinia concentrica* (Bolt.: Fr.) Ces. & De Not. on *Fraxinus* (ash) (Gaskell, 1995).

The presence of individual species of Xylariaceae as a dominant part of the endophytic flora of tropical plants has been shown in a number of studies (Rodrigues, 1992; Rodrigues & Samuels, 1990). *Xylaria cubensis* was found to be the second most frequent species isolated from leaves of *Licuala ramsayi* (Muell.) Domin. (Rodrigues & Samuels, 1990) and an unidentified species of *Xylaria* was a frequent isolate from leaves of *Stylosanthes guianensis* Sw. (Pereira, Azevedo & Petrini, 1993). Rodrigues investigated leaf endophytes of the tropical palm, *E. oleraceae* and found *X. cubensis* to be numerically the most important species with isolates of the xylariaceous genera, *Anthostomella* Sacc., *Daldinia* Ces. & De Not., *Hypoxylon* and *Ustulina* also being recovered (Rodrigues, 1994). A total of 11 species of Xylariaceae were named with a further 15 separate xylariaceous taxa recognised but only identified to generic level (Table 7.)

Table 7. Xylariaceous fungi isolated from the leaves of *Euterpe oleracea* (Rodrigues, 1994)

As pointed out by Whalley (1996) it would be unwise to think that all, or even the majority of Xylariaceae live as endophytes. Those species of *Xylaria* which form their teleomorphs on fallen leaves, petioles and fruits might be expected to be endophytic. However, current evidence suggests that many are not and that the leaves and fruits act as baits in the litter layer. For example *X. carpophila* (Pers.)Fr. only occurs on fallen beech cupules (Rogers, 1979) although it is widely distributed in the British Isles and Europe (Watling & Whalley; 1977; Dennis, 1981) and elsewhere (Rogers, 1979). Incubation of beech cupules collected directly from the tree or captured in suspended litter nets always failed to develop stromata although cupules collected from the litter layer frequently developed stromata of *Xylaria* after several weeks incubation in a damp chamber (Whalley, 1996). Similarly, Laessøe & Lodge (1994) were unable to demonstrate the ability of *X. axifera* Mont., a species specifically associated with fallen Araliaceae petioles, to invade attached petioles. They failed to isolate *X. axifera* from either diseased or healthy senescent petioles collected shortly before, or after, abscission or from healthy non-senescent petioles obtained from the canopy. They concluded that it is probable that the

petioles are colonized by *Xylaria* after they have fallen to the ground (Laessøe & Lodge, 1994). In the same study they suggested that *X. guareae* Laessøe & Lodge, a species host specific to *Guarea guidonia* (L.) Sleumer, might be endophytic. Clearly there is a need to investigate other leaf, petiole or fruit inhabiting species of *Xylaria.*

Nevertheless the Xylariaceae occur as endophytes in many plants on a worldwide basis but they appear to show a greater presence in the tropics (Rodrigues, 1994; Rodrigues & Samuels, 1990; Petrini *et al.*, 1995). It is still not known why or how they come to occur in the tissue of such a wide range of different host plants or why they occur in the tissue of hosts in which they are not known to produce their teleomorph (Whalley, 1996). Rodrigues *et al.,* (1993) used somatic incompatibility testing and gel electrophoresis of isoenzymes of thirty strains of *X. cubensis* which had been isolated from leaves of the Brazilian rainforest palm *E. oleracea* and found a high degree of genetic diversity. They concluded that this diversity demonstrated that the endophytic isolates of *X. cubensis* had been established by ascospores (Rodrigues *et al.,* 1993). Gowan & Vilgalys (1991) used ribosomal DNA length polymorphisms to study populations of *X. magnoliae* J.D. Rogers and concluded that the populations are mostly highly clonal and that it seemed likely that conidia or ascospores serve for long term dispersal. This they concluded would account for the high level of variability found within narrow populations and also the same phenotype occurring in distant localities. *Xylaria magnoliae* is, however, a fruit-inhabiting species and does not appear to be an endophyte. The suggestion that the conidia may provide an infection inoculum in this and in *X. cubensis*, which is a common endophyte, is of considerable interest. Greenhalgh & Roe (1984) had shown that conidia of a wide range of xylariaceous taxa, including *Xylaria* species, contained different types of nuclei and concluded that "it seems likely that the conidia of these fungi possess the ability to germinate but that certain conditions, as yet unknown, are required" (Greenhalgh & Roe, 1984). It is possible that an intricate and specific host fungus recognition system occurs in conidia in a similar way to the eclosion phenomenon observed by Chapela *et al.* (1990, 1991,1993) for ascospores of *Hypoxylon fragiforme.*

1. 8. Methodology

Many early studies on leaf and stem endophytes were based largely on cytological examinations (Stone, 1987; Suske & Acker, 1987). Microscopic observations of cleared, stained samples have been used to detect the presence of fungal endophytes. Microscopic estimation of infection frequencies of *Rhabdocline parkeri* on Douglas-fir needles (*Pseudotsuga menziesii* (Mirbel) Franco) was performed by a clearing and staining process consisting of clearing with KOH solution, bleaching in alkaline H_2O_2, staining with trypan blue in lactophenol, followed by dehydration with xylene-ethanol (Stone, 1987). Detection of endophytes, however, under the microscope can be a laborious and difficult task, particularly when they occur intracellulary in recalcitrant tissues such as leaf epidermis or wood. When tissue is colonized by two or more endophytes simultaneously, visual detection of the separate species may be uncertain unless characteristic patterns of colonization have been shown for each endophyte species individually (Petrini, 1986).

Endophytes cause symptomless infections, and therefore their presence within host tissues cannot be determined simply by casual inspection. Censuses of endophytic fungi have typically relied on rigorous surface sterilisation of samples of host tissue followed by incubation of the sterile samples on agar medium, with scoring and pure culturing of the fungi which grow out (Dreyfuss & Petrini, 1984; Fisher *et al.*, 1994; Johnson & Whitney, 1992; Petrini, 1984; Rodrigues, 1994). Surface-sterilization techniques which have been widely used for isolation of endophytic fungi involve a sequence of alcohol and sodium hypochlorite : they are first immersed in 70-96% alcohol for 1 min then soaked in sodium hypochlorite (3-6 % available chlorine) for 2-10 min with a second immersion in 70-96% alcohol for 30s-1 min then washed with sterile distilled water (Dreyfuss & Petrini, 1984; Petrini, 1986; Fisher & Petrini, 1987; Bertoni & Cabral, 1988; Johnson & Whitney, 1989a; Rodrigues & Samuels, 1990; Sieber *et al.* 1991; Fisher *et al.* 1993; Bayman *et al.*, 1997). In a few studies, mercuric chloride ($HgCl_2$) or formaldehyde 37-40% were used for isolation of endophytes (Schulz *et al.*, 1993). Advantages and disadvantages of the isolation and culturing techniques have been discussed by Petrini, (1986). Careful investigations of the biology of particular host-endophyte systems require both culture work and cytological examination. The great majority of endophytes, however, are known only from culture work

(Petrini, 1986). For broad censuses of the endophyte mycoflora, pure culture work will continue to be the method of choice (Carroll, 1990; Petrini, 1986). Modern techniques have been used to understand endophyte-host interactions. Scanning and Transmission electronmicroscopes have been used to observe internal hyphae of endophytes in symptomless needles which are then correlated with cultural investigations (Bernstein & Carroll, 1977; Sherwood-Pike *et al.*, 1986; Suske & Acker, 1987; Johnson & Whitney, 1989 b).

1. 9. Objectives of the project

In this study endophytic fungi were isolated from leaves of *Tectona grandis* L. (teak) to investigate how endophyte assemblages differ between natural forest teak trees and plantation trees. Teak was chosen to extend knowledge on fungal endophytes of tropical plants and also because teak is economically important in Southeast Asia. Certain taxonomic problems which emerged from the endophyte isolations are discussed and in particular novel methods for the identification of Xylariaceous endophytes are presented. Since members of the Xylariaceae proved to be frequent isolates in this study, and in other studies of tropical plants, the inability to identify many of them has been a major problem in assessing endophyte diversity. Comparisons of endophyte assemblages on young and mature leaves, between areas of isolation from leaves i. e. leaf lamina, vein, midrib and between geographical locations were made. These biological and ecological aspects can be used to develop sampling strategies to maximise efficiency of recovery of fungal endophytes from plants. This is of importance for further studies on fungal biodiversity, biology and ecology and for the provision of suitable cultures for screening by the pharmaceutical industry.

Chapter 2

Materials and Methods

2. 1. Locations

Leaves of teak were collected from two different geographical sites for the isolation of endophyte assemblages. One site was a natural stand as part of a mixed deciduous forest. It is located in Mae Rim District, Chiang Mai, Thailand, at 900 m. above sea level. The other site was a teak plantation in Chiang Dao District, Chiang Mai, at 650 m. above sea level. It consists of an approximate area of teak covering two hectares and surrounded by a mixed deciduous forest. Sampled trees were selected from the middle of the plantation. The two sampling sites were about 80 kilometres distance from each other (Fig. 1.)

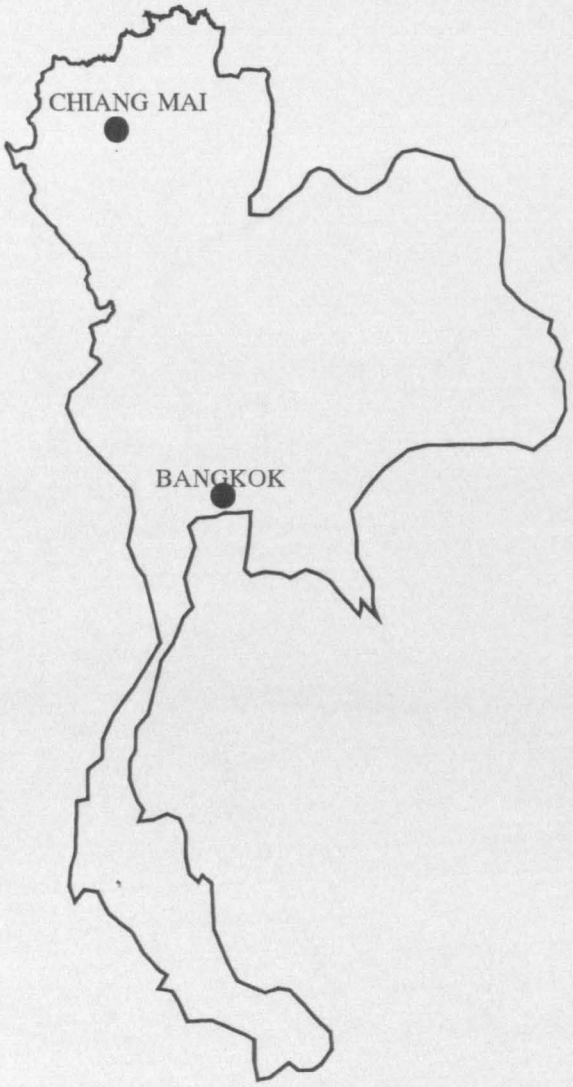
2. 2. Sampling and subsampling

The leaves were collected twice a year from each location, with both young and mature leaves being taken, except in the year 1995 when only mature leaves were collected. Young leaves were collected in May while mature leaves were collected in November. A total of five leaves were obtained from five trees at each sampling site. All leaves were processed within one week and during that time the leaves were preserved in a refrigerator at 5°C (except in 1995 when leaves were dried one week before isolation). Sampling units on each leaf were from 10 areas : 2 in midrib, 2 in veins, and another 6 in lamina (Fig 2.). Each area measured 1x1 cm^2 and was then divided into 4 equal pieces. There were therefore, 40 sampling units on each leaf measuring 5 x 5 mm^2.

2. 3. Media

Malt extract agar (MA, Merck) was selected for the endophyte isolation as being a medium proven to isolate a diverse taxonomic population (Petrini, 1986). Potato dextrose agar (PDA, Difco), oat meal agar (OA, Difco) and MA were selected for morphology observations

Figure 1. Map of sampling sites

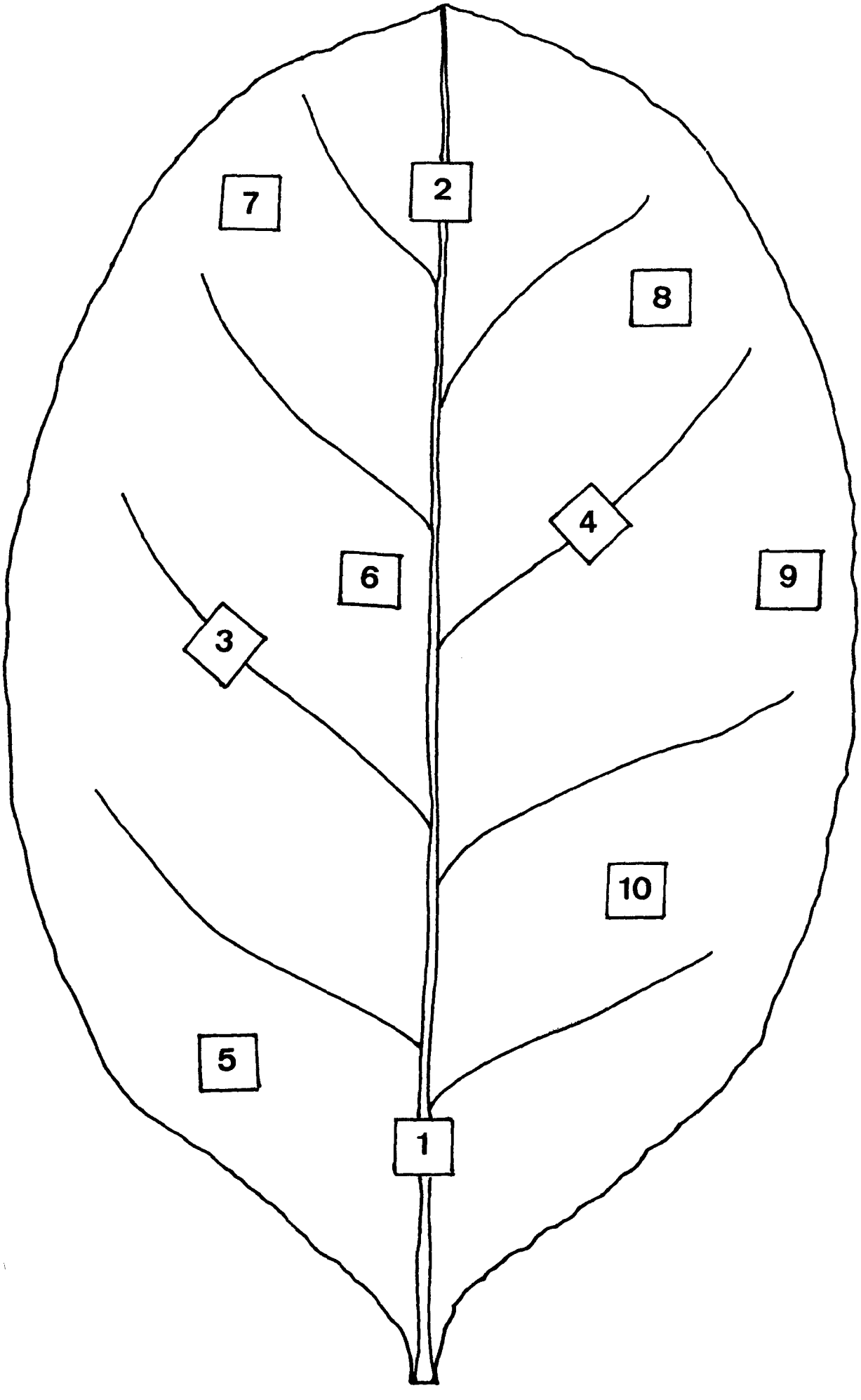


MAP OF THAILAND

SAMPLING SITES IN CHIANG MAI

Figure 2. Sampling units on leaf

Area 1, 2	Midrib
Area 3, 4	Veins
Area 5, 6,7, 8, 9, 10	Leaf lamina


7
2
8
4
6
9
3
10
5
1

and PDA was used to produce the inoculum for teleomorph induction (Booth, 1971). For isolation of endophytic fungi, the antibiotics, streptomycin sulphate and oxytetracycline (Sigma) were added at a final concentration of 50 μg/ml to prevent bacterial contamination.

2. 4. Isolation and culture methods

Surface sterilisation techniques followed those of Petrini (1986). The leaf pieces first had the hairs on the lower surface of epidermis removed by a razor blade before being submerged in 96% ethanol for 1 minute. They were then transferred to sodium hypochlorite (5% available chlorine) solution containing 1 drop per 15 ml of Tween 80 for 5 minutes and then transferred to 96% ethanol for 30 seconds. They were finally washed in sterile distilled water and the sterilised leaf pieces were then surfaced dried with sterile paper and immediately placed on the agar medium. Four pieces from the same sampling area were placed in a 9.0 cm Petri dish containing 2% malt extract agar with streptomycin and oxytetracycline. All the inoculated plates were incubated at room temperature (25-30°C) for at least 1 month with daily examination for fungal growth. Developing fungal colonies were transferred to fresh PDA medium plate and, if pure, to a fresh agar slant. The fungi were induced to sporulate under near UV light and were identified to genus or species by the characteristics of their spores and /or structures. Impression plates of surface sterilised leaf pieces were made to test the success of the surface sterilisation protocol (Schulz *et al.*, 1993).

2. 5. Identification and nomenclature of organisms

2. 5. 1. Microscopical features

The microscopical analyses were based on observations by bright field and Differential Interference Contrast (DIC) light microscopy on an Olympus BH2 research microscope using a x40 and x60 dry objective and by scanning electron microscopy (SEM) using a Jeol SEM 840 with cryo techniques for the observation of anamorphs. Specimens for light microscopy were mounted in water, lactophenol or acid fucshin for observation of spore and other characteristics, and then identified. Nomenclature of the fungi follows Hawksworth *et al.*, (1995). Xylariaceous anamorphs were separated from others for further study by induction of teleomorphs (see 2.6.).

Once teleomorphs were obtained using the special techniques described below, identification to species of Xylariaceae was undertaken. Ascospores in 10% aqueous KOH were observed for dehiscence or indehiscence of the perispore (Ju & Rogers, 1996). Measurements of ascospores from squash mounts in distilled water or Melzer's iodine reagent were made, Ascospore dimensions were based on 20 fully mature spores, or 50 where new taxa were proposed. The ascus apical apparatus was examined for its amyloid or dextrinoid reaction following mounting in Melzer's iodine reagent and shape and size were determined. Photographic records were made using Agfa ASA 25 fine grain monochrome or Kodak Ectachrome film.

2. 5. 2. Macroscopical features

Characters such as shape, size, colour, type of stromal surface, shape and size of perithecia, type and size of ostioles were studied using an Olympus stereomicroscope (SZ 60) fitted with the Olympus automatic camera (SC 35). Stromal surface details were based on examination by SEM using a Jeol-SEM 840 following coating in gold applied by a Polaron splutter coater. Colour was determined by comparison with the Rayner Mycological Colour Chart (Rayner, 1970) and extracts of colour from *Hypoxylon* and *Daldinia* were made using 10% potassium hydroxide (KOH-w/v) and then compared after 1 minute with the colour chart (Ju & Rogers, 1996).

2. 5. 3. Identification of deuteromycetous fungi

Isolates were cultured on 9.0 cm MA plates. Observations were based on morphological appearance of colony, morphology and size of conidia and fruiting structures. Measurements of conidia were made from mounts in water or lactophenol. Conidial dimensions were based on 20 conidia, or more for those with varied morphology.

2. 6. Technique for induction of teleomorph

In a study of some European Xylariaceae in culture, Petrini & Petrini (1985), provided a key for identification by a careful comparison of suspected endophytic xylariaceous colonies with single ascospore isolates from known freshly collected xylariaceous teleomorphs. The

present study raises the possibility of inducing teleomorphs from pure cultures of anamorphs by inoculating fungi onto wood which is then incubated to produce teleomorphs. Some cultures isolated in the year 1995-96 had been previously identified as belonging to the genus *Xylaria* on the basis of their upright stromata which were sometimes covered with their anamorphs. Although characteristic *Xylaria*-type stromata often developed in culture however they never developed to maturity. Some isolates only produced anamorphic structures. In the absence of a mature teleomorph, it is rarely possible to identify these isolates to species and therefore a special technique was developed to induce teleomorph formation.

1. The cultures with young stromata and anamorphs were separated from the others using cultural morphology and characteristics of anamorphs following Callan & Rogers (1986, 1990), Rogers (1985), Rogers & Callan (1987), Thienhirun (1997).

2. The separated isolates were inoculated on to PDA in bottles and incubated for 10 days at room temperature (25-28°C).

3. Twigs * of about 3 cm diameter were freshly cut into 8 cm long pieces. They were put in autoclavable bags and then autoclaved (15 lb/inch ^{2}) for 1 hour. One piece of sterilised twig was put vertically on the surface of each culture in the bottle and the cultures were kept at room temperature for 1 month (Fig. 3a.).

4. The twigs that were well colonised by the fungi were transferred from the bottles to sterile bags with moist sand to a depth of 4 cm at the bottom (Fig. 3b.). The bags were tied with a rubber band and kept at a temperature of 28-30°C for one to two months. Stromata production was observed at this stage.

5. The inoculated twigs which failed to develop stromata in some of the bottles or produced immature stromata after 2 months were transferred to a natural forest site to continue their development.

6. If the twigs failed to develop any stromata or were still immature after being in the forest for some time, the twigs were transferred back to laboratory and kept in a moist condition.

7. The infected twigs lacking any noticeable stromata and which had been recovered from the forest were transferred to moist sand baskets covered with moist paper and incubated for 1 to 2 months at ambient temperature. Once the stromata were produced, they were transferred to a moist sand pot covered with a polyethylene sheet to conserve moisture (Fig. 3d)

while the others without stromata were incubated further without being covered and water was applied once or twice a week as necessary to maintain suitable moisture levels.

8. Alternatively, teleomorph induction was performed under shade in moist conditions (Fig. 3c.) The twigs with young stromata (in 2.6.4) were transferred to moist sand pots and watered daily. The infected twigs without any noticeable stromata were incubated further in polyethylene bags for one more month before the bags were cut open horizontally (Fig. 3e.) at which stage they were incubated further with water application once or twice a week as necessary. (summarised in Fig. 4.)

* twigs used in the experiment were from five different trees species.

1. Mango (*Mangifera indica* L.)
2. Litchi (*Litchi chinensis* Sonn.)
3. Teak (*Tectona grandis* L.)
4. Longan (*Dimocarpus longan* Lour.)
5. East-Indian Walnut [*Samanea saman* (Jaeq.) Merr.]

2. 7. Surveys of Xylariaceae in sampling sites and the surrounding forest

Surveys were carried out in August to November 1997. All specimens of Xylariaceae were collected in the forest and plantation where teak leaves had been collected for isolation of endophytic fungi. Surveys were also performed in the forest near to the sampling sites. All the fungi collected were identified to genus and, in most cases, to species.

2. 8. Isolation of cultures and production of anamorphs

Teleomorphs derived from the wood inoculation techniques were used to isolate cultures in order to make cultural comparison with the initial ones that had been isolated from teak leaves. Cultures were also obtained from air-dried specimens or fresh collections of known species from the forest and these were also compared with endophyte isolates. The methods used followed those of Kendrick *et al.* (1979) and Callan & Rogers (1990). In general, multispore isolations were made. Colonies were incubated on 9.0 cm. Petri dishes at around 25-

Figure 3. **Special technique was developed for induction teleomorphs of Xylariaceae isolates**

a A sterilised twig was put vertically on surface of PDA in a bottle

b A well colonised twig was transferred to moist sand bag

c The shade which was provided for the conditions appropriated for xylariceous isolates

d Twigs with young stromata were transferred to moist sand pots

e The bags were cut open horizontally for incubation of *Hypoxylon* and *Daldinia*


a
b
c
d
e

30°C on the bench with alternating light and darkness. The cultures obtained are stored by Biotec (Bangkok) under liquid nitrogen.

2. 9. Secondary metabolite comparisons

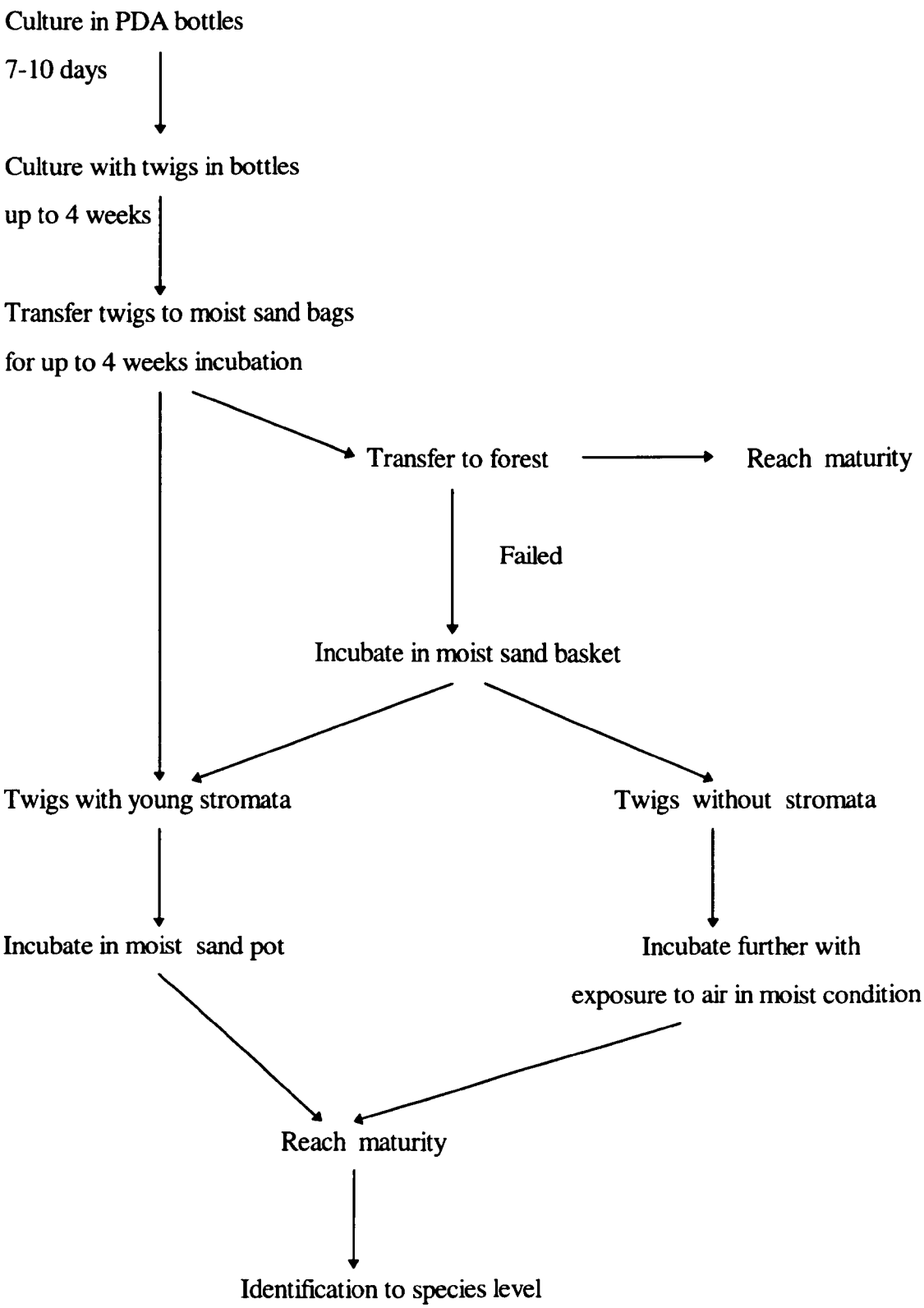
In attempting to identify xylariaceous endophytic isolates, secondary metabolites profiles have been used. The Xylariaceae are prolific producers of secondary metabolites which can be assigned to chemical classes such as dihydroisocoumarins, butyrolactones, punctaporanins, cytochalasins, succinic acid derivatives and other compounds (Whalley & Edwards, 1995; Whalley, 1996). It has been possible to use presence or absence of particular compounds in identification. Miss Pattama Pittayakhajonwut (University of Bradford) has investigated metabolite profiles from known species of *Xylaria* and from teak endophytes. In a number of cases correlation of metabolite profiles has enabled identification to be made. All cultures were grown in 3% malt extract medium sometimes amended with 10% glucose. Incubation at 25°C was for 6-8 weeks under static conditions. Following extraction of metabolites using continuous extraction with ethyl acetate as previously described (Edwards, Maitland & Whalley, 1991). Individual compounds were separated by thin layer chromatography and visualised by spraying with diazotised p-nitroaniline or anisaldehyde (Edwards *et al.*, 1991)

2. 10. Statistical analysis

Incubated leaf pieces were examined daily for fungal colonisation during the first 2 weeks and once a week for 2 weeks thereafter. Single and multiple colonisation were scored for each individual leaf piece and for each leaf. Percentage frequency of fungal colonisation by each taxon recovered in each leaf was computed by using the number of each taxon found divided by total number of isolates from a given leaf and multiplied by 100. Percentage of isolation was computed by dividing the total number of colonised pieces by the total number of leaf pieces incubated then multiplied by 100. Statistical analysis was performed with Statistix 3.5 for Windows. Krukal-Wallis one-way nonparametric AOV was used to detect differences of colonisation frequencies and number of fungal species recovered on leaves in a given year from each site (Johnson & Whitney, 1989 a,b; Anderson & Sclove, 1986). The Wilcoxon signed rank test was used to compare paired results such as endophytes of midrib and leaf lamina, veins and

leaf lamina, midrib and vein. To measure the diversity of endophytes from two sites, the Sorenson index was used. $Cs = 2j / (a+b)$, where j = the number of species in both sites and a = the number of species in site A with b the number of species in site B. The index is designed to equal 1 in cases of complete similarity (that is where the two sets of species are identical) and 0 if the sites are dissimilar and have no species in common (Wu, 1997).

Figure 4. Procedure for induction of teleomorphs of xylariaceous fungi.



Chapter 3

Diversity of leaf endophytic fungi of teak

3.1 Introduction

Mycofloristic surveys of a range of plant hosts which have appeared during the past 20 years have demonstrated that endophytic fungi colonise algae, ferns, lichens, mosses and flowering plants including herbaceous annuals, deciduous and evergreen perennials and that their presence is ubiquitous (Boullard, 1951,1957,1979; Schuster, 1966; Carroll, 1986; Petrini, 1996; Petrini *et al.*, 1992, Fisher *et al.*, 1994,1995; Schmidt, 1994; Pelaez *et al.*, 1998). In many plants the diversity of fungal taxa is considerable (Petrini, 1996; Bills & Polishook, 1992). Moreover the endophyte assemblages vary between plants in both their frequency of recovery and number of fungal species. It is generally considered that this may be the outcome of biochemical, cytological and morphological differences between plant species, and the environmental conditions in which the plants grow although little appears to be known about these factors (Clay, 1986,1988; Carroll, 1986; Petrini, 1986,1991; Fisher & Petrini, 1988; Fisher *et al.*, 1995). It is also generally agreed that for any single plant the distribution pattern of endophytic fungi within different tissues or organs is not homogeneous and is therefore not regarded as tissue- or organ-specific (Carroll *et al.*, 1977; Fisher & Petrini, 1988; Petrini, 1991). Most studies also show that endophyte diversity is related to other factors such as geographical location of the sampling site (Fisher & Petrini, 1990; Fisher *et al.*, 1993; Fisher *et al.*, 1994) and to environmental influences including temperature, rainfall, or even pollution (Petrini, 1991; Helander *et al.*, 1993 a,b; Wilson & Carroll, 1994). Geographical variation is the only one which has been well investigated and it has been shown that for the same plant species the endophytic assemblages can differ between different localities, especially those which are far apart. These fungi are therefore regarded as site-specific endophytes (Fisher & Petrini, 1990; Fisher *et al.*, 1994).

The majority of endophytic studies have been undertaken in temperate areas and there have been very few studies dealing with endophytic fungi from tropical areas where a

much higher diversity of fungi is expected (Petrini & Dreyfuss, 1981; Dreyfuss & Petrini, 1984; Rodrigues & Samuels, 1990, 1992; Bettucci & Saravay, 1993; Pereira *et al.*, 1993; Fisher *et al.*, 1994, 1995; Rodrigues, 1994; Hyde & Hawksworth, 1997). Some isolations have demonstrated spatial variation of diversity of endophytic fungi from certain plants during one year (Widler & Müller, 1984; Helander *et al.*, 1994) although most of the endophytic fungi do not appear to be subject to seasonal changes (Widler & Müller, 1984).

The combination of biological and ecological studies on diversity of endophytes from different plants, host and/or tissue specificity, geographical distribution and site preference/specificity are important contributions to endophyte knowledge. The information can also be used to develop sampling strategies to increase recovery of endophytic fungi for commercial activities as well as to progress knowledge about endophytes themselves. Specific questions to be addressed here are: 1), how endophyte assemblages differ between leaves of teak trees from different localities; 2), how endophyte assemblages of teak leaves vary from season to season; 3), how endophyte assemblages differ between young and mature leaves; 4), how endophytic fungi relate to tissue site within leaves, and 5), host specificity, if any, of the endophytic fungi.

3.2. Results

3.2.1. Endophytes of mature leaves from natural forest trees.

Endophytes from mature teak leaves were isolated from leaves collected at Mae Rim in November 1995, 1996 and 1997. The total range of species identified together with their isolation frequencies for each year are presented in Tables 8, 9 and 10. The total number of taxa and number of isolates recorded obtained from different parts of the leaf, i.e lamina, midrib and vein for each leaf examined are shown in Figs. 5. and 6. A total of 15 genera and 41 species of fungi were identified but there were 38 cultures which remained sterile although these could be grouped into 4 species based on cultural features and this raised the total of isolated fungi to 45 species. Representatives of the Xylariaceae, species of *Colletotrichum* and *Phomopsis* were the most frequently isolated fungi from Mae Rim obtained from the annual sampling of mature leaves over the 3 year period (Table 11 and Fig. 7.). There were, however, differences in the species isolated and the frequency of their isolation between the years.

Table 8. Endophytic fungi isolated from mature leaves at Mae Rim forest (1995).

Taxa	Leaves					Number of isolates	Percentage frequency
	1	2	3	4	5		
1. Cladosporium sp.	0	0	0	2	1	3	1.30
2 .Colletotrichum sp.	6	2	0	1	0	9	3.90
3. Curvularia sp.	0	0	0	0	2	2	0.87
4. Daldinia eschscholzii	8	7	13	8	8	44	19.05
5. Diplodia sp.	1	0	0	0	0	1	0.43
6. Fusarium sp.	3	1	0	0	1	5	2.16
7. Humicola sp.	1	1	0	0	5	7	3.03
8. Hypoxylon cf. subrutilum	1	5	2	2	3	13	5.63
9. H. subrutilum	6	6	6	2	0	20	8.66
10. Hypoxylon sp. 188	0	4	0	0	0	4	1.73
11. H. haematostroma	9	2	3	4	3	21	9.09
12. Nemania subannulata	0	3	7	4	0	14	6.06
13. Nigrospora sp.	1	0	0	1	1	3	1.30
14. Phoma sp.	3	0	0	0	0	3	1.30
15. Phomopsis sp.1	1	2	1	0	6	10	4.33
16. Phomopsis sp.2	1	2	2	1	8	14	6.06
17. Phomopsis sp.3	1	9	3	4	0	17	7.36
18. Phomopsis sp.4	0	0	0	0	2	2	0.87
19. Septonema sp.	0	0	2	2	0	4	1.73
20. Xylaria sp. 271,342	0	0	2	0	0	2	0.87
21. Xylaria sp. 347	0	0	0	0	1	1	0.43
22. Xylaria sp.564	0	0	0	0	1	1	0.43
23. X. grammica	0	0	3	0	0	3	1.30
24. X. cubensis	2	0	0	0	0	2	0.87
25. X. feejeensis	1	0	0	0	0	1	0.43
26. X. juruensis var. microspora	0	3	2	12	0	17	7.36
27. Trichoderma sp.	0	0	0	0	1	1	0.43
28. Sterile sp.1	3	0	0	0	2	5	2.16
29. Sterile sp.3	0	1	0	0	0	1	0.43
30. Sterile sp.6	0	0	0	1	0	1	0.43
Number of isolates	**48**	**48**	**46**	**44**	**45**	**231**	
Number of species	**16**	**14**	**13**	**13**	**15**		

Table 9. Endophytic fungi isolated from mature leaves at Mae Rim forest (1996).

Taxa	Leaves					Number of isolates	Percentage frequency
	1	2	3	4	5		
1. Cladosporium sp.	0	1	0	0	0	1	0.35
2. Colletotrichum sp.	7	0	6	4	1	18	6.25
3. Daldinia eschscholzii	10	20	10	8	7	55	19.10
4. Humicola sp.	2	1	0	0	1	4	1.39
5. Hypoxylon cf. anthochroum	0	0	0	1	2	3	1.04
6. H. cf. subrutilum	3	4	1	2	4	14	4.86
7. H. subrutilum	0	4	3	3	4	14	4.86
8. Hypoxylon sp.188	1	0	2	0	2	5	1.74
9. H. haematostroma	6	1	2	2	5	16	5.56
10. Nemania subannulata	1	5	2	2	4	14	4.86
11. Penzigia sp.	1	0	0	0	0	1	0.35
12. Phomopsis sp.1	6	7	2	3	3	21	7.29
13. Phomopsis sp.2	2	8	6	8	0	24	8.33
14. Phomopsis sp.3	0	5	10	9	1	25	8.68
15. Phomopsis sp.4	3	0	0	4	0	7	2.43
16. Septonema sp.	1	0	1	0	0	2	0.69
17. Xylaria sp.5	0	1	0	0	0	1	0.35
18. Xylaria sp.7	0	1	0	0	0	1	0.35
19. Xylaria sp.9	0	0	0	1	0	1	0.35
20. Xylaria sp.15	0	1	0	0	0	1	0.35
21. Xylaria sp.24	0	1	0	0	0	1	0.35
22. Xylaria sp.40	0	1	0	0	0	1	0.35
23. Xylaria sp.64	0	0	0	0	1	1	0.35
24. Xylaria sp.69	1	0	0	0	2	3	1.04
25. Xylaria sp.73	0	0	0	0	1	1	0.35
26. Xylaria sp.76	0	0	0	0	1	1	0.35
27. X. grammica	0	0	2	0	0	2	0.69
28. X. aristata	0	1	2	2	2	7	2.43
29. X. cf. allantoidea	1	0	0	0	0	1	0.35
30. X. cubensis	6	7	3	4	0	20	6.94
31. X. juruensis var microspora	2	5	1	1	2	11	3.82
32. X. feejeensis	0	1	0	1	2	4	1.39
33. Sterile sp.1	0	0	1	0	1	2	0.69
34. Sterile sp.6	2	0	0	0	3	5	1.74
Number of isolates	**55**	**75**	**54**	**55**	**49**	**288**	
Number of species	**17**	**19**	**16**	**16**	**19**		

Table 10. Endophytic fungi isolated from mature leaves at Mae Rim forest (1997).

Taxa	Leaves					Number of isolates	Percentage frequency
	1	2	3	4	5		
1. Cladosporium sp.	0	1	0	0	0	1	0.32
2. Colletotrichum sp.	8	8	6	9	12	43	13.69
2. Daldinia eschscholzii	7	11	2	7	6	33	10.51
4. Diplodia sp.	1	2	0	0	3	6	1.91
5. Humicola sp.	0	1	1	1	0	3	0.96
6. Hypoxylon cf. subrutilum	0	4	3	3	1	11	3.50
7. H. subrutilum	4	3	2	4	4	17	5.41
8. Hypoxylon sp.188	0	0	2	2	0	4	1.27
9. H. cf. anthochroum	0	2	0	0	0	2	0.64
10. H. haematostroma	1	2	1	3	0	7	2.23
11. Nemania subannulata	0	7	3	5	6	21	6.69
12. Nigrospora sp.	1	0	0	0	0	1	0.32
13. Phomopsis sp.1	2	4	1	2	4	13	4.14
14. Phomopsis sp.2	5	0	0	0	6	11	3.50
15. Phomopsis sp.3	3	0	0	9	4	16	5.10
16. Phomopsis sp.4	4	0	0	3	1	8	2.55
17. Pycnidia	8	0	3	1	2	14	4.46
18. Septonema sp.	1	0	0	2	0	3	0.96
19. Xylaria sp.36	2	0	4	0	1	7	2.23
20. Xylaria sp.69	0	2	4	1	2	9	2.87
21. X. grammica	0	0	4	0	2	6	1.91
22. Xylaria sp.294	0	2	0	0	0	2	0.64
23. X. aristata	6	4	7	5	3	25	7.96
24. X. cubensis	4	3	4	2	4	17	5.41
25. X. juruensis var. microspora	1	2	4	2	1	10	3.18
26. Sterile sp.1	2	1	1	3	2	9	2.87
27. Sterile sp.2	0	1	0	0	0	1	0.32
28. Sterile sp. 3	1	1	3	0	0	5	1.59
29. Sterile sp. 6	2	2	5	0	0	9	2.87
Number of isolates	**63**	**63**	**60**	**64**	**64**	**314**	
Total of species	**19**	**20**	**19**	**19**	**18**		

Figure 5. Maximum taxa found on each area of mature leaves at Mae Rim (1995-1997)

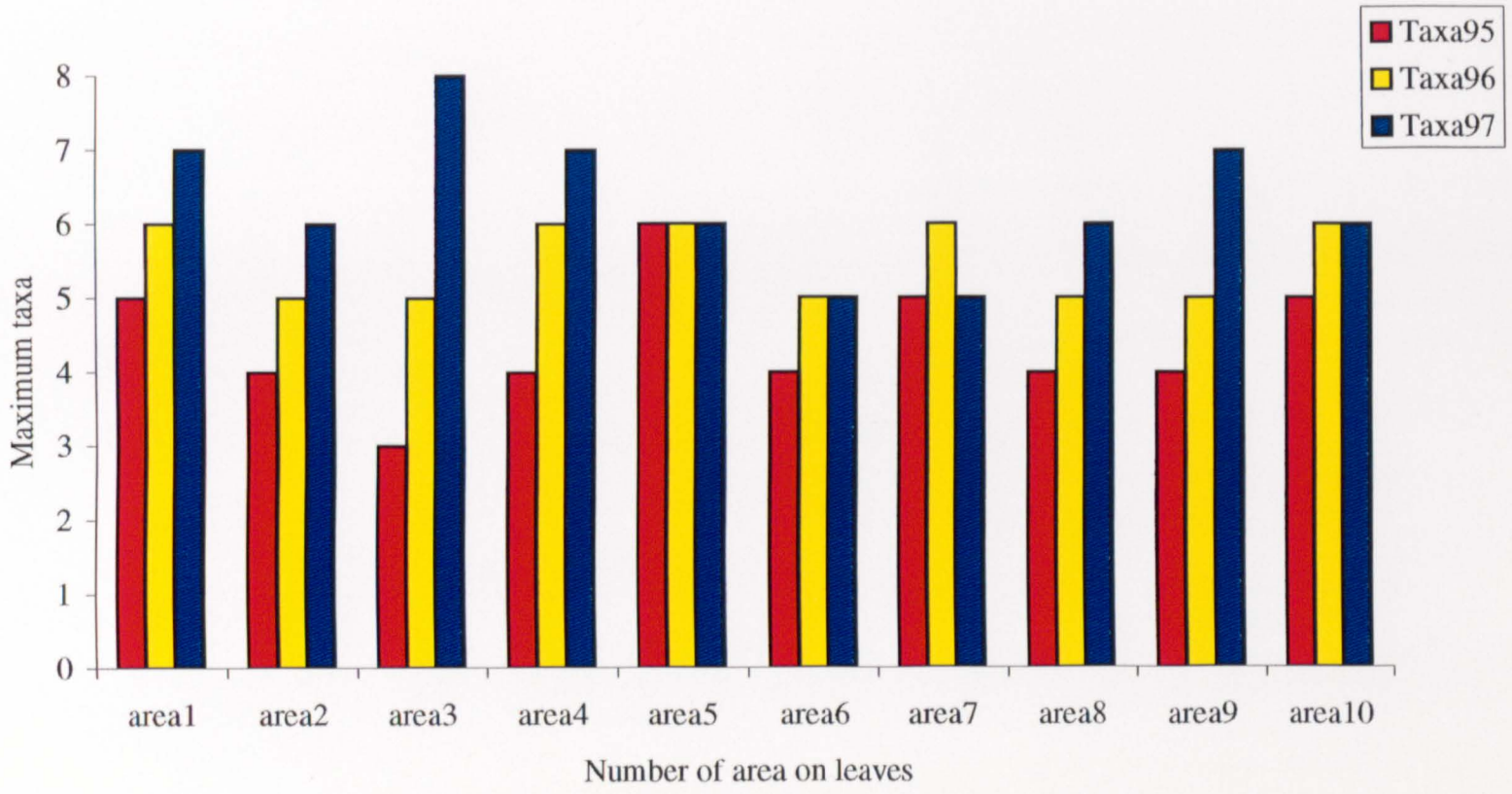
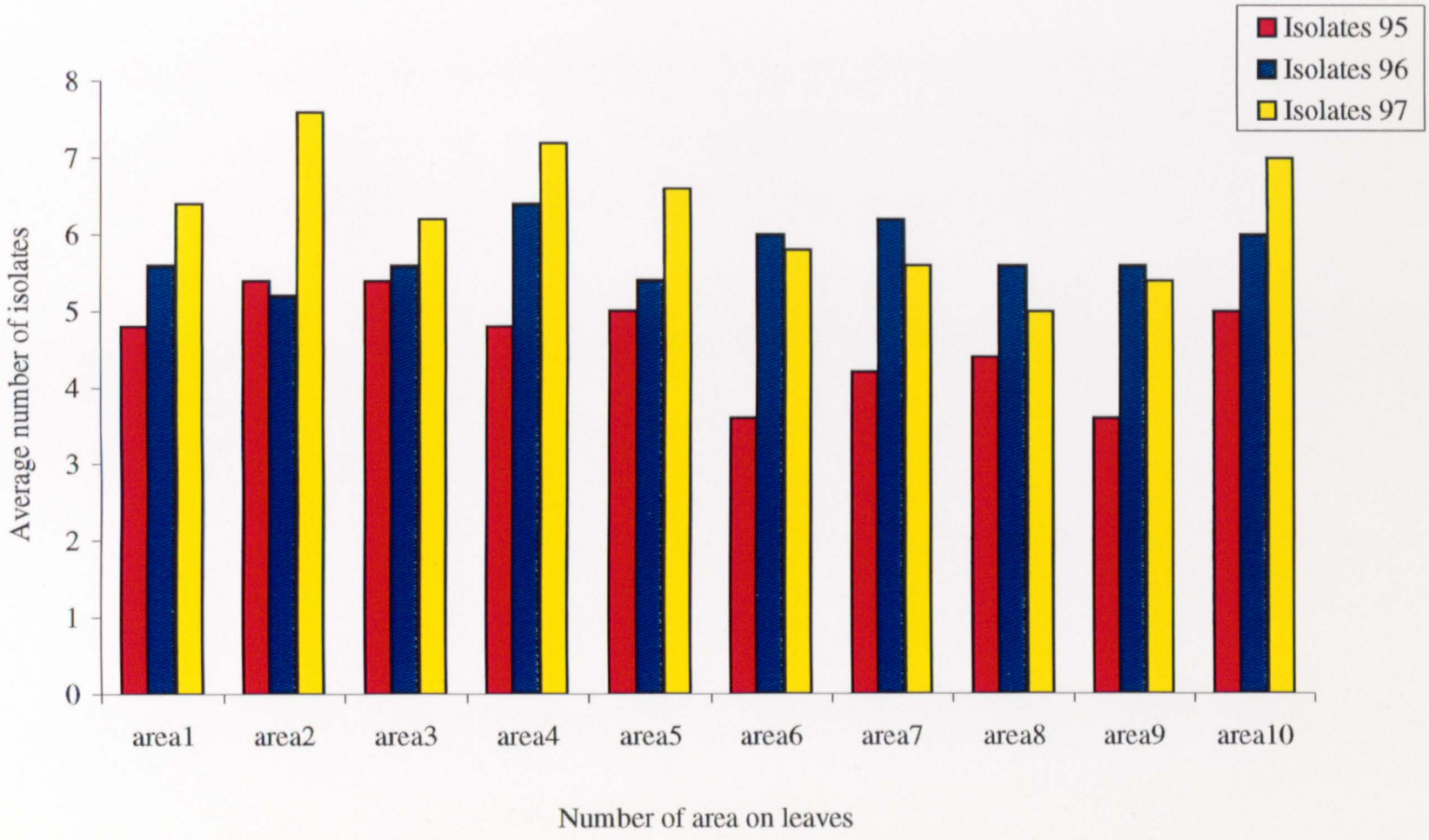


Figure 6. Number of isolates found on each area of mature leaves at Mae Rim (1995-1997)



In 1995 a total of 30 fungal species were recognised with members of the Xylariaceae exhibiting a dominant presence with 61.9% of the total isolates (Table 8. and Fig. 8.). *Daldinia eschscholzii* with 44 isolates or 19.05% was by far the most frequently recovered taxon and accounted for 30.77% of all the xylariaceous taxa recovered (Table 12.). This is perhaps not unexpected since *D. eschscholzii* is a common species throughout Thailand (Thienhirun, 1997) and has been found in the vicinity of the sampling site at Mae Rim. *Daldinia* is also one of the genera of the Xylariaceae known to occur regularly as endophytes (Petrini & Petrini, 1985). *Daldinia eschscholzii*, in common with most species of *Daldinia,* is a prolific producer of ascospores (Ju, Rogers & San Martin, 1997) and it is the ascospores which are likely to provide the inoculum for the endophytic isolates (Whalley, 1996). In Thailand *D. eschscholzii* is widespread and is commonly associated with log piles or fallen trunks in more open parts of the forest (Thienhirun, 1997). *Hypoxylon haematostroma* (9.09%), a species of *Hypoxylon subrutilum* (8.66%), *N. subannulata* (6.06%), *X. juruensis var. microspora* (7.36%) and *Phomopsis sp.3* (7.36%) and *Phomopsis sp.2* (6.06%) were the other most frequently recorded species (Table 8.). It is interesting to note that in 1995 *X. cubensis*, a species frequently isolated as an endophyte from tropical palms (Rodrigues, 1994), was only isolated on 2 occasions and then both isolates were obtained from a single leaf.

Whilst some of the Xylariaceae show consistency in isolation from different leaves others are much less evenly distributed. *Nemania subannulata* at 9.79% of all Xylariaceae isolated was not recovered from 2 of the 5 leaves sampled and *X. juruensis var. microspora* (11.89%) was recorded at this high frequency because of its very high occurrence in one of the 5 leaves, 12 out of 17 isolates obtained from a single leaf (Table 12.).

In 1996, a total of 34 species were recovered including 2 which remained sterile (Table 9.). Again the Xylariaceae dominated showing 63.54% frequency of isolation, followed by species of *Phomopsis spp.* and *Colletotrichum. Daldinia eschscholzii* (19.10%), *H. haematostroma* (5.56%), *N. subannualata* (4.86%), *Hypoxylon subrutilum* and *H. cf. subrutilum*, both with frequency of isolation of 4.86%, maintained the general trend indicated in the results for 1995 (Table 9. and Fig. 8.). However *X. cubensis* was now recovered at a frequency of 6.94% which is more in line with the results obtained by Rodrigues (1994) for her palm endophytes from Brazil. It is also notable that in 1996 a

Table 11. Percentage frequency of the most common taxa isolated from mature leaves at Mae Rim forest (1995-1997).

Taxa	1995	1996	1997
Daldinia eschscholzii (A)	**19.05**	**19.10**	**10.51**
Hypoxylon haematostroma (B)	**9.09**	5.56	2.23
H. subrutilum (C)	**8.66**	4.86	**5.41**
Phomopsis sp. 3 (D)	**7.36**	**8.68**	5.10
X. juruensis var microspora (E)	**7.36**	3.82	3.18
Phomopsis sp.2 (F)	**6.06**	**8.33**	3.50
N. subannulata (G)	**6.06**	4.86	**6.69**
X. cubensis (H)	0.87	**6.94**	**5.41**
Colletotrichum sp. (I)	1.30	**6.25**	**13.69**
X. aristata (J)	0.00	2.43	**7.96**
Number of genera	15	10	12
Number of species	27	32	25
Number of sterile species	3	2	4

Grand total of fungal assemblages in 1995-1997 at Mae Rim forest
Number of genera = 15
Number of species = 41
Number of sterile species = 4
Number of sterile isolates = 38

Figure 7. Colonization by the most common taxa found from mature leaves at Mae Rim forest (1995-1997).

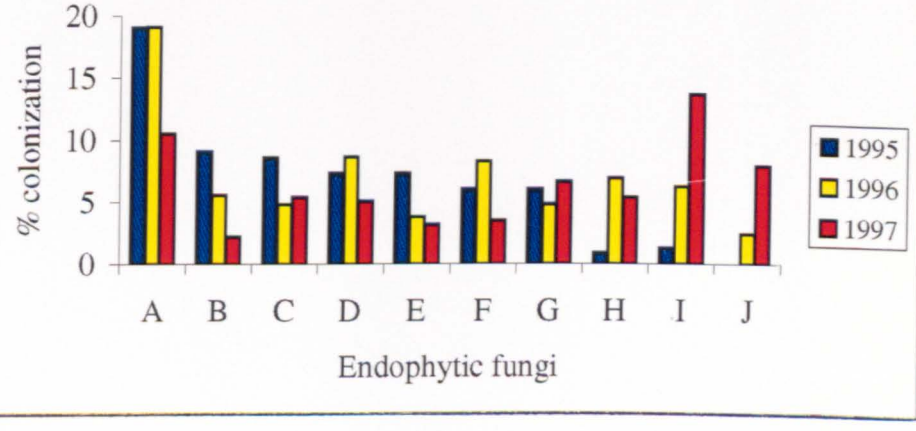


Figure 8. Endophytic fungi isolated from mature leaves at Mae Rim forest (1995-1997)

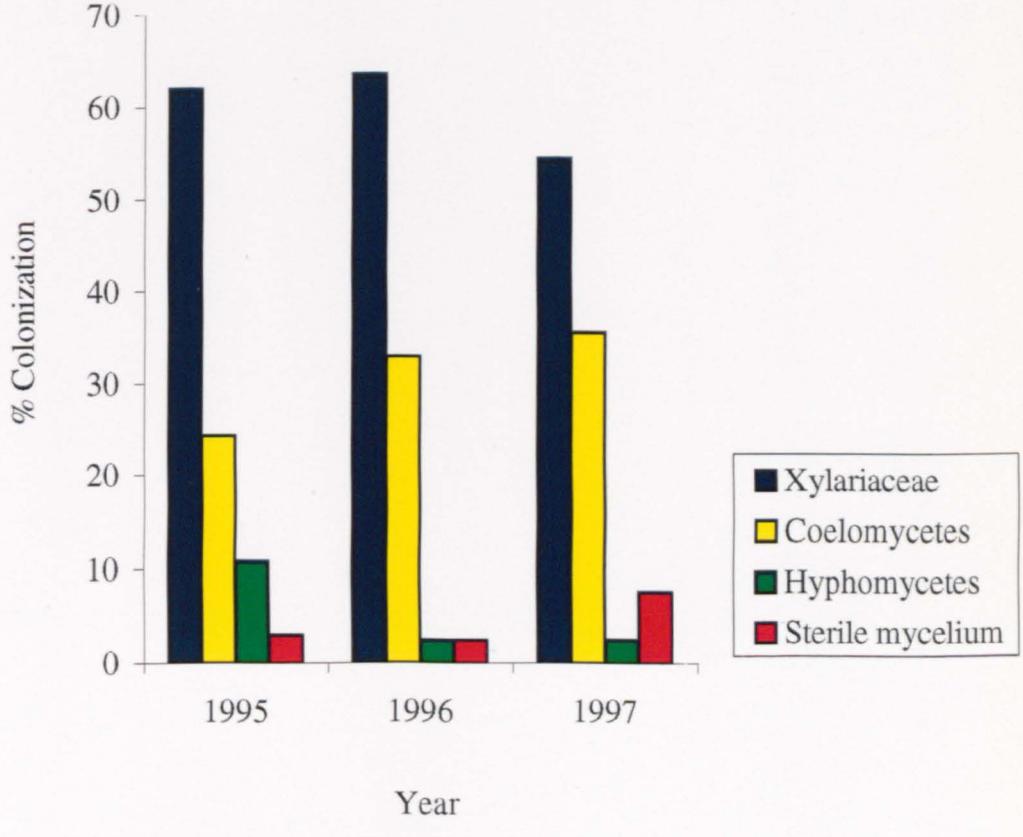


Table 12. Number of xylariaceous isolates obtained from mature leaves at Mae Rim forest (1995).

Taxa	Leaves					Total isolates	Percentage frequency
	1	2	3	4	5		
1. Daldinia eschscholzii	8	7	13	8	8	44	30.77
2. Hypoxylon cf. subrutilum	1	5	2	2	3	13	9.09
3. H. subrutilum	6	6	6	2	0	20	13.99
4. Hypoxylon sp. 188	0	4	0	0	0	4	2.80
5. H. haematostroma	9	2	3	4	3	21	14.69
6. Nemania subannulata	0	3	7	4	0	14	9.79
7. Xylaria sp. 271	0	0	1	0	0	1	0.70
8. Xylaria sp. 342	0	0	1	0	0	1	0.70
9. X. grammica	0	0	3	0	0	3	2.10
10. Xylaria sp.347	0	0	0	0	1	1	0.70
11. Xylaria sp.564	0	0	0	0	1	1	0.70
12. X. cubensis	2	0	0	0	0	2	1.40
13. X. feejeensis	1	0	0	0	0	1	0.70
14. X. juruensis var. microspora	0	3	2	12	0	17	11.89
Total isolates	**27**	**30**	**38**	**32**	**16**	**143**	

greater range of species of *Xylaria* were obtained although most of these were based on a single isolate (Tables 13.). Comparison of frequency of isolation of the xylariaceous species clearly shows *D. eschscholzii* (30.05%) to be the dominant isolate with *H. haematostroma* (8.74%), *N. subannulata* (7.65%), *Hypoxylon subrutilum* and *H. cf. subrutilum* (7.65% each) and *X. cubensis* (10.93%) having a substantial presence (Table 13.). In 1996 the total number of isolates at 288 was higher than the 231 recovered in 1995 and similarly the number of Xylariaceae at 183 isolates exceeded the number of 143 obtained in 1995.

In 1997, 29 species, including 4 sterile species, were recognised. A total of 314 isolates were obtained making this the highest recovery for the 3 years sampled (Table 10.). At 54.46% the Xylariaceae were still dominant but at the lowest frequency for the 3 years (Fig 8.). Following the established pattern of previous years *D. eschscholzii* (19.30%), *N. subannulata* (12.28%) and *H. haematostroma* (4.09%) were the common xylariaceous isolates with *X. cubensis* again at around 10% (Table 14.). The high percentage recovery of *X. aristata* (14.62%) is of interest since this is one of the species of *Xylaria* associated with fallen leaves and petioles (Gonzalez & Rogers, 1989) and might therefore be predicted to be endophytic. *Xylaria aristata* has also been recorded from Thailand including Doi Inthanon National Park in Chiang Mai Province (Thienhirun, 1997) but it was not found in the vicinity of the sampling sites during the present study.

Examination of the most frequently isolated species each year (Table 11. and Fig. 7.) shows that there is considerable difference in frequency of isolation of some species although over the 3 years the high dominance of *Daldinia eschscholzii* is maintained and only in 1997 was it replaced by a *Colletotrichum* species as the most frequently recorded taxon overall.

3.2.1.1. Tissue specificity of endophytes from mature leaves from natural forest trees

Taxa and isolates obtained from leaf lamina, midrib and veins for each leaf were recorded separately (Appendix 3.: Tables 1. to 6.). The number of taxa isolated from lamina, midrib and veins exhibited little variation ranging from 1 to 5 in 1995, 1 to 6 in 1996 and 2-8 in 1997. Maximum taxa found on each area of mature leaves at Mae Rim natural forest in 1995-1997 are shown in Fig 5. There were no significant differences in

Table 13. Number of xylariaceous isolates found from mature leaves at Mae Rim forest (1996).

Taxa	Leaves					Total isolates	Percentage frequency
	1	2	3	4	5		
1. Daldinia eschschlozii	10	20	10	8	7	55	30.73
2. Hypoxylon cf. anthochroum	0	0	0	1	2	3	1.68
3. H. cf. subrutilum	3	4	1	2	4	14	7.82
4. H. subrutilum	0	4	3	3	4	14	7.82
5. Hypoxylon sp. 188	1	0	2	0	2	5	2.79
6. H. haematostroma	6	1	2	2	5	16	8.94
7. N. subannulata	1	5	2	2	4	14	7.82
8. Penzigia sp.	1	0	0	0	0	1	0.56
9. Xylaria sp.5	0	1	0	0	0	1	0.56
10. Xylaria sp.7	0	1	0	0	0	1	0.56
11. Xylaria sp.9	0	0	0	1	0	1	0.56
12. Xylaria sp.15	0	1	0	0	0	1	0.56
13. Xylaria sp.24	0	1	0	0	0	1	0.56
14. Xylaria sp.40	0	1	0	0	0	1	0.56
15. Xylaria sp.64	0	0	0	0	1	1	0.56
16. Xylaria sp.69	1	0	0	0	2	3	1.68
17. Xylaria sp.73	0	0	0	0	1	1	0.56
18. Xylaria sp.76	0	0	0	0	1	1	0.56
19. X. grammica	0	0	2	0	0	2	1.12
20. X. aristata	0	1	2	2	2	7	3.91
21. X. cf. allantoidea	1	0	0	0	0	1	0.56
22. X. cubensis	6	7	3	4	0	20	11.17
23. X. juruensis var microspora	2	5	1	1	2	11	6.15
24. X. feejeensis	0	1	0	1	2	4	2.23
Total isolates	**32**	**53**	**28**	**27**	**39**	**179**	

Table 14. Number of xylariaceous isolates obtained from mature leaves at Mae Rim forest (1997).

Taxa	Leaves					Total isolates	Percentage frequency
	1	2	3	4	5		
Daldinia eschscholzii	7	11	2	7	6	33	19.30
Hypoxylon cf. subrutilum	0	4	3	3	1	11	6.43
H. subrutilum	4	3	2	4	4	17	9.94
Hypoxylon sp.188	0	0	2	2	0	4	2.34
H. cf. anthochroum	0	2	0	0	0	2	1.17
H. haematostroma	1	2	1	3	0	7	4.09
Nemania subannulata	0	7	3	5	6	21	12.28
Xylaria sp.36	2	0	4	0	1	7	4.09
Xylaria sp. 69	0	2	4	1	2	9	5.26
Xylaria sp. 294	0	2	0	0	0	2	1.17
X. grammica	0	0	4	0	2	6	3.51
X. aristata	6	4	7	5	3	25	14.62
X. cubensis	4	3	4	2	4	17	9.94
X. juruensis var. microspora	1	2	4	2	1	10	5.85
Total isolates	**25**	**42**	**40**	**34**	**30**	**171**	

number of taxa recovered from each area on leaves in 1995,1996 and 1997 (Kruskal-Wallis, number of taxa 1995, 1996 and 1997, X^2 = 6.78, 7.60 and 3.79 respectively, P>0.01). Number of isolates obtained from leaf lamina, midrib and veins also exhibited little variation ranging from 3.6-5.4 in 1995, 5.2-6.4 in 1996 and 5.0-7.6 in 1997 (Fig 6.). There were no significant differences in number of isolates recovered from each area on leaves in 1995,1996 and 1997 (Kruskal-Wallis, number of isolates, P>0.01). Thus there is greater variation between sampling years than between position of isolation from the leaf.

3.2.2. Endophytes of mature leaves from plantation trees.

Endophytes were isolated from 5 mature leaves sampled from plantation trees at Chiang Dao in November 1995, 1996 and 1997. The total range of species identified together with their frequency of isolation is shown in Tables 15, 16, and 17 and Fig. 9. The maximum taxa and average number of isolates obtained from different parts of the leaf, i.e lamina, midrib and vein for each leaf examined are shown in Figs. 10. and 11. A total of 17 genera and 34 species of fungi were identified with a further 3 species which remained sterile resulting in a grand total of 37 species recovered from the plantation site (Table 18). Species of *Phomopsis* followed by members of the Xylariaceae, *Nigrospora* sp and *Humicola sp.* were the most frequently isolated taxa over the 3 year period. There were, however, differences in the species isolated, and particularly in the frequency of isolation, between the years.

In 1995, 16 genera consisting of a total of 26 species to which a further 3 sterile species can be added, were recorded (Table 18). Species of *Phomopsis* demonstrated major dominance with 61.63% frequency of isolation (Table 15). *Phomopsis sp. 1* with 38.38% of the total was by far the most common endophyte for that year (Table 18 and Fig. 12.). The Xylariaceae with 11.44% were the next most frequently recorded endophytic fungi and *D. eschscholzii* was the most common at 38.71% of all xylariaceous species (Table 19). The majority of other Xylariaceae, however, were only recovered once or twice (Table 19).

In 1996 only 9 genera consisting of 22 species were recorded. A further 2 species which remained sterile were also obtained (Table 18). *Phomopsis,* although still dominant,

Table 15. Endophytic fungi isolated from mature leaves at Chiang Dao plantation (1995).

Taxa	Leaves					Number of isolates	Percentage frequency
	1	2	3	4	5		
1. Cladosporium sp.	0	1	2	1	1	5	1.85
2. Colletotrichum sp.	0	1	2	0	0	3	1.11
3. Curvularia sp.	1	0	1	0	0	2	0.74
4. Daldinia eschscholzii	3	2	3	2	2	12	4.43
5 .Diplodia sp.	2	2	1	4	0	9	3.32
6. Fusarium sp.	2	1	0	0	3	6	2.21
7. Humicola sp.	1	3	2	3	3	12	4.43
8. Hypoxylon cf. subrutilum	0	2	1	0	0	3	1.11
9. H. subrutilum	2	0	0	0	0	2	0.74
10. H. haematostroma	2	2	0	0	0	4	1.48
11. Nemania subannulata	0	0	0	2	0	2	0.74
12. Nigrospora sp.	2	3	2	2	6	15	5.54
13. Pestalotia sp.	2	0	0	0	0	2	0.74
14. Phialophora sp.	0	0	0	0	1	1	0.37
15. Phoma sp.	0	0	0	1	0	3	1.11
16. Phomopsis sp.1	26	21	25	20	12	104	38.38
17. Phomopsis sp.2	3	3	12	7	6	31	11.44
18. Phomopsis sp.3	5	7	4	3	2	21	7.75
19. Phomopsis sp.4	0	1	3	2	5	11	4.06
20. Septonema sp.	0	0	0	0	1	1	0.37
21. Xylaria sp. 294	0	0	0	1	1	2	0.74
22. X. juruensis var. microspora	0	0	1	0	0	1	0.37
23. Xylaria sp.564	0	0	0	1	1	2	0.74
24. X. cubensis	0	0	2	0	0	2	0.74
25. X. feejeensis	0	0	0	1	0	1	0.37
26. Trichoderma sp.	0	0	0	1	0	1	0.37
27. Sterile sp.1	3	0	0	0	0	3	1.11
28. Sterile sp.3	0	0	2	0	5	7	2.58
29. Sterile sp.6	0	3	0	1	0	4	1.48
Number of isolates	**55**	**52**	**63**	**52**	**49**	**271**	
Total of species	**13**	**14**	**15**	**16**	**13**		

Table 16. Endophytic fungi isolated from mature leaves at Chiang Dao plantation (1996).

Taxa	Leaves					Total isolates	Percentage frequency
	1	2	3	4	5		
1. Colletotrichum sp.	1	4	3	1	1	10	3.42
2. Daldinia eschscholzii	11	16	12	6	14	59	20.21
3. Humicola sp.	0	0	1	0	2	3	1.03
4. Hypoxylon cf. subrutilum	4	4	4	2	4	18	6.16
5. Hypoxylon sp.188	0	0	0	2	2	4	1.37
6. Hypoxylon subrutilum	4	4	4	3	4	19	6.51
7. H. cf. anthochroum	2	1	3	2	0	8	2.74
8. H. haematostroma	3	5	4	2	4	18	6.16
9. N. subannulata	5	5	5	0	4	19	6.51
10. Nigrospora sp.	1	0	0	0	0	1	0.34
11. Phomopsis sp.1	9	2	6	11	4	32	10.96
12. Phomopsis sp.2	1	4	5	4	6	20	6.85
13. Phomopsis sp.3	1	1	5	12	3	22	7.53
14. Phomopsis sp.4	0	1	1	0	0	2	0.68
15. Septonema sp.	0	0	0	0	1	1	0.34
16. Xylaria sp.7	0	1	0	0	0	1	0.34
17. Xylaria sp.29	1	0	0	0	2	3	1.03
18. Xylaria sp.36	0	0	0	0	1	1	0.34
19. X. grammica	1	0	0	0	1	2	0.68
20. X. aristata	2	1	1	3	2	9	3.08
21. X. cubensis	5	0	6	3	5	19	6.51
22. X. juruensis var. microspora	2	1	2	0	3	8	2.74
23. Sterile sp.3	2	2	0	1	1	6	2.05
24. Sterile sp.4	0	2	2	0	3	7	2.40
Number of isolates	**55**	**54**	**64**	**52**	**67**	**292**	
Number of species	**17**	**16**	**16**	**13**	**19**		

Table 17. Endophytic fungi isolated from mature leaves at Chiang Dao plantation (1997).

Taxa	Leaves					Number of isolates	Percentage frequency
	1	2	3	4	5		
1. Colletotrichum sp.	0	0	2	3	2	7	2.37
2. Daldinia eschscholzii	7	5	9	3	8	32	10.85
3. Diplodia sp.	0	0	1	0	1	2	0.68
4. Humicola sp.	0	1	7	6	2	16	5.42
5. Hypoxylon cf. subrutilum	4	4	4	3	5	20	6.78
6. Hypoxylon subrutilum	3	4	4	3	3	17	5.76
7. Hypoxylon sp.188	3	0	0	1	0	4	1.36
8. H. haematostroma	3	0	1	4	4	12	4.07
9. Nemania subannulata	8	5	5	6	4	28	9.49
10. Phomopsis sp.1	5	10	2	2	5	24	8.14
11. Phomopsis sp.2	0	3	2	2	4	11	3.73
12. Phomopsis sp.3	2	9	2	3	6	22	7.46
13. Phomopsis sp.4	8	6	9	4	2	29	9.83
14. Pycnidia	0	1	3	2	0	6	2.03
15. Septoria sp.	0	1	0	0	0	1	0.34
16. Xylaria sp.32	0	0	1	0	0	1	0.34
17. Xylaria sp.69	6	4	3	5	0	18	6.10
18. Xylaria sp.36	0	0	2	0	0	2	0.68
19. X. aristata	1	1	0	2	0	4	1.36
20. X. cubensis	6	4	5	5	7	27	9.15
21. X. juruensis var. microspora	0	0	0	1	0	1	0.34
22. Sterile sp.1	3	2	2	0	3	10	3.39
23. Sterile sp.3	0	0	0	1	0	1	0.34
Number of isolates	**59**	**60**	**64**	**56**	**56**	**295**	
Number of species	**13**	**15**	**18**	**18**	**14**		

Figure 9. Endophytic fungi isolated from mature leaves at Chiang Dao plantation (1995-1997)

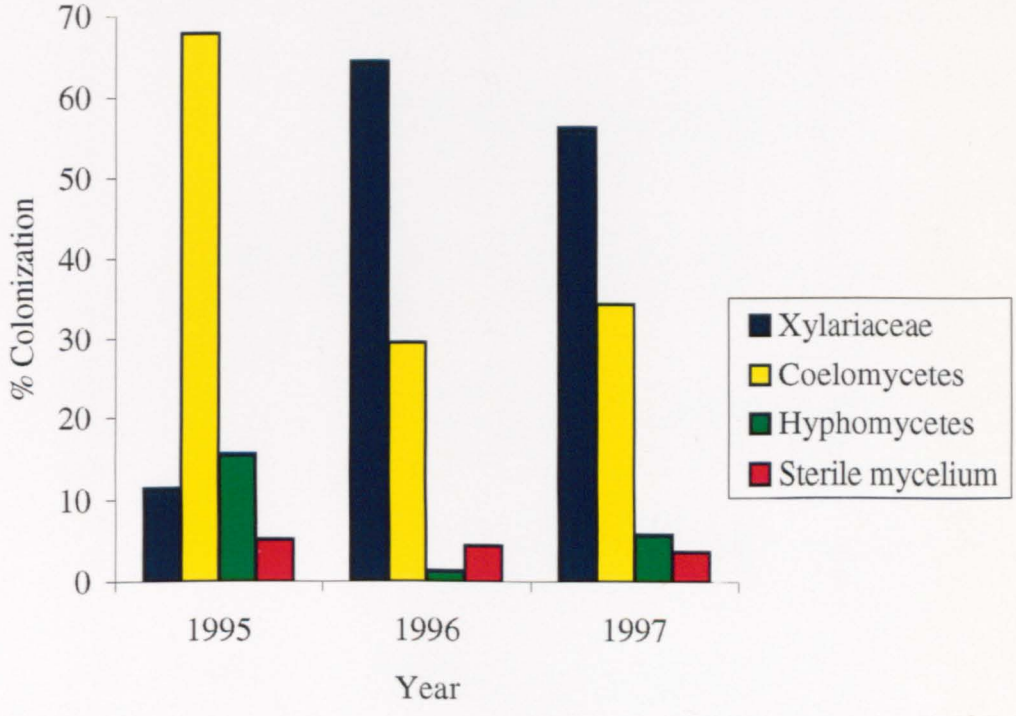


Figure 10. Maximum taxa found on each area of mature leaves at Chiang Dao (1995-1997)

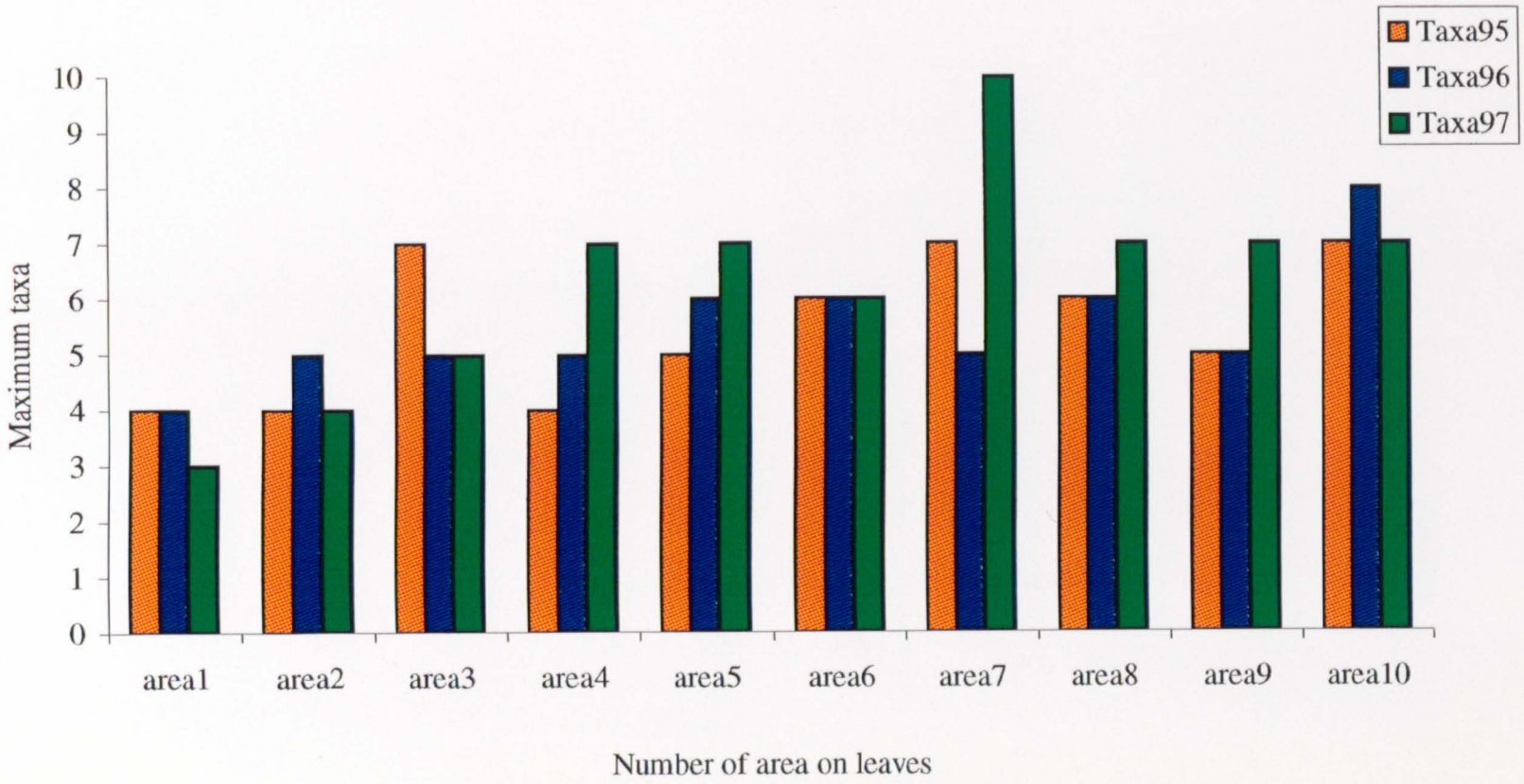


Figure 11. Number of isolates found on each area of mature leaves at Chiang Dao (1995-1996)

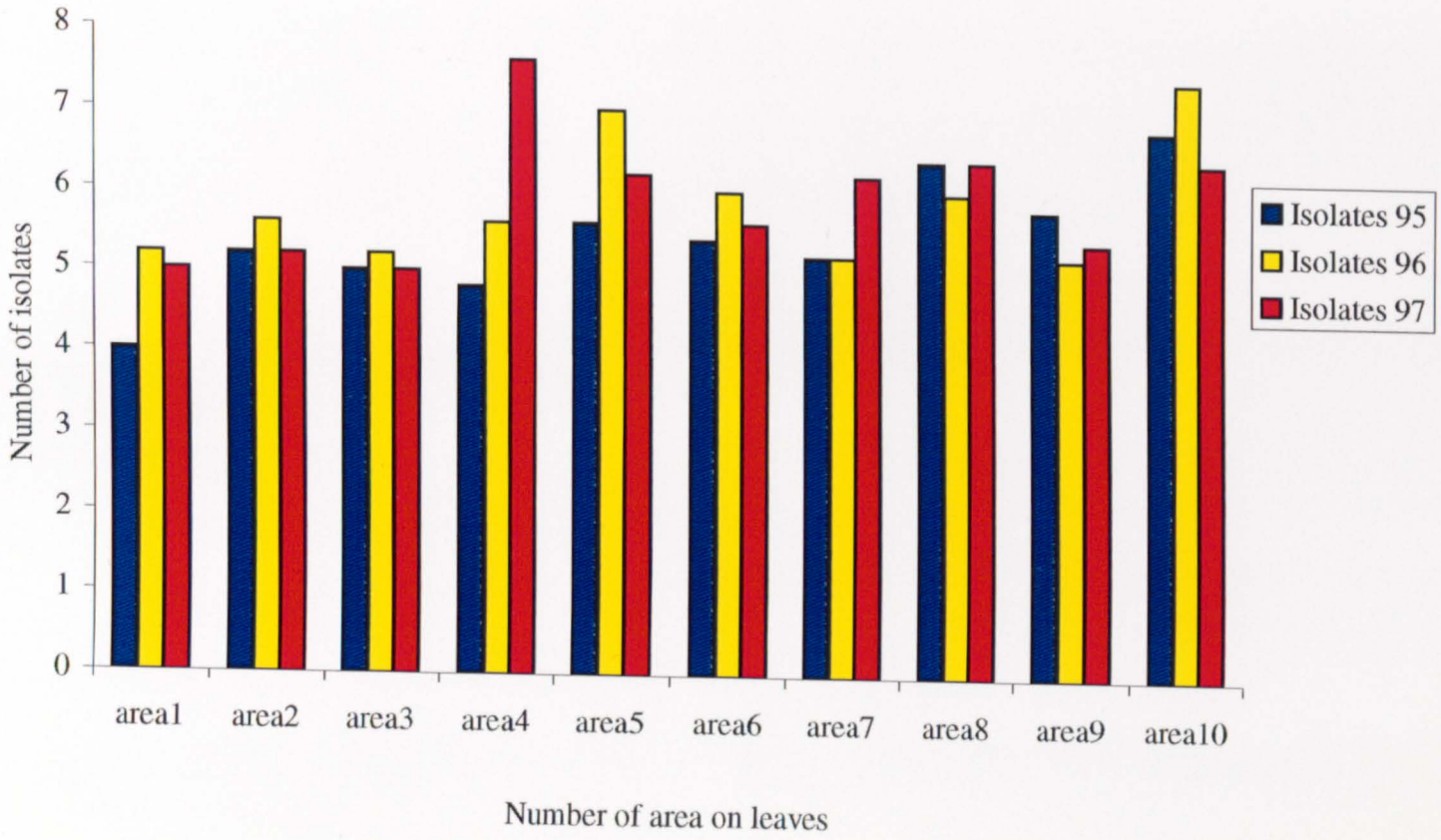
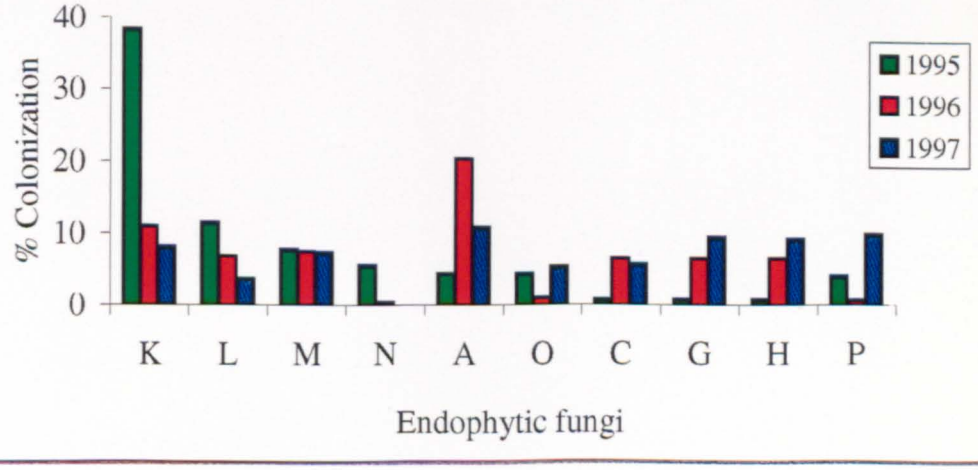


Table 18. Percentage frequency of the most common taxa isolated from mature leaves at Chiang Dao plantation (1995-1997)

Taxa	1995	1996	1997
Phomopsis sp.1 (K)	**38.38**	**10.96**	**8.14**
Phomopsis sp.2 (L)	**11.44**	6.85	3.73
Phomopsis sp.3 (M)	**7.75**	7.53	7.46
Nigrospora sp. (N)	**5.54**	0.34	0.00
Daldinia eschscholzii (A)	**4.43**	**20.21**	**10.85**
Humicola sp. (O)	**4.43**	1.03	5.42
Hypoxylon subrutilum (C)	0.74	**6.51**	5.76
Nemania subannulata (G)	0.74	**6.51**	**9.46**
Xylaria cubensis (H)	0.74	**6.51**	**9.15**
Phomopsis sp. 4 (P)	4.06	0.68	**9.83**
Number of genera	16	9	10
Number of species	26	22	21
Number of sterile species	3	2	2

Grand total of fungal assemblages in 1995-1997 at Chiang Dao plantation
Number of genera = 17
Number of species = 34
Number of sterile species = 3
Number of sterile isolates = 38

Figure 12. Colonization by the most common taxa found from mature leaves at Chiang Dao plantation (1995-1997).



was isolated at a much lower frequency than in 1995 with a total for the 4 species recognised of 26.02% (Table 16). In 1996 *D. eschscholzii* at 20.21% of the total of all taxa recorded was the single dominant species. Other Xylariaceae such as *H. haematostroma, N. subannulata* and *X. cubensis* resulted in an overall recovery of Xylariaceae of 64.38% (Table 20). Unlike the previous year most species of *Xylaria* were recovered from more than one leaf (Tables 19 & 20). The 1997 isolates showed an increase in the overall frequency of *Phomopsis* (29.16%) with *Phomopsis sp. 1* being the most frequent. However, *D. eschscholzii* (10.85%) was the most frequently isolated individual species (Table 17). Species of *Xylaria* such as *X. cubensis* (9.15%) and *Xylaria sp.* 69 (6.10%) were also numerically important isolates. The frequency of occurrence of members of the Xylariaceae (Table 21) illustrates the regular presence of members of the family with strong representation from *Xylaria.* Furthermore, *Daldinia eschscholzii, H. haematostroma* and *N. subannulata* appear to be more or less constantly present as teak leaf endophytes at this site. Although *X. cubensis* was recorded as teleomorphic material in the general vicinity of the plantation *Xylaria sp. 69* and the other *Xylaria* species isolated were not found in natural field situations during the study. It can also be seen that only 21 species belonging to 10 genera together with a further 2 sterile species were obtained. Thus 1997 was similar to 1996 with a lower fungal diversity than for the first year, 1995 (Table 15). The total number of endophytic isolates isolated from the plantation leaves ranged from 271 in 1995 to 292 in 1996 with the highest number of 295 in 1997.

3.2.2.1 Tissue specificity of endophytes from mature leaves from plantation trees.

Taxa and average isolates obtained in 1995 from leaf lamina, midrib and veins for each leaf were recorded separately (Appendix 3.: Table 7-12.). Generally all tissues provided a suitable number of isolates representing the taxa identified. On the basis of taxa recovered from the different regions in 1995 leaf lamina sections produced the highest mean number of taxa, followed by veins and then midrib. A similar result was obtained for 1996 although for this year the differences were less clear. There were no significant difference in number of taxa recovered from each area on leaves in 1995 and 1996

Table 19. Number of xylariaceous isolates obtained from mature leaves at Chiang Dao plantation (1995).

Taxa	Leaves					Total isolates	Percentage frequency
	1	2	3	4	5		
Daldinia eschscholzii	3	2	3	2	2	12	38.71
Hypoxylon cf. subrutilum	0	2	1	0	0	3	9.68
Hypoxylon subrutilum	2	0	0	0	0	2	6.45
H. haematostroma	2	2	0	0	0	4	12.90
Nemania subannulata	0	0	0	2	0	2	6.45
Xylaria sp.294	0	0	0	1	1	2	6.45
Xylaria sp.564	0	0	0	1	1	2	6.45
X. cubensis	0	0	2	0	0	2	6.45
X. juruensis var. microspora	0	0	1	0	0	1	3.23
X. feejeensis	0	0	0	1	0	1	3.23
Total isolates	**7**	**6**	**7**	**7**	**4**	**31**	

Table 20. Number of xylariaceous isolates obtained from mature leaves Chiang Dao plantation (1996).

Taxa	Leaves					Total isolates	Percentage frequency
	1	2	3	4	5		
Daldinia eschscholzii	11	16	12	6	14	59	31.38
Hypoxylon cf. subrutilum	4	4	4	2	4	18	9.57
Hypoxylon sp.188	0	0	0	2	2	4	2.13
H. subrutilum	4	4	4	3	4	19	10.11
H. cf. anthochroum	2	1	3	2	0	8	4.26
H. haematostroma	3	5	4	2	4	18	9.57
N. subannulata	5	5	5	0	4	19	10.11
Xylaria sp.7	0	1	0	0	0	1	0.53
Xylaria sp.32	1	0	0	0	2	3	1.60
Xylaria sp.36	0	0	0	0	1	1	0.53
X. grammica	1	0	0	0	1	2	1.06
X. aristata	2	1	1	3	2	9	4.79
X. cubensis	5	0	6	3	5	19	10.11
X. juruensis var. microspora	2	1	2	0	3	8	4.26
Total isolates	**40**	**38**	**41**	**23**	**46**	**188**	

Table 21. Number of xylariaceous isolates obtained from mature leaves at Chiang Dao plantation (1997).

Taxa	Leaves					Total isolates	Percentage frequency
	1	2	3	4	5		
Daldinia eschscholzii	7	5	9	3	8	32	19.28
Hypoxylon cf. subrutilum	4	4	4	3	5	20	12.05
H. subrutilum	3	4	4	3	3	17	10.24
Hypoxylon sp.188	3	0	0	1	0	4	2.41
H.haematostroma	3	0	1	4	4	12	7.23
Nemania subannulata	8	5	5	6	4	28	16.87
Xylaria sp. 32	0	0	1	0	0	1	0.60
Xylaria sp. 69	6	4	3	5	0	18	10.84
Xylaria sp. 36	0	0	2	0	0	2	1.20
X. aristata	1	1	0	2	0	4	2.41
X. cubensis	6	4	5	5	7	27	16.27
X. juruensis var. microspora	0	0	0	1	0	1	0.60
Total isolates	**41**	**27**	**34**	**33**	**31**	**166**	

(Kruskal-Wallis, number of taxa 1995 and 1996, X^2 = 15.09 and 15.22, respectively, P>0.01). In 1997, however, there was a significant difference in the results for midrib and veins (Wilcoxon signed rank test, P<0.05); the results for lamina and vein taxa were similar but number of taxa isolated from the midrib was lower (Fig. 10.). Thus for the plantation leaves the overall highest recovery, in terms of taxa recorded, was from the lamina, followed by veins and finally the midrib. Average number of isolates found on each area of mature leave were exhibited in Fig. 11. There were little variation ranging from 4-6.8 in 1995, 5.2-7.4 in 1996 and 5.0-7.6 in 1997. No significant difference found on the basis of isolates on each area of mature leaves at Chiang Dao plantation (Kruskal-Wallis, number of isolates, P>0.01).

3.2.3. Endophytes of young leaves from natural forest trees

Examination of young leaves collected in May 1996 from Mae Rim (Table 22) immediately shows that there is a much lower diversity of fungal species recorded with only 8 species, including 3 sterile species, recognised. *Colletotrichum* at 51.85% was clearly the dominant isolate followed by an unidentified pycnidial species and then *Phomopsis*. The complete lack of any xylariaceous taxa is of special interest. Comparison of tissue sites associated with the isolations indicates a slight preference for midrib and vein as opposed to leaf lamina (Wilcoxon signed rank test, P>0.05). This is shown by reference to both total isolates recorded and species identified (Fig. 13.). The sample collected in May 1997 also exhibited similar results with 9 species, including 1 sterile isolate, recorded (Table 23). Again *Colletotrichum* at 66.67% isolation frequency was most dominant genus followed by species of *Phomopsis*. *Daldinia eschscholzii* was recorded for this year but only at the very low percentage frequency of 2.22%. No other xylariaceous taxa were found. Comparison of tissue sites associated with the isolations and taxa identified indicated similar preference of tissue sites (Fig. 13.) (Wilcoxon signed rank test, P>0.05). There is therefore a clear difference between the fungal populations of young and mature leaves sampled over 2 years at the natural forest site of Mae Rim (Wilcoxon signed rank test, P<0.05). The differences are both qualitative and quantitative since in mature leaves the percentage infection was always 100% but in young leaves it ranged from 7.5% to 42.5% in 1996 and from 20% to 60% in 1997. The mean infection percentage for 1996 was 27% and 44.5% for 1997 (Table 24).

Table 22. Species identified from young leaves at Mae Rim forest (1996).

Taxa	Leaves					Total isolates	Percentage frequency
	1	2	3	4	5		
1. Colletotrichum sp.	9	0	3	9	7	28	51.85
2. Phomopsis sp.1	0	0	0	3	1	4	7.41
3. Phomopsis sp.2	1	0	0	1	0	2	3.70
4. Pycnidia	0	3	5	0	0	8	14.81
5. Nigrospora sp.	0	0	0	0	1	1	1.85
6. Sterile sp.5	2	0	0	2	0	4	7.41
7. Sterile sp.6	3	0	0	0	0	3	5.56
8. Sterile sp.7	0	0	0	2	2	4	7.41
Total isolates	12	3	8	15	9	54	
Total species	4	1	2	5	4		
Total genera	5(+3)						

Table 23. Species identified from young leaves at Mae Rim forest (1997).

Taxa	Leaves					Total isolates	Percentage frequency
	1	2	3	4	5		
1. Colletotrichum sp.	7	7	21	14	11	60	66.67
2. Daldinia eschscholzii	0	1	1	0	0	2	2.22
3. Phomopsis sp.1	4	0	2	0	3	9	10.00
4. Phomopsis sp.2	5	0	0	0	0	5	5.56
5. Phomopsis sp.3	2	0	0	0	0	2	2.22
6. Phomopsis sp.4	2	0	0	3	0	5	5.56
7. Pycnidia	0	0	0	3	0	3	3.33
8. Nigrospora sp.	0	0	0	1	0	1	1.11
9. Sterile sp.7	0	0	0	0	3	3	3.33
Total isolates	20	8	24	21	17	90	
Total species	5	2	3	4	3		
Total genera	8(+1)						

Figure 13. Number of taxa and isolates found from young leaves at Mae Rim forest (1996-1997).

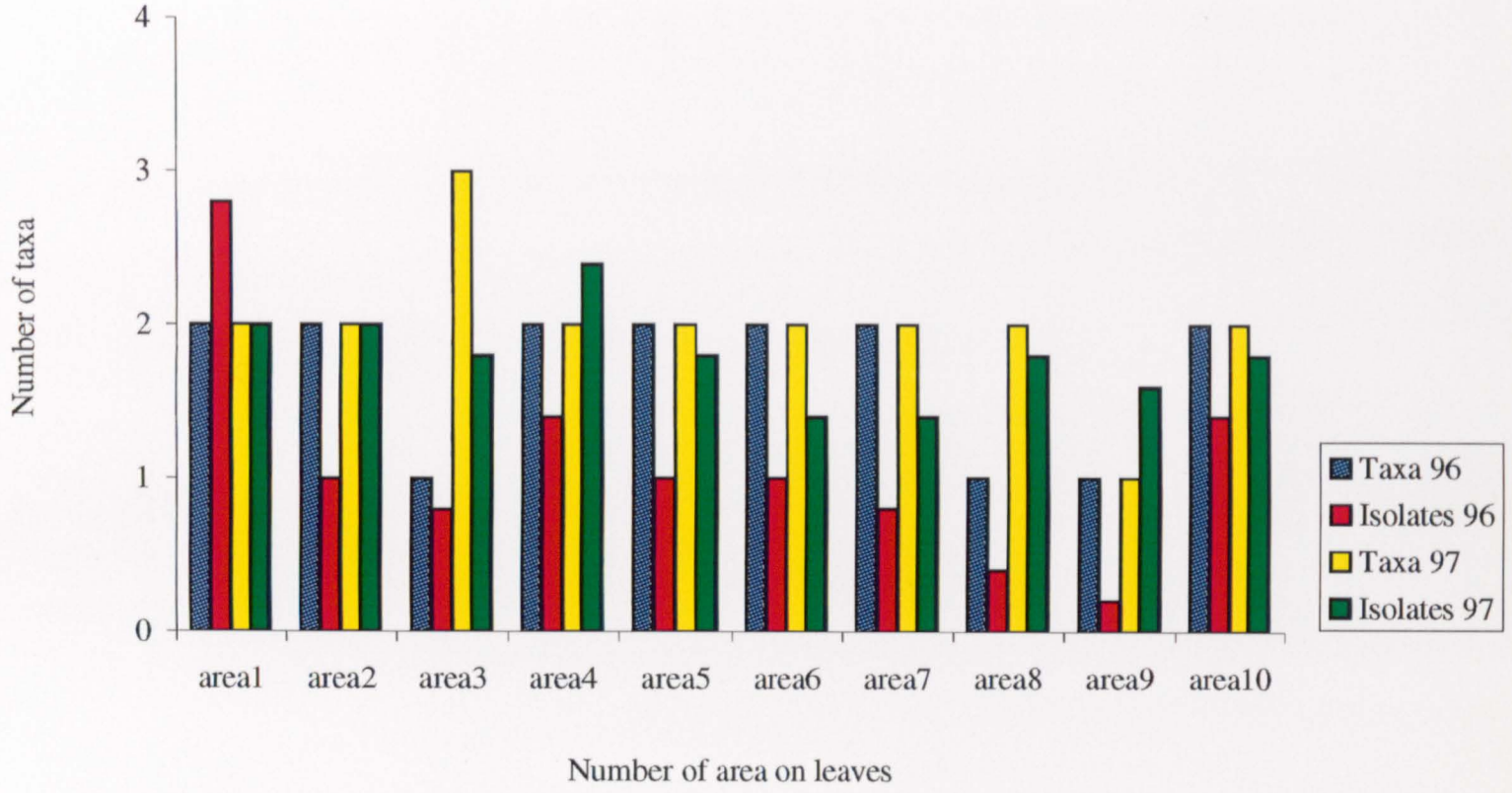


Table 24. Common species found from young leaves at both sites (1996-1997).

Taxa	Mae Rim		Ching Dao	
	1996	1997	1996	1997
1. Colletotrichum sp.	51.85	66.67	62.03	39.57
2. Phomopsis sp.1	7.41	10.00	13.92	23.74
3. Phomopsis sp.2	3.70	5.56	10.13	16.55
4. Phomopsis sp.4	0.00	5.56	0.00	10.07
5. Pycnidia	14.81	3.33	0.00	0.00
6. Sterile sp.2	7.41	0.00	6.33	0.00
%of isolation	27	44.5	39	66.5

Table 25. Species identified from young leaves at Chiang Dao plantation (1996).

Taxa	Leaves					Total isolates	Percentage frequency
	1	2	3	4	5		
1. Colletotrichum sp.	2	20	7	12	8	49	62.03
2. Dalinia eschscholzii	0	0	0	1	0	1	1.27
3. Phomopsis sp.1	2	2	4	3	0	11	13.92
4. Phomopsis sp.2	0	3	0	0	5	8	10.13
5. N. subannulata	0	1	0	0	0	1	1.27
6. Sterile sp.1	0	0	0	2	0	2	2.53
7. Sterile sp.2	0	0	0	2	3	5	6.33
8. Sterile sp. 3	0	0	0	0	2	2	2.53
Total isolates	4	26	11	18	13	79	
Total species	2	4	2	5	4		
Total genera	**4(+3)**						

Table 26. Species identified from young leaves at Chiang Dao plantation (1997).

Taxa	Leaves					Total isolates	Percentage frequency
	1	2	3	4	5		
Colletotrichum sp.	7	10	14	13	11	55	39.57
Diplodia sp.	1	0	0	0	0	1	0.72
Phomopsis sp.1	5	7	6	9	6	33	23.74
Phomopsis sp.2	5	7	5	1	5	23	16.55
Phomopsis sp.3	3	1	1	2	4	11	7.91
Phomopsis sp.4	5	0	3	2	4	14	10.07
Sterile sp. 5	0	0	0	2	0	2	1.44
Total isolates	**26**	**25**	**29**	**29**	**30**	**139**	
Total species	**6**	**4**	**5**	**6**	**5**		
Total genera	**3(+1)**						

3.2.4. Endophytes of young leaves from plantation trees.

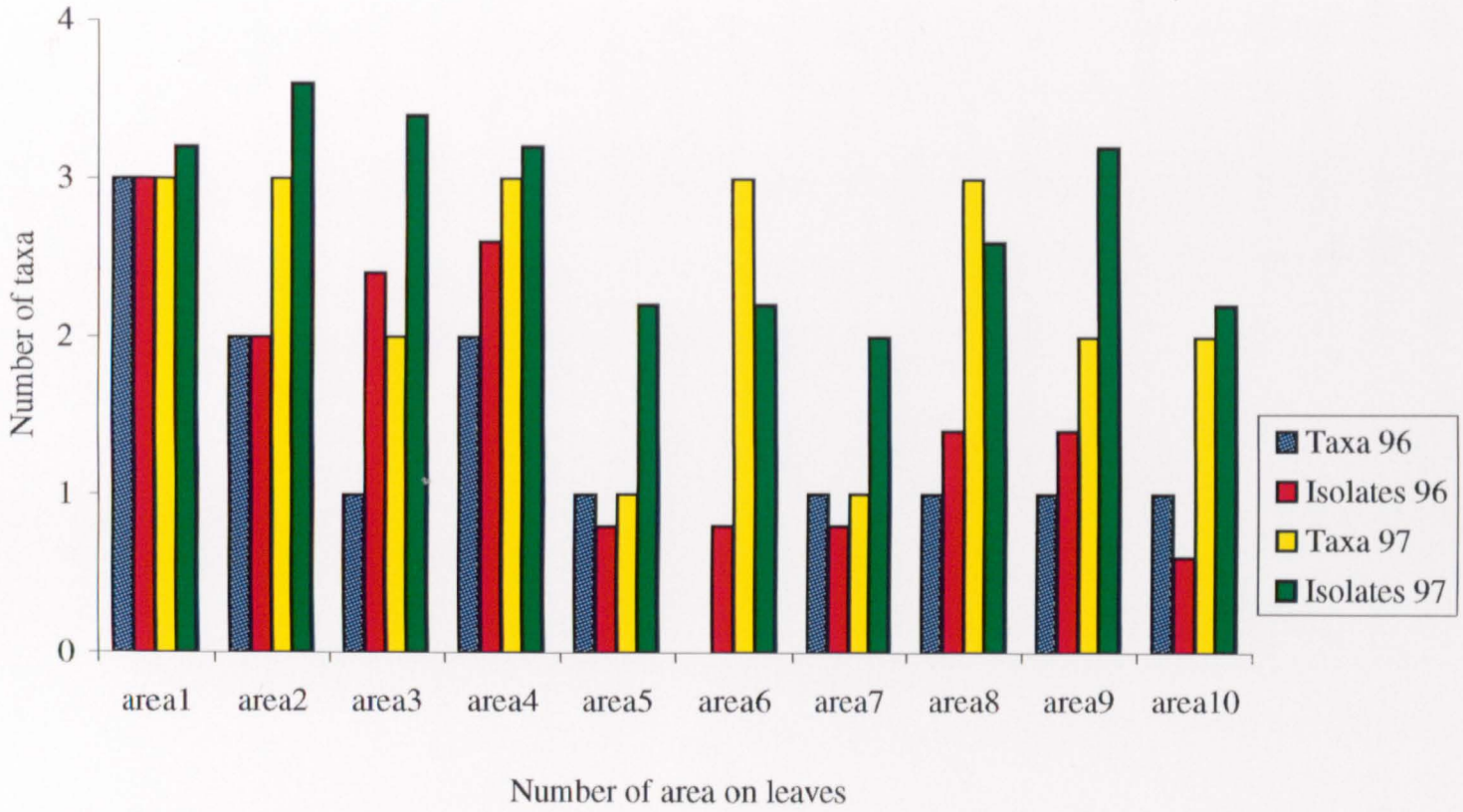
In 1996, 8 species of endophytes were recognised including 3 which remained sterile. *Colletotrichum* at 62.03% frequency was by far the most commonly isolated fungus followed by 2 species of *Phomopsis* at the lower frequencies of 13.92% and 10.13% respectively (Table 25). Two common endophytes recorded from mature leaves, *D. eschscholzii* and *N. subannulata* were isolated but only at the very low levels of 1.27%. In 1997 only 7 species were recorded and again *Colletotrichum* (39.57%) was dominant but 4 species of *Phomopsis* were present at appreciable levels (Table 26). In this year no members of the Xylariaceae were isolated. Again it is interesting to note that the isolation frequency from midrib and veins was slightly greater than from leaf lamina (Fig 14.) although with no significant difference was found (Wilcoxon signed rank test, $P>0.05$). Overall infection frequency was also low at 39% mean for 1996 and 66.5% for 1997 (Table 24).

Comparison of species isolated and frequency of infection between the natural forest sample and the plantation tree sample (Table 24) illustrates the major dominance of *Colletotrichum* at both sites and for both years. Species of *Phomopsis* are the other regular and numerically significant taxa. The very low occurrence or complete absence of xylariaceous species is of considerable interest since this contrasts most strongly with the situation occurring in mature leaves. The low overall infection percentage for the young leaves compared to mature leaves regardless of site is in keeping with results reported in many other studies of endophytic fungi (Bernstein & Carroll, 1977; Fisher *et al.*, 1986, 1993; Wu, 1997).

3.2.5. Host specificity of endophytes

Evidence obtained during this study indicates a low level of host specificity regarding the fungal endophytes of teak leaves. In general most of the genera reported are commonly found as endophytes in a wide range of hosts e.g. *Cladosporium, Colletotrichum, Fusarium and Phomopsis* (Petrini & Müller, 1979; Widler & Müller,1984; Sieber, 1988; Bills & Polishook, 1992; Fisher *et al.*, 1993, 1994). However with regards to teak leaves a number of endophytic isolates are likely to be previously undescribed species

Figure 14. Number of taxa and isolates found from young leaves at Chiang Dao plantation (1996-1997).



which may be shown to be host specific in the future. Nevertheless most of the data obtained strongly suggests that host specificity is not usual. In the Xylariaceae most of the endophytic isolates identified represent taxa known to occur as teleomorphic material in the wild when they are known to be associated with a range of hosts. *Xylaria cubensis*, for example, is widely distributed throughout the tropics (Van der Gucht & Whalley, 1996) and is a common and widespread species in Thailand and is known from the general vicinity of the sampling sites (Thienhirun, 1997). *Xylaria cubensis* is also a regular, endophyte of palm (Rodrigues, 1994; Hyde, Frohlich & Tayler, 1997).

3.3. Discussion

3.3.1. Geographical distribution and site orientation of endophytic fungi

The endophyte assemblages from the same plant species growing in different locations may be quite different. A number of factors might cause this phenomenon including vegetation in the vicinity and environmental condition such as temperature, rainfall and humidity. There can also be effects on the mycoflora resulting from human activities. Endophytic fungi have been isolated from many different plant species from different geographical sites and these studies have shown that geographical location strongly influences the endophyte assemblages (Petrini, 1986,1991; Fisher & Petrini, 1990; Fisher *et al.*, 1993, 1994). It is also well known that the mycoflora in tropical and subtropical areas are more complex and diverse than those in temperate areas and that the composition is also different (Hawksworth, 1993; Hyde & Hawksworth, 1997). However, very few studies have been done in the tropics and it is therefore not possible to compare endophytic fungi in these situations (Petrini & Dreyfuss, 1981; Dreyfuss & Petrini, 1984; Rodrigues & Samuels, 1990,1992; Rodrigues, 1994; Petrini *et al.*, 1995; Bills, 1996). One of the few substantive investigations was made by Rodrigues & Samuels 1990, 1992; Rodrigues, 1994 on endophytic fungi from palm leaves in tropical Brazil when they recognised a number of xylariaceous taxa and also described a number of new species.

In this investigation of fungal endophytes, teak leaves were sampled from two geographically separated sites where the trees from one site were growing in a natural forest and from the second site which was in a plantation. A range of fungal species were recorded. At Mae Rim (natural forest) over the 3 years of sampling, a total of 41 species

assignable to 15 genera were isolated and identified with a further 4 species which remained sterile (Table 11.). The most frequently recorded taxa were *D. eschscholzii, Hypoxylon haematostroma, Hypoxylon subrutilum, Phomopsis sp.2 and sp.3, X. cubensis, X. aristata, X. juruensis var. microspora, N. subannulata* and *Colletotrichum sp.* although there were variations in frequency between the years. At Chiang Dao (plantation) 34 species assignable to 17 genera together with 3 sterile species were obtained. *Phomopsis spp., Hypoxylon subrutilum, Humicola sp., Nigrospora sp., Nemania subannulata* and *X. cubensis* were the most frequently isolated endophytes (Table 18.). Again, variation in frequency of isolation was observed between the different years. Application of the Sorenson index to assess diversity of endophytes from the two sites indicated a high level of similarity between Mae Rim and Chiang Dao. The index values for 1995, 1996 and 1997 were 0.80, 0.69 and 0.79 respectively. As the Sorenson index is designed to equal 1 in cases of complete similarity and 0 if the sites are dissimilar then the values obtained for 1995 and 1997 of 0.80 and 0.79 indicate a high level of site similarity regarding species diversity. The lower value obtained for 1996 (0.69) still indicated however a substantial level of common species diversity. The reason for this is unknown but it could be related to climatic differences occurring that year. It is perhaps noteworthy that at Mae Rim in 1996 an unusually high number of *Xylaria* species (16) were obtained.

Since both sampling sites are tropical there are few published accounts with which the data can be compared. However examination of most frequently recorded endophytic genera from conifer trees (Table 27.) and broad leaf trees (Table 28.) shows that a number of the genera isolated in Thailand are well known as endophytes in temperate regions. Thus, *Colletotrichum, Cladosporium, Phomopsis* and members of the Xylariaceae have been reported from coniferous plants (Table 27.) as well as from teak during the present study. Comparison with broad-leaf trees (Table 28.) extends this range with *Phoma, Diplodia, Fusarium* and *Phialophora*. Wu (1997) investigated leaf endophytes from 86 different plants species from China and apart of *Cladosporium, Colletotrichum, Phoma, Phomopsis, Nigrospora* and numbers of the Xylariaceae in common also recorded (Table 29.). It would therefore seem likely that, at least at generic level, a number occur widely as endophytes regardless of plant species, geographical locality or even climatic zone. Wu (1997) also noted that there were some differences in the composition of fungi isolated from temperate

Table 27. Most commonly isolated fungal genera as endophytes from 47 conifer trees

This table is based on data from publications by Carroll *et al.* (1977); Carroll & Carroll (1978); Petrini & Muller (1979); Petrini & Carroll (1981); Butin (1986); Sieber (1988, 1989); Sieber-Canavesi & Sieber (1988); Johnson & Whitney (1989a,b); Bills & Polishook (1992); Helander *et al.* (1994); Wu (1997).

Table 28. Most commonly isolated fungal genera as endophytes from 40 broad-leaf trees.

This table is based on data from publications by Luginbuhl & Müller (1980); Petrini *et al.* (1982); Petrini (1984, 1985); Wider & Müller (1984); Fisher *et al.* (1986); Sieber & Hugentobler (1987); Bertoni & Cabral (1988); Fisher & Petrini (1990); Sieber *et al.* (1991); Fisher *et al.* (1992); Toti *et al.* (1992); Fisher, Petrini & Sutton (1993); Bissegger & Sieber (1994); Rodrigues (1994) and Wu (1997).

Table 29. Most commonly isolated fungal genera as leaf endophytes from 86 different plant species from China (Wu, 1997).

(UK) and subtropical areas of China. *Colletotrichum spp.* could be isolated from temperate plants but always at a low isolation frequency where as in subtropical areas their isolation frequency was high and they could be isolated from a wide variety of plants. In the current study *Colletotrichum* was a frequent isolate. Wu (1997) also concluded that to obtain isolates of more than one species from the same genus such as *Phyllosticta, Colletotrichum, Phomopsis* and *Septoria* from the same plant species is rare in temperate areas but it is not uncommon in the subtropics. It is therefore noteworthy that *Phomopsis, Hypoxylon* and *Xylaria* were usually well represented in the teak leaves with as many as 16 species of *Xylaria* being recognised from mature leaves from Mae Rim in 1996. In a single leaf the maximum number of isolates obtained as 75 isolates and this comprised of identified 19 species. This finding was found at Mae Rim in 1996. It is also of special interest to note that a number of new species of *Hypoxylon* and *Xylaria* can now be recognised from their endophytic isolates (see Chapter 4)

Sieber-Canavesi & Sieber (1988) found that there can be considerable differences in species richness and distribution of selected fungal species between populations of the same plant-host species growing in different sites. This was regarded as site-specificity of endophytes. However, the isolations from teak leaves suggest that in this case site-specificity is low. The distance between the two sampling sites is approximately 80 Km and apart from a single year species diversity in common was high. However, a number of taxa have not been identified to species level and future studies may show that some of these are site-specific. Sieber-Canavesi & Sieber (1988) cited the nature of surrounding vegetation and microclimate differences as the major reasons for site specific endophyte assemblages. Therefore in spite of the apparent major difference in surrounding vegetation, Mae Rim (natural forest), Chiang Dao (plantation) the high number of species in common is impressive and was unexpected. However it is very clear that in the surrounding forest vegetation at Chiang Dao plantation the Xylariaceae (14 taxa) were well represented and it can be speculated that in relation to provision of inoculum there is probably not a great difference between the two sites.

3.3.2. Nature of leaf in relation to species diversity and frequency of infection

Comparison of isolates made from young leaves sampled in May 1996 and 1997 at both sites with isolates obtained from mature leaves sampled in November of the same years indicated significant differences. The Wilcoxon signed rank test was applied to data from both sites for both years and a P-value of 0.0312 demonstrates a significant difference between species diversity and isolation frequency for young and mature leaves. Thus, at both sites far fewer isolates and species were recovered from the young leaves than from fully mature ones. This finding is in general agreement with conclusions from other studies (Bernstein & Carroll, 1977; Fisher *et al.*, 1986)

A major difference in the presence of xylariaceous taxa was found between young and mature leaves. Apart from the low occurrence of *D. eschscholzii* and *N. subannulata* at 1.27% frequency in Chiang Dao (1996) other members of Xylariaceae were absent. *Colletotrichum* dominated in young leaves from each site in both years with a highest percentage of 66.67% (Table 33). There are therefore significant differences in both level of infection and species present between young and mature leaves. All the identified taxa from young leaves were also recorded in mature leaves but at a reduced frequency and in the presence of greater species diversity.

3.3.3 Tissue specificity of endophytic fungi

Tissue specificity was first considered by Carroll *et al.* (1977) during their studies of endophytes from European conifer trees. It was shown that many of the fungi isolated from the petioles of the needles were restricted to that area and were infrequently isolated from more distal portions of the needle. Further studies have confirmed this (Carroll & Carroll, 1978; Petrini & Müller, 1979; Petrini & Fisher, 1986,1988; Fisher & Petrini, 1987, 1988; Stone 1987, 1988; Bertoni & Cabral, 1988; Petrini & Carroll, 1981; Sieber *et al.*, 1991; Fisher *et al.*, 1992, 1994; Hata & Futai, 1995; Lodge, Fisher & Sutton, 1996). Luginbuhl & Müller (1980) investigated endophytic fungi from *Buxus, Hedera, Ilex* and *Ruscus* and found that they were restricted to the cortex within stems and within a leaf. They were concentrated near veins with a very high recovery from near midrib. The seeds were not infected (Luginbuhl & Müller, 1980). According to Stone (1987) *Rhabdocline parkeri* and

Phyllostricta sp. coexisted in needles of *Pseudotsuge menziesii* (Mirbel) Franco with the *R. parkeri* confined to epidermal and hypodermal cells and the *Phyllostricta* occurring intercellularly in the mesophyll.

In teak leaves endophytes were freely isolated from leaf lamina, midrib and veins and with the exception of samples from mature leaves from Mae Rim in 1995 no significant differences were found between these areas based on the Wilcoxon sign test (e.g. P-value 0.9374 for Mae Rim 1996). There were differing results, since in Mae Rim 1995 a significant difference between 2 areas on the leaf lamina compared to the other areas (P-value 0.0312) was found. It is apparent, however, that tissue specificity does not occur for the endophytic fungi from teak leaves. Wu (1997) found *Virgariella sp.* (probably a xylariaceous anamorph) was more commonly isolated from midrib on *Rhododendron* spp. However, in teak leaves the xylariaceous representatives occur commonly on lamina, midrib and vein without showing any clear preference. Petrini *et al.* (1992) considered each plant organ and tissue to represent distinct microhabitats each having different microecological and physiological conditions and this they suggested might be the reason for organ or tissue specificity. In the case of teak this is not the case and distribution of endophytes appears to be on a random or haphazard basis. Carroll (1995) and Lodge *et al.* (1996) advocated the use of many subsampling units per leaf but on the basis of 40 individual segments of 0.5 cm^2 each from 5 leaves for 3 years it is unlikely that further sampling will greatly change the pattern reported here.

Chapter 4

Identification of teleomorphs of endophytic xylariaceae isolated from teak leaves

4.1. Introduction

In reviewing the taxonomy of endophytic fungi (Petrini, 1985; Petrini *et al.*, 1992) attention was drawn to the fact that most endophytic fungi recovered from different plants are Ascomycetes and their anamorphs. They also stressed that identifying endophytic fungi to species level is a major problem because of the shortage of comparative data and frequently the absence of a distinctive sporing structure. Except for a few species, which produce distinctive characters allowing identifications to be made, most endophytic fungi are either not named or are named with assumptions based on the host plants. This assumes host specificity which might be incorrect. Various approaches have been undertaken to overcome this problem by using biochemical and molecular techniques in some common endophytic genera including *Xylaria, Phyllosticta, Leptostroma* (Brunner & Petrini, 1992; Rodrigues, *et al.*, 1993; Sieber-Canavesi *et al.*,1991) but with limited success. Members of the Xylariaceae are notoriously difficult since only a few produce a teleomorph in culture and most do not produce sufficiently distinctive cultures or anamorphs to enable confident identification to be made (Whalley, 1996). A few species have been determined by comparison of cultures obtained from free living teleomorphs with endophytic isolates (Rodrigues & Samuels 1990, 1992; Rodrigues, 1994; Laessøe & Lodge, 1994). Since the Xylariaceae are common as endophytes in tropical plants (Rodrigues, 1994) and since members of the Xylariaceae were regular and sometimes dominant isolates from teak leaves attempts at identification to species level were considered to be important. Two approaches were undertaken, one to induce the formation of mature teleomorphs and two to use secondary metabolite profiles from endophytes and to match these with those obtained from known species.

4.2. Induction of teleomorph formation

All isolates considered to be xylariaceous were separated for experimentation following the broad procedure previously used (Mekkamol *et al.*, 1996). To induce teleomorph formation pure cultures of individual xylariaceous isolates were grown on PDA in glass bottles 11 cm tall and 6 cm diam. until the surface of the agar was completely covered by the fungus. Pieces of cut twig approx. 8 cm long x 3 cm diam. of teak and 4 other tree species were introduced following sterilization. After appropriate incubation the twigs were removed and subjected to special environmentally devised incubation to allow teleomorph formation (Chapter 2; Figs. 3. and 4.).

4.2.1. Results

Following appropriate incubation regimes mature teleomorphs were produced in over 60% of the isolates (Table 30. and Fig 15.). Species of *Xylaria* (including one Penzigoid form) were obtained together with *D. eschscholzii, N. subannulata* and 5 species of *Hypoxylon.* Differences in time for development to maturity and ability to mature were observed (Tables 30 and 31). *Daldinia escheschol zii* readily developed to maturity with 100% success within 14-16 weeks. A number of species of *Xylaria*, including *X. aristata, X. cubensis, X. feejeensis* and *X. juruensis var. microspora* were named on the basis of their mature teleomorph. *Xylaria cubensis* is well known as an endophyte in the tropics (Rodrigues, 1994) but *X. aristata*, a leaf inhabitant is recorded as an endophyte for the first time. Similarily, *X. juruensis var. microspora* var.nov. (Thienhirun, 1997) is found for the first time as an endophyte. *Hypoxylon haematostroma* is known from free living teleomorphic material in Thailand (Thienhirun, 1997). It is also a widespread and common species which is known from Chiang Mai province (Thienhirun, 1997) and was found occuring naturally in the forest near to both of the sampling sites. *Daldinia eschscholzii* and *N. subannulata* were also found in the vicinity (Chapter 5). *Hypoxylon sp.* 188 is considered to be a new species not listed in Ju & Rogers (1996). Whether it occurs in a free living condition near by or elsewhere remains to be seen. One collection of *Hypoxylon sp.* 51 was

Table 30. Teleomorphs obtained from twig culture of endophytic Xylariaceae from teak leaves.

Taxa	Number of isolates	Number reaching maturity
Daldinia eschscholzii (Ehrenb.:Fr.) Rehm.	35	35
Hypoxylon sp. 188 (new species)	7	5
H. cf. anthochroum Berk.& Broome.	5	1
H. haematostroma Mont.	20	20
H. subrutilum Starb.	20	12
H. cf. subrutilum Starb.	20	15
Nemania subannulata (Henn. & E. Nyman)	15	10
Penzigia sp. (new species)	1	1
Xylaria cf. allantoidea (Berk.) Fr.	1	1
X. aristata Mont.	10	9
X. cubensis (Mont.) Fr.	25	24
X. feejeensis (Berk.) Fr.	8	6
X. juruensis var. microspora sp. nov.	10	10
Xylaria sp. 294 (new species)	2	2
Xylaria sp. 29 (new species)	3	3
X. grammica (Mont.) Fr.	4	3[a]
7 Xylaria spp. Isolates	11	6[a]
Non-stromatic isolates	77	
Total	240	163

[a] immature stromata

Figure 15. Appropriate incubation regimes require for production of teleomorph of xylariaceous endophytes

a Colonised twigs were incubated in steriled moist sand in shade condition

b Wet condition require for teleomorph production of *Xylaria* and *Nemania*

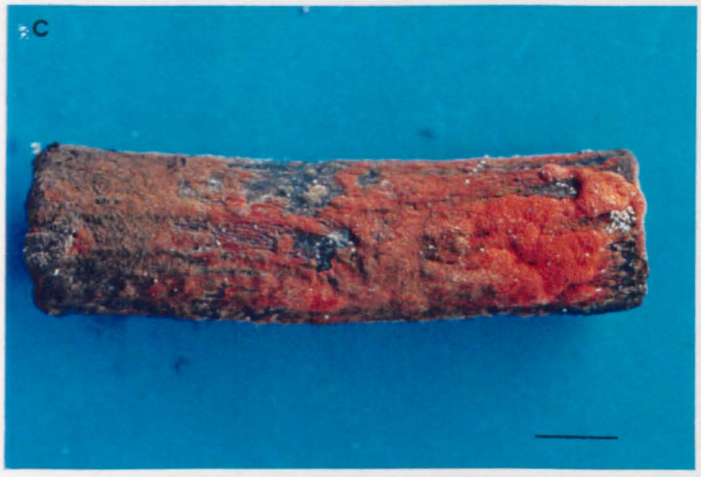
c Mature teleomorph of *Hypoxylon haematostroma* (Bar = 1 cm)

d Mature teleomorph of *Xylaria sp. 294* (Bar = 1 cm)

e Mature teleomorph of *Xylaria cubensis* (Bar = 1 cm)


a


b


c


d

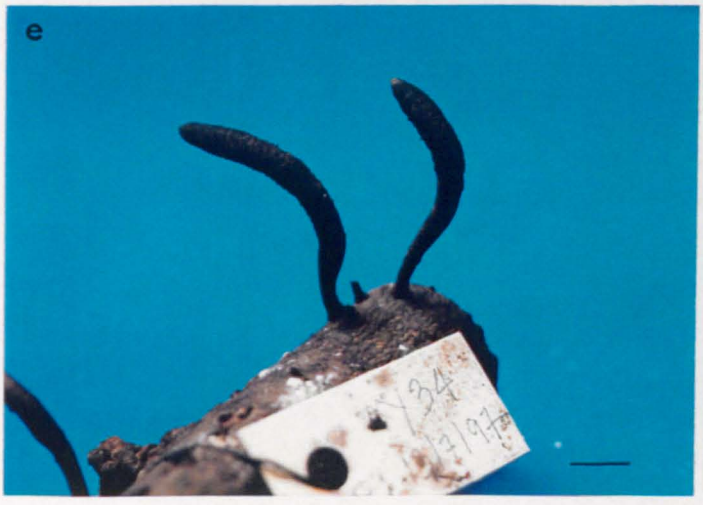

e

Table 31. Specific length of time required to reach maturity for some endophytic Xylariaceae from teak leaves.

Taxa	Weeks*
Daldinia eschscholzii (Ehrenb.:Fr.) Rehm.	14-16
Hypoxylon sp. 188	21-25
H. cf. anthochroum Berk.& Broome.	25
H. haematostroma Mont.	20-24
H. subrutilum Starb.	20-21
H. cf. subrutilum Starb.	25-27
Nemania subannulata (Henn. & E. Nyman)	14-16
Penzigia sp.	12-13
Xylaria cf. allantoidea (Berk.) Fr.	14-16
X. aristata Mont.	13-15
X. cubensis (Mont.) Fr.	13-16
X. feejeensis (Berk.) Fr.	18-20
X. juruensis var. microspora sp. nov.	14-26
Xylaria sp.29	30-32
Xylaria sp.294	30-32

* after twig inoculation and incubation under appropriated environment conditions

identified as *H. subrutilum* (Ju & Rogers, pers. comm) but examination of the spore shape and cultural characteristics suggest that it is likely to be new. A number of *Xylaria* species failed to develop to maturity but several of these have been identified by other means. It is also interesting to note that a number of common species, *D. eschscholzii, N. subannulata, X. cubensis*, attained maturity in 14-16 weeks (Table 31). The *Hypoxylon* species were in general slower to develop taking between 20-27 weeks depending on the species. The two new species of *Xylaria* sp. 29 and sp. 294 were late in maturing requiring 30-32 weeks incubation before mature asci and ascospores were formed (Table 31).

4. 3. Secondary metabolite profiles

Since secondary metabolites of Xylariaceae have been the subject of extensive investigation and since species specific compounds and characteristic secondary metabolite profiles occur (Whalley & Edwards, 1995; Whalley, 1996) they could provide means of identifying unknown endophytic xylariaceae. Compounds identified to date include dihydroisocoumarins (Anderson, Edwards & Whalley, 1983), butyrolactones (Edwards & Whalley, 1979; Anderson, Edwards & Whalley, 1982), sesquiterpene alcohols (Anderson *et al.*, 1984 a,b; Poyser *et al.*, 1986; Edward, Poyser & Whalley, 1988), cytochalasins (Edwards, Maitland & Whalley, 1989), succinic acid and derivatives (Anderson, Edwards & Whalley, 1985) cubensic acid (Edwards, Maitland & Whalley, 1991), globoscinic acid and globoscin (Adeboya *et al.*, 1995a), berteric, cameronic and malaysic acids (Adeboya *et al.*, 1995b). Cultures of selected endophytes were grown in liquid malt extract under static conditions for 6-8 weeks followed by solvent extraction with ethyl acetate, column and thin layer chromatography and chemical analysis using NMR and Infrared spectroscopy

4. 3.1. Results

The identity of several endophytic isolates was obtained by matching their secondary metabolite profiles on TLC and through identification of key compounds. A typical profile is shown in Fig. 16. and on the basis of further chemical investigations *X. grammica* and a new species of *Xylaria* have been confirmed. *Xylaria grammica* (Fig. 17.) is known to produce

gramicin, 4-hydroxymellein and γ- pyrone 3- methyl acetate (Anderson *et al.*, 1983). Whilst isolates 294, 564 and 587 produce corioxin and are believed to represent a new species related to *Xylaria obovata.*

4. 4. Discussion

The problems associated with identification of endophytic Xylariaceae have been noted by several authors (Petrini & Petrini, 1985; Rodrigues, 1994; Wu, 1997; Whalley, 1996; Petrini *et al.*, 1995; Rodrigues & Petrini, 1997). Since few xylariaceae develop stromata in culture the failure of many isolates to produce a distinctive anamorph in culture has, in the majority of cases, made accurate identification extremely difficult (Whalley, 1996). Furthermore very few species produce sufficiently distinctive cultures to enable naming. *Xylaria cubensis* is, however, one species which produces a flabelliforme anamorph in culture which appears to be a species characteristic (Rogers, 1984; Van der Gucht, 1995). In the absence of a mature teleomorph, suitably distinctive anamorph, or characteristic culture, other approaches are neccessary to make identifications. Generally it is possible to segregate cultures into genera on the basis of overall cultural characteristics and type of anamorph when produced. For example most species of *Daldinia* and *Hypoxylon* produce a *Nodulisporium* or related anamorph and *Nemania* typically forms a *Geniculosporium* anamorph (Laessøe, 1994; Whalley, 1996). *Camillea* species are the only ones known to date to produce a *Xylocladium* anamorph (Laessøe, Rogers & Whalley, 1989; Whalley, 1996) although as yet no *Camillea* has been isolated as an endophyte (Petrini *et al.*, 1995). However a part from these general groupings identification to species is rarely possible. Attempts have been made using isoenzyme patterns of *Xylaria* species (Brunner & Petrini, 1992; Rodrigues *et al.*, 1993) or by comparison of cultures obtained as endophytes with those obtained from authenticated teleomorphic Xylariaceae (Petrini & Petrini, 1985; Rodrigues, 1994). Since cultures of closely related species may be very similar identification on this basis is not entirely reliable (Whalley, 1996; Mekkamol *et al.*, 1996). Ideally formation of mature teleomorphs would reslove the problem but few species produce teleomorphs in culture and although many species of *Xylaria* develop rudimentary stromata these never develop to maturity (Callan & Rogers, 1990).

Inoculation of pure cultures into twigs of teak and other woody hosts, followed by incubation to simulate natural conditions is one way identification might be achieved.

Figure 16. Metabolite profiles on TLC of some xylariaceous endophytes

Isolate 497	unknown
Isolates 327, 503, 488	*Xylaria juruensis var. microspora*
Isolates 587, 564, 294	*Xylaria new species*
Isolates 598, 420	*Xylaria cubensis*
Isolate M271	unknown
Isolate 342	unknown
Isolates 248, 515, 263	*Xylaria feejeensis*
Isolates 306, 307, 536	*Xylaria grammica*

Age of fungal culture: 8 weeks

Growth medium: 3% MA

Conditions for TLC:

- incubation at 25°C under static condition
- solvent: ethyl acetate
- visualisation spay: Anisaldehyde

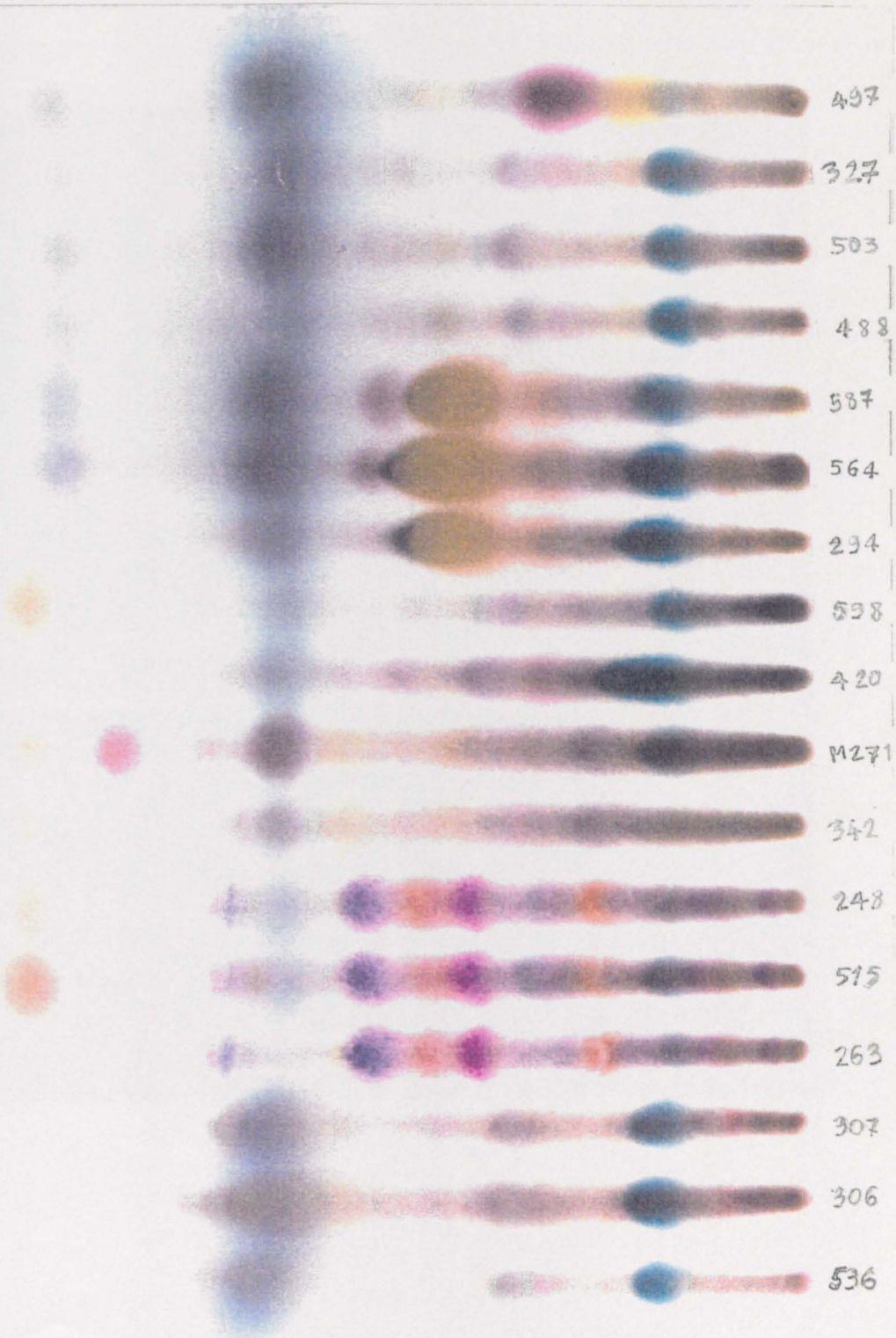

497
327
503
488
587
564
294
538
420
M271
342
248
515
263
307
306
536

Figure 17. ***Xylaria grammica*** **(Mont.) Fr.**

a Mature culture on PDA showing characteristic carbonaceous regions

b Developing stromata on agar surface (do not attain maturity) (Bar = 1 cm)

c Developing stromata on twig (Bar = 1 cm)

d Almost mature teleomorphic stromata with distinctive longitudinal striations (Bar = 1 cm)

However, in the current study attempts to produce teleomorphs or to initiate mature teleomorph development by placing inoculated twigs in the forest was not successful. This was due to the drying out of specimens and the loss of material to termites and monkeys. However, by placing inoculated twigs in damp sand either in bags, open or sealed, or in earthware pots it was possible to produce mature stomata from many of the isolates. In some cases mature stromata were readily formed e.g. *D. eschscholzii, N. subannulata, H. haematostroma, X. cubensis and X. aristata.* In other species stromata developed but required longer incubation periods and then not all isolates of what were considered to be the same fungus developed to maturity. Nevertheless, it was possible to identify 14 species of Xylariaceae with a success rate of over 60% in induction of teleomorph development. It is significant that at least one new species of *Hypoxylon* and 2 or 3 new species of *Xylaria* have been isolated as endophytes and mature stromata produced.

In an entirely different approach to identification of endophytes grouped as *Xylaria* species secondary metabolite profiles and the presence of specific chemical markers were investigated. The Xylariaceae are well known producer of a wide range of secondary metabolites but these appear to be species specific in the majority of cases (Whalley & Edwards, 1995; Whalley, 1996). On the basis of profiles *X. grammica* was recognised to be endophytic and others were grouped one the basis of the chemicals present. It seems likely that chemical profiles from endophytes can be used more widely to identify the species concerned by matching of profiles with those from known species. On the basis of this limited study the technique looks very promising. As Rodrigues & Petrini (1997) stated "The identification of xylariaceous endophytes is difficult because they very seldom form the teleomorph in culture". A correct identification of endophytic Xylariaceae is problematic even after direct comparison with cultures derived from ascospores of known teleomorphs (Petrini & Petrini, 1985) and a combination of morphological characters with biochemical analyses is probably the only way to achieve satisfactory identification of species (Rodrigues *et al.*, 1993)". A combination of teleomorph induction and secondary metabolite profiles in the absence of molecular techniques would therefore seem to be more accurate and useful at the present time. In this study a wide range of Xylariaceae were identified using combined techniques which enabled a greater rate of identification to be made compared with the earlier study by Rodrigues (1994) on *Euterpe oleracea* (Table 32).

Table 32. Xylariaceous fungi isolated from leaves of *Euterpe oleracea* (Rodrigues, 1994) and from leaves of *Tectona grandis*.

Euterpe oleracea	***Tectona grandis***
Daldinia eschscholzii (Ehrenb.) Rehm.	*Daldinia eschscholzii* (Ehrenb.) Rehm.
Hypoxylon quisquiliarum (Mont.) Mont.	*Hypoxylon sp.* 188 (new sp.)
H. serpens (Pers.: Fr.) Kickx	*H. cf. anthochroum* Berk.& Broome
H. stygium (Lev.) Sacc.	*H. haematostroma* Mont.
Penzigia ?berteri (Mont.) J. H. Miller	*H. subrutilum* Starb.
Xylaria adscendens (Fr.) Fr.	*H. cf. subrutilum* Starb.
X. allantoidea (Berk.)	*Nemania subannulata* (Henn. & E. Nyman)
X. cf. anisopleuria (Mont.) Fr.	*Penzigia sp.*(new sp.)
X. arbuscula Sacc.	*Xylaria sp. 29* (new sp.)
X. cf. castorea Berk.	*Xylaria sp. 294* (new sp.)
X. coccophora Mont.	*X. cf. allantoidea* (Berk.) Fr.
X. cubensis (Mont.) Fr.	*X. ariatata* (Mont.) Fr.
X. curta Fr.	*X. cubensis* (Mont.) Fr.
X. cf. microceras (Mont.) Fr.	*X. feejeensis* (Berk.) Fr.
X. cf. multiplex (Kunze) Fr.	*X. grammica* (Mont.) Fr.
X. cf. obovata (Berk.)	*X. juruensis var microspora* sp. nov.
X. cf. palmicola Winter	7 *Xylaria spp.*
X. cf. telfairii Berk. & Fr.	

Chapter 5

Taxonomy of endophytic species and relevant Xylariaceae

5.1. Taxonomic aspects of Xylariaceae

Introduction

The species composition of members of the Xylariaceae collected during surveys of the natural forest at Mae Rim and from the plantation and nearby forest at Chiang Dao are presented in Table 33. In both areas a range of Xylariaceae were recorded and application of the Sorenson Index (Chapter 2) shows a high level of shared species. Fifteen species of Xylariaceae isolated as endophytes were identifed on the development of mature stromata (Chapter 4).

5.1.1. Description of the species

Daldinia bambusicola Y.-M. Ju, J.D. Rogers & San Martin. Fig. 18.

Stromata globose, sessile or subsessile, smooth, 1-2.0 cm diam x 0.5-1.5 cm high; surface brown vinaceous or sepia, blackened and somewhat varnished in age; dull reddish brown granules immediately beneath surface, with KOH-extractable pigments dark livid; the tissue between perithecia white to greyish brown, pithy to woody; the tissue below the perithecial layer composed of alternating zones, the darker zones dark brown, pithy to woody, 0.1-0.2 mm thick, the lighter zones white to greyish brown, pithy to woody, persistent, 1.2-2.0 mm thick.

Perithecia obovoid to tubular, 1.2-1.5 mm high x 0.7-1.0 mm diam.

Ostioles slightly papillate.

Table 33. Xylariaceae collected in natural forest during August-November 1997

Site A	Site B	Site C
Daldinia bambusicola	*Daldinia eschscholzii*	*Daldinia bambusicola*
Daldinia eschscholzii	*Hypoxylon investiens*	*Daldinia eschscholzii*
Hypoxylon anthochroum	*H. moriforme*	*Hypoxylon anthochroum*
H. crocopeplum	*X. feejeensis*	*H. haematostroma*
H. fuscum		*H. monticulosum*
H. haematostroma		*H. perforatum*
H. lividicolor		*H. subgilvum*
H. rubiginosum		*N. subannulata*
N. subannulata		*Xylaria allantoidea*
Xylaria badia		*X. badia*
X. cubensis		*X. culleniae*
X. culleniae		*X. feejeensis*
X. feejeensis		*X. juruensis var. microspora*
X. multiplex		*Xylaria sp.nov 1(on pod)*
X. schweinitzii		*Xylaria sp.nov2(yellow flesh)*
Xylaria sp.nov2(yellow flesh)		

Site A : Mae Rim natural forest
Site B : Chiang Dao plantation
Site C : forest near to plantation

Figure 18. ***Daldinia bambusicola*** **Y.-M. Ju, J. D. Rogers & Martin**

a Stromata on bamboo (Bar = 10 cm)

b Stroma showing concentric zones (Bar = 1 cm)

c Ascus (Bar = 10 µm)

d Ascospores with straight germ slit spore-length (arrowed) (Bar = 10 µm)

Asci 115-120 µm total length x 5-7 µm broad, the spore-bearing parts 45-55 µm long, the stipe 70-75 µm long, with apical apparatus, discoid, 0.6 µm high x 2.0 µm broad, blueing in Melzer's iodine reagent.

Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrow rounded ends, 8.8-10.0 x 4.4-5.0 µm with straight germ slit spore-length on convex side; perispore dehiscent in 10% KOH, inconspicuous or conspicuous coil ornamentation; epispore smooth.

Habitat on bamboo

Daldinia eschscholzii (Ehrenb.: Fr.) Rehm., Ann. Mycol. 2: 175. 1904. Fig. 19.

Lectotype (selected by Ju *et al.*, of *Daldinia luzonensis*) Philippines: Luzon, Laguna Prov., Los Banos, Baker, C.F. 516, comm. via Merrill, E.D., wood (BPI, 716999 [Lloyd herb. 12401]).

Synonyms (following Ju *et al.*, 1997)

Sphaeria eschscholzii Ehrenb.: Fr., Fung. Chamisso Coll.,pl 18, fig. 8. 1820.

Sphaeria concentrica Bolton: Fr. var. *eschscholzii* (Ehrenb.: Fr) Fr., Syst. Mycol. II, p. 331. 1823.

Daldinia concentrica (Bolton: Fr.) Ces. & De Not. var. *eschscholzii* (Ehrenb.: Fr.) Starb., Bih. Kongl. Svenska Vetensk.-Akad. Handl. 27,3: 5. 1901.

Hypoxylon stratosum Sacc., Syll. Fung. IX, p. 544. 1891.

Daldinia stratosa (Sacc.) Sacc. & Trott., Syll. Fung. XXII, p. 327. 1913.

Daldinia luzonensis Rehm, Philipp. J. Sci., Bot. 8: 260. 1913.

Stromata turbinate to placentiform, sessile or with short, stout stipe, solitary to infrequently aggregated, smooth, 1.5-4 cm diam x 1-3 cm high; surface brown, vinaceous, dark brick, sepia, greyish sepia or vinaceous grey, blackened and varnished in age; dull reddish brown granules immediately beneath surface, with KOH-extractable pigments livid purple, dark livid or vinaceous purple; the tissue between perithecia brown, pithy to woody; the tissue below the perithecial layer composed of alternating zones, The darker zones dark brown, pithy to woody, 1-0.2 mm thick, the lighter zones white, grey or greyish brown, gelatinous and very hard when dry, becoming pithy to woody, persistent, 0.3-1 mm thick.

Figure 19. ***Daldinia eschscholzii*** **(Ehrenb.: Fr.) Rehm.**

a Culture on PDA

b Anamorphic stromata on wood (Bar = 1 cm)

c Mature teleomorphic stromata with spore discharge (arrowed) (Bar = 1 cm)

d Cut stroma showing zonation (Bar = 1 cm)

e Long tubular perithecia (Bar = 1 mm)

f Ascospores with ornamentation (Bar = 1 μm)

g Ascus with apical apparatus (arrowed) and ascospores with straight germ slit spore-length (arrowed) (Bar = 10 μm)

Perithecia tubular, 0.8-1.6 mm high x 0.3-0.4 mm diam

Ostioles obsolete or slightly papillate.

Asci 95-125 µm long, with apical apparatus, discoid, 0.5 µm high x 2-2.5 µm broad blueing in Melzer's iodine reagent.

Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 11.3-12.5 x 5.0-6.3 µm, with straight germ slit spore-length on convex side; perispore dehiscent in 10% KOH, conspicuous coil ornamentation; epispore smooth.

Cultural characteristics

Colonies on malt extract agar, under 12 h light and 12 h darkness, reaching edge of a 9 cm diam. Petri dish in 2 weeks; at first white to orange-white in the centre, becoming pale to dull yellow, floccose, finally in patches orange-grey to brownish grey in the centre, greyish yellow and olive-brown towards the margins, and in between, brown woolly with coarse texture. Reverse at first pale yellow to greyish yellow and finally fairly uniform dark greyish brown. Exudate orange to reddish brown. Agar staining yellowish brown to brown. Odour ether-like with a sweet component. Hyphae sparingly branched, septate, at first hyaline, later light brown, smooth or slightly verrucose, 2-3 µm diam. *Conidiogenous structures* formed after 3-4 days, at first in the centre, later abundant throughout the whole colony, directly on the mycelium. *Conidiophores* mononematous, di-or trichotomously branched, especially towards the apex, regularly septate, at first hyaline and smooth, later light brown and verrucose, up to 280 µm long x 2-3 µm diam. *Conidiogenous cells* terminal, cylindrical, at first hyaline and smooth, later light brown and verrucose, 10-20 x 2-3 µm, bearing circular refractile to more or less denticulate conidial scars in the somewhat flattened apices. *Conidia* acrogenous, ellipsoid to obovoid, with a flattened circular abscission scar at the base, hyaline, smooth by L.M. (4)4.5-7(8) x 2-3(3.5) µm.

Habitat endophyte, on dead wood, usually logs, lying on the forest floor in lowland rain forest, mostly in sun-exposed sites like man-made fellings (e.g. for gardening) or natural clearing (tree fall areas), or in localities bordering gardens or roads, and in plantations, sometimes on very dry sites.

Hypoxylon anthochroum Berk. & Broome, J. Linn. Soc., Bot. 14: 122. 1873. Fig. 20.

Hypoxylon murcidum Berk. & Broome, J. Linn. Soc., Bot. 14: 123. 1873.

Hypoxylon albostigmatosum Speg. Anales Soc. Ci. Argent. 18: 271.1884.

Hypoxylon rubiginosum (Pers.:Fr.)fr.f. *albostigmatosum* (Speg.) Theiss., Ann. Mycol 7; 147. 1909.

Hypoxylon guarapiense Speg., Anales Soc. Ci. Argent. 18: 272. 1884.

Hypoxylon fuscopurpureum (Schwien.:Fr.) M.A. Curtis f. *corticola* Starb., Bih. Kongl. Svensk Vetensk.-Akad. Handl. 27, 3: 9. 1901.

Stromata effused-pulvinate, plane or with inconspicuous perithecial mounds, 0.3-4(10) cm long x 0.3-3 cm broad x 0.5-1.1 mm thick; surface sepia, dark vinaceous; dull reddish brown or blackish granules immediately beneath surface and between perithecia, with KOH-extractable pigments isabelline olivaceous grey or amber; the tissue below the perithecial layer inconspicuous.

Perithecia obovoid to tubular, 0.2-0.4 mm diam x 0.3-0.6 mm high.

Ostioles lower than the stromatal surface.

Asci 90-140 µm total length x 7.5-8.0 µm broad, the spore-bearing parts 65-95 µm long, the stipes 30-50 µm long, with apical ring blueing in Melzer's iodine reagent, discoid, 1.3 µm high x 2.5 µm broad.

Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 12.5-15.0 x 6.3-7.5 µm, with straight to slightly sigmoid germ slit spore-length; perispore dehiscent in 10% KOH, smooth or with inconspicuous to conspicuous coil-like ornamentation; epispore smooth.

Fits description of *H. anthrochroum* (Ju & Rogers, 1996) except for larger spores 12.5-15 x 6.3-7.5 µm compared to 8.5-13.5 x 4-6 µm (Ju & Rogers page 88). However, Ju & Rogers (1996) refer to a collection from Taiwan (Ping-Tung Co., Heng-Chun, Ken-ting. 11.9.1991. Ju, Y-M. 80091101 which appears to be identical with the Chiang Dao collection (sp. for 80091101 = 12-14(-15) x 6-7 µm.)

Colonies on OA covering Petri dish in 2 weeks, at first whitish, becoming olivaceous with sporulation. velvety, azonate, with diffuse margins; reverse uncoloured. Sporulating regions

Figure 20. *Hypoxylon anthochroum* Berk. & Broome

a Stromata on bark (Bar = 1 cm)

b Surface of stroma (Bar = 1 mm)

c Ostiole lower than the stromal surface (Bar = 10 µm)

d Asci (Bar = 10 µm)

e Ascospores with ornamentation (Bar = 10 µm)

f Ascospores with straight germ slit spore-length and perispore dehiscent in 10% KOH (arrowed) (Bar = 10 µm)

scattered over entire surface of colony, olivaceous. Conidiogenous structure *Nodulisporium*-like, hyaline to yellowish, smooth to finely roughened. Conidiogenous cells hyaline, smooth, 8.2 x 3-4.5 μm. Conidia yellowish, smooth, ellipsoid, 4.5-7 x 3-4 μm.

Habitat endophyte, on bark and logs.

Hypoxylon crocopeplum Berk. & M. A. Curtis apud Berk., Grevillea 4: 49. (1875). Fig. 21.

Hypoxylon crocatum Mont. Apud C. Gay, Fl. Chilena VII, p. 343. 1850.

Hypoxylon ochraceofulvum Berk. & Cooke apud Cooke, Grevillea 11: 133. 1883.

Hypoxylon polyporoideum Berk. ex Cooke, Grevillea 12: 53. 1883.

Hypoxylon haematostroma Mont. Subsp. *haematozonum* Sacc., Syll. Fung. IX, p. 549. 1891.

Hypoxylon ferrugineorufum Henn., Hedwigia 39: 138. 1900.

Stromata effused-pulvinate, plane or with inconspicuous to conspicuous perithecial mounds, 2.5-5 cm long x 2-3 cm broad x 0.5-0.6 cm thick; surface brown vinaceous to sepia; orange red granules immediately beneath surface and between perithecia, with KOH-extractable pigment sienna or ochraceous; the tissue below the perithecial layer inconspicuous to 1 mm thick, black.

Perithecia spherical, obovoid to long tubular, 0.2-0.3 mm high x 0.2-0.7 mm diam.

Ostiole lower, or at the same level as, the stromatal surface.

Asci 115-150 μm total length x 6-7 μm, the spore part 70 x 85 μm long, the stipe 45 x 65 μm, with apical apparatus slightly discoid, 0.6 x 2.5 μm broad, blueing in Melzer's iodine reagent.

Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 11-13.8(15.6) x 5.0-6.3 μm, with straight or slightly sigmoid germ slit spore-length; perispore dehiscent in 10% KOH, with inconspicuous to conspicuous coil-like ornamentation; epispore smooth.

Cultural characteristics and anamorph

Anamorph typical for this species, Ju and Rogers (1996) reported the anamorph as *Virgariella*-like.

Figure 21. ***Hypoxylon crocopeplum*** **Berk. & M. A. Curtis apud Berk.**

a Surface of stroma with orange red granules immediatedly beneath surface (arrowed) (Bar = 1 mm)

b Ascospores with ornamentation (Bar = 10 μm)

c Ascospore with straight germ slit spore-length (arrowed) (Bar = 10 μm)

Habitat on wood and bark

Hypoxylon fuscum (Pers.:Fr) Fr., Summa Veg. Scand. II, p.384.1849. Fig. 22. (d-e)

Sphaeria fusca Pers., Ann. Bot (Ursteri) 11: 22. 1794; Per.: Fr., Syst. Mycol. II, p. 332. 1823.

Peripherostoma fuscum (Pers.: Fr.) S. F. Gray, Nat. Arr. Brit. Pl. l, p.513. 1821.

Stromatosphaeria fusca (Pers.: Fr.) Grev., Fl. Edinburgh, p. 356. 1824.

Sphaeria fragiformis Hoffm., Veg. Crypt. l, p.20. 1787; non Pers. [: fr.], 1794; [nom.rejic., ICBN Art. 13.1 (d)]. Fide Persoon (1801), Fries (1823), and Tulasne and Tulasne (1863).

Sphaeria confluens Willd., Fl. Berol. Prodr., p. 416. 1787; non Tode [:Fr.], 1791; [nom.rejic., ICBN Art. 13.1 (d)]. Fide Persoon (1801), Fries (1823), and Tulase and Tulasne (1863).

Hypoxylon fuscum (Pers.: Fr.) Fr. var. *confluens* (Willd.) J. Kicdx fil., Fl. Crypt. Fland. l, p. 307. 1867. *Hypoxylon confluens* (Willd.) Wettst., Verh. Zoo.-Bot.. K.K. Ges. Wien 35: 592. 1885; non (Tode: Fr.) Westend., 1845; [nom. rejic., ICBN Arts. 13.1 (d) & 64.1].

Sphaeria tuberculosa Bolton, Hist. Fung. Halifax, III, p. 123. 1789; non Sowerby, 1803; nec Schwein. [:Fr.], 1822; [nom. rejic., ICBN Arts. 13.1 (d)] Fide Persoon (1801), Fries (1823), and Tulasne and Tulasne (1863).

Sphaeria castorea Tode, Fung. mecklenb. Sel. II, p. 28. 1791. Fide Fries (1823), and Tulasne and Tulasne (1863).

Sphaeria fragiformis Pers.: Fr. var. *castoreaj*(Tode) Pers., Syn. Meth. Fung., p. 10. 1801.

Hypoxylon glomerulatum Bull., Hist. champ. France I, p. 178. 1791; non Theiss., 1908. Fide Fries (1823), and Tulasne and Tulasne (1863).

Sphaeria glomerulata (Bull.) DC. & Lam., Fl. Franc. II, p. 287. 1805.

Sphaeria effusa Sowerby, Col. Fig. Engl. Fung. III, Pl. 374, Fig. 8. 1803. Fide Fries (1849).

Sphaeria coryli DC. & lam., Fl. Franc.II. 287. 1805; non Batsch. [:FR.], 1786; [nom. rejic., ICBN Arts. 13.1 (d)] Fide Fries (1823), and Tulasne and Tulasne (1863).

Hypoxylon vinosum Mont., Ann. Sci. Nat. Bot., ser.II, 13: 356. 1840.

Figure 22. (a-c) *Hypoxylon perforatum* (Schwein.: Fr.) Fr.

a Stromata on bark (Bar = 10 mm)

b Surface of stroma with inconspicuous perithecial mounds (Bar = 1 mm)

c Ascospores with perispore dehiscent in 10% KOH (Bar = 10 µm)

Figure 22. (d-e) *Hypoxylon fuscum* (Pers. :Fr.) Fr.

d Surface of stroma with inconspicuous perithecial mounds (Bar = 1 mm)

e Ascospores with perispore dehiscent in 10% KOH (Bar = 10 µm)

Hypoxylon purpureum Nitschke, Pyren. Germ., p. 37. 1867.

Hypoxylon commutatum Nitschke subsp. *holwayanum* Sacc. & Ellis apud Sacc., Michelia 2: 570. 1882.

Hypoxylon bicolor Ellis & Everh., J. Mycol. 2: 88. 1886; non Berk. & M.A. Curtis, 1875; [nom. rejic., ICBN Arts. 64.1].

Hypoxylon bicoloratum P. Martin, J. S. African Bot. 42; 72. 1976.

Hypoxylon subchlorinum Ellis & Calk., J. Mycol. 4: 86. 1888.

Hypoxylon lianincolum Rehm, Leafl. Philipp. Bot. 6: 1944. 1913.

Hypoxylon pruinatoides Kauffm., Pap. Michigan Acad. Sci. 11: 169. 1930.

Hypoxylon oregonense Kauffm., Pap. Michigan Acad. Sci. 11: 169. 1930.

Illustration: Miller (1961, Figs. 4,5, & 37).

Stromata usually hemispherical to pulvinate, sometimes effused-pulvinate, plane or with inconspicuous perithecial mounds, 1-6 cm long x 0.3-3 cm broad x 0.3-2 cm thick; surface brown vinaceous or dark vinaceous; buff, dull orange, dull orange-brown, or dull reddish brown granules immediatedly beneath surface and between perithecia, with KOH-extractable pigments amber, isabelline; the tissue below the perithecial layer dark brown to blackish brown, inconspicuous to 1.5 mm thick

Perithecia spherical to obovoid, 0.1-0.4 mm diam x 0.2-0.5 mm high

Ostioles lower than the stromatal surface.

Asci 130-170 µm total length x 6-10 µm broad, the spore-bearing parts 86-109 µm long, the stipes 45-60 µm long, with apical ring blueing to lightly blueing in Melzer's iodine reagent, discoid, 1-1.3 µm high x 3.8-5 µm broad .

Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 15.0-20.0 µm x 6.3-8.1 µm, with slightly sigmoid germ slit spore-length; perispore dehiscent in 10% KOH, smooth or with inconspicuous coil-like ornamentation; epispore smooth.

This fits in with the concept of *H. fuscum* given by Ju & Rogers (1996) as an effused form. Colour similar to European collections on *Alnus* but not on *Corylus*.

Colonies on OA covering Petri dish in 3 weeks, at first white, remaining white, or becoming smoke gray, pale luteous, or hazel, velvety, azonate, with diffuse margins, with

radiate furrows from centre; reverse fawn. Sporulating regions in patches, vinaceous buff. Conidiogenous structure *Virgariella-like,* hyaline, smooth to finely roughened. *Conidiogenous cells* hyaline smooth, 8-15 x 3-4.5 µm. *Conidia* hyaline, smooth, ellipsoid, 5-7.5 x 3-4 µm.

Habitat on bark and wood

Hypoxylon haematostroma Mont. apud Sagra, Hist. Phys. Pol. Nat. Cuba IX, p.334.(1845). Fig. 23.

Hypoxylon vividum Berk.& Broome, J. Linn.Soc., Bot.14: 122.1873.

Hypoxylon veracrucis Berk.& Cooke, Grevillea 11: 129. 1883.

Hypoxylon haematites Lev. ex Cooke, Grevillea 11: 133. 1883; non Lev. ex Thesis., 1909.

Hypoxylon lucidum Ellis & Everh., Bull. lowa Univ. Lab. Nat. Hist. 4:72. 1896.

Hypoxylon stjanianum Ferd. & Winge, Bot. Tidsskr. 29: 14. 1908.

Stromata hemispherical to effused-pulvinate, plane or with inconspicuous to conspicuous perithecial mounds, 3 mm thick; surface fulvous, sienna, or rust; orange red granules immediately beneath surface and between perithecia, with KOH-extractable pigments orange or scarlet; the tissue below the perithecial layer black, 0.5-1 mm thick.

Perithecia long tubular, 1.6-2.5 mm high x 0.2-0.5 mm diam.

Ostioles lower than the stromatal surface.

Asci 190-255 µm total length x 8-10 µm broad, the spore-bearing parts 65-80 µm long, the stipe 120-170 µm, with apical apparatus, discoid, 2.5-3 µm high x 1.3-1.5 µm broad.

Ascospores brown dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 15.0-17.0 x 5.0-6.3 µm, with straight germ slit spore-length; perispore dehiscent in 10% KOH, smooth; epispore smooth.

Cultural characteristics and anamorph

Produces a strongly zonate culture with orange and dull brown rings, reverse green, anamorph *Periconiella-like.* This is in close agreement with the findings of Ju and Rogers (1996).

Habitat on wood; endophyte

Figure 23. ***Hypoxylon haematostroma*** **Mont.**

a Stromata on wood (Bar = 1 cm)

b Long tubular perithecia (Bar = 1 mm)

c Surface of stroma with conspicuous perithecial mounds (Bar = 10 mm)

d Ascospores with straight germ slit spore-length (arrowed) (Bar = 10 μm)

e Ascospores with perispore dehiscent in 10% KOH (arrowed) (Bar = 10 μm)

f Culture on PDA

Hypoxylon investiens (Schwein.) M.A.Curtis, Geol. Nat. Hist. Surv. North Carolina, pt. III, p.140. 1867. Fig. 24.

Sphaeria investiens Schwein., Trans. Amer. Philos. Soc., n . ser., 4: 193. 1832.
Hypoxylon concurrens Berk. & M. A. Curtis apud Berk., Grevillea 4: 93. 1876.
Hypoxylon microspora Pande, Bull. Bot. Surv. India 15: 8. 1973; non *microsporum* Ces., 1879; [nom. rejic., ICBN Art. 64.1].

Stromata effused-pulvinate, plane or with inconspicuous perithecial mounds, 1-5.0 cm long x 1.2-1.5 cm broad x 0.2-0.5 mm thick; surface brown vinaceous; black granules immediately beneath surface and between perithecia, with KOH-extractable pigments sienna, umber becoming isabelline; the tissue below the perithecial layer inconspicuous to conspicuous.

Perithecia tubular to long tubular, 0.2-0.4 mm diam x 0.5-1.0 mm high.

Ostioles lower than the stromatal surface, beneath the perforated opening of the overlying stromatal layer.

Asci 100-140 µm total length x 4-6 µm broad, the spore-bearing parts 70-80 µm long, the stipes 30-65 µm long, with apical ring blueing in Melzer's iodine reagent, discoid, 1.3 µm high x 2.5 µm broad.

Ascospores light brown to brown, unicellular, ellipsoid, nearly equilateral, with broadly rounded ends to infrequently narrowly rounded ends, 9.4-10.0 x 3.8 µm with faint, straight germ slit less than to slightly less than spore-length; perispore indehiscent in 10% KOH; epispore smooth.

Colonies on OA covering Petri dish in 2 weeks, at first greenish olivaceous near the centre, grading through apricot and amber to the white edge, becoming honey, hazel to isabelline on hyphal tufts, velvety but with loose hyphal tufts, azonate, with diffuse margins; reverse honey to hazel. Sporulating regions scattered over entire surface of colony, pale mouse gray.

Habitat on wood

Figure 24. ***Hypoxylon investiens*** **(Schwein.) M. A. Curtis**

a Stromata on wood (Bar = 1 mm)

b Ostiole lower than the stromal surface (Bar = 10 μm)

c Ascospores (Bar = 1 μm)

d Ascospores with straight germ slit slightly less than spore-length (arrowed) (Bar = 10 μm)

Hypoxylon lividicolor Y.-M. Ju & J. D. Rogers, A Revision of the genus *Hypoxylon*: 144-145 (1996) Fig. 25.

Stromata effused-pulvinate, with inconspicuous perithecial mounds, 1.0-4.0 cm diam x ca. 2 mm thick; surface chestnut, dark brick; black granules immediately beneath surface and between perithecia, with KOH-extractable pigments greyish lavender to dark livid; the tissue below the perithecial layer conspicuous, black, ca. 2 mm thick.

Perithecia obovoid to long tubular, 0.2-0.4 mm diam x 0.6-1.5 mm high.

Ostioles lower than the stromatal surface.

Asci 140-160 μm total length x 6-7 μm broad, the spore-bearing parts 75-85 μm long, the stipes 65-80 μm long, with apical ring blueing in Melzer's iodine reagent, discoid, 1.3 μm high x 2.5 μm broad.

Ascospores dark brown, unicelluar, ellipsoid-inequilateral, with narrowly rounded ends, (8.8)10.0-12.5 x 3.8-5.0 μm, with straight to slightly sigmoid germ slit spore-length; perispore dehiscent in 10% KOH, smooth to with inconspicuous coil-like ornamentation; epispore smooth.

Colonies on OA not reaching the edge of Petri dish, at first whitish, with diffuse margins; reverse uncoloured. Sporulating regions scattered over entire surface of colony, white. Conidiogenous structure *Sporothrix-like*, hyaline, smooth, with denticulate conidial secession scars, ca. 0.8 μm diam. Conidia hyaline, smooth, ellipsoid 6-10.5 x 3-3.5 μm.

Habitat on wood

Hypoxylon monticulosum Mont., Syll. Gen. Sp. Crypt., p.214.1856. Fig 26.

Hypoxylon epiphaeum Berk. & M.A. Curtis apud Berk., Grevillea 4: 52. 1875.

Hypoxylon investiens (Schwein.) M.A. Curtis var. *epiphaeum* (Berk. & M. A. Curtis) J.H. Miller, Monogr. of the World Species of *Hypoxylon,* p. 52. 1961.

Hypoxylon mascariensis Berk. ex Cooke, Grevillea 11: 131. 1883.

Hypoxylon berkeleyi Sacc., Syll. Fung. II, p. xxii. 1883; [nom. rejic., ICBN Art. 63. 1].

Hypoxylon glomeratum Cooke, Grevillea 11: 134. 1883.

Hypoxylon subnigricans Speg., Anales Soc. Ci.Argent. 18: 273. 1884.

Hypoxylon antracoderma Speg., Anales Soc. Ci.Argent. 26: 30. 1888.

Hypoxylon nuttallii Ellis & Everh., Proc. Acad. Nat. Sci Philadelphea 1894: 346. 1894.

Figure 25. ***Hypoxylon lividicolor*** **Y.-M. Ju & Rogers**

a Stromata on wood (Bar = 1 cm)

b Long tubular perithecia (Bar = 0.1 mm)

c Surface of stroma showing conspicuous perithecial mounds (Bar = 1 mm)

d Perithecia with ostioles (Bar = 0.1 mm)

e Ascospores with ornamentation (Bar = 1 μm)

f Ascospores with perispore dehiscent in 10% KOH (Bar = 10 μm)

g Ascus with apical apparatus (arrowed) (Bar = 10 μm)

Hypoxylon bakeri Earle, Bull. Torrey Bot. Club 26: 633. 1899.

Hypoxylon rubigineoareolatum Rehm var. *bakeri* (Earle) J.H. Miller apud Chardon & toro, J. Agric. Univ. Puerto Rico 14: 273. 1930.

Hypoxylon multiforme (Fr.: Fr.) Fr. f. minor Starb., Bih. Kongl. Svenska Vetensk.-Akad. Handl. 27, 3.9.1901.

Hypoxylon rubigineoareolatum Rehm var. *microspora* Theiss., Ann. Mycol. 6: 345.1908.

Hypoxylon merrillii Syd. & P. Syd., Ann. Mycol. 15: 212. 1917.

Hypoxylon cupricolor Petch, Ann. Roy. Bot. Gard. (Peradeniya) 8: 158. 1924.

Stromata pulvinate to effused-pulvinate, with inconspicuous to conspicuous perthecial mounds, 0.5-5.0 cm long x 0.3-1.7 cm broad x 0.5-1.5 cm thick; surface brown vinaceous; blackish woody to carbonaceous tissue immediately beneath surface and between perithecia, without apparent KOH-extractable pigments; the tissue below the perithecial layer inconspicuous to 0.5 mm thick.

Perithecia spherical to obovoid, 0.2-0.5 mm diam x 0.3-0.5 mm high.

Ostioles higher than the stromatal surface, minutely papillate,

Asci 95-105 µm total length x 4.5-5 µm broad, the spore-bearing parts 55-65 µm long, the stipes 35-40 µm long, with apical ring blueing in Melzer's iodine reagent, discoid, 1.3 µm high x 1.3 µm broad.

Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 7.5-8.8(10.0) x 3.8-4.4 µm with sigmoid germ slit spore-length; perispore dehiscent in 10% KOH, smooth or with inconspicuous coil-like ornamentation; epispore smooth.

Cultural characteristics and anamorph

Typically produces a *Virgariella-like* anamorph (Ju & Rogers, 1996).

Habitat on bark and wood

Figure 26. *Hypoxylon monticulosum* Mont.

a Stromata on bark (Bar = 1 cm)

b Surface of stroma with conspicuous perithecial mounds (Bar = 1 mm)

c Ostiole higher than the stromal surface (Bar = 10 μm)

d Obovoid perithecia (Bar = 1 mm)

e Ascus with ascospores sigmoid germ slit spore-length (arrowed) (Bar = 10 μm)

Hypoxylon moriforme Henn., Bot. Jahrb. Syst. 23: 287. 1896. Fig. 27.

Stromata glomerate, hemispherical, to effused-pulvinate, with perthecial mounds 1/3-1/2 exposed, and not covered by outermost stromatal layer, 1-7 cm long x 0.5-3 cm broad x 1-3 mm thick; surface blackish with greenish tones; blackish granules immediately beneath surface, with KOH-extractable pigments greenish glaucous; the tissue below the perthecial layer conspicuous, blackish.

Perithecia spherical 0.6-0.8 mm diam.

Ostioles papillate, encircled with a truncatum-type disc 0.1-0.2 mm diam.

Asci 112-128 µm total length x 4-5 µm broad, the spore-bearing parts 75-80 µm long, the stipes 37-48 µm long, with apical ring blueing or not blueing in Melzer's iodine reagent, discoid, 0.5-1 µm high x 1-1.5 µm broad.

Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly to broadly rounded ends, 7.5-10.0 x 3.1-3.8 µm, with straight germ slit spore-length; perispore of at least some spores dehiscent in 10% KOH, smooth; epispore smooth.

Cultural characteristics and anamorph

Produces a *Nodulisporium-like* anamorph (Ju & Rogers, 1996).

Habitat on wood and twig

Note This fits *H. moriforme* but with smaller discs 0.1-0.2 mm. There is *H. moriforme* var. *microdiscus* but in that taxa the spores are bigger 10-12 x 4-5 µm. Although the stromal surface has a greenish tone as in *H. moriforme* it appears more reddish brown than usual. The related *H. archeri* has spore dimensions of 9-10.5 x 4-5 µm with perithecia of 0.5 mm or more. It is not *H. microcarpum.*

Figure 27. ***Hypoxylon moriforme*** **Henn.**

a Stromal surface with perithecial mounds 1/3-1/2 exposed (Bar = 1mm)

b Ostioles with a truncatum-type disc (Bar = 0.1 mm)

c Ascus with ascospores straight germ slit spore-length (arrowed) (Bar = 10 µm)

Hypoxylon perforatum (Schwein.: Fr) Fr., Summa Veg. Scand. II: 384.(1849). Fig. 22.(a-c)

Sphaeria perforata Schwein., Schriften Naturf. Ges. Leipzig 1: 31. 1822; Schwein.: Fr., Syst. Mycol. II, p.340. 1823.

Hypoxylon rubiginosum (Pers.: Fr) Fr. var. *perforatum* (Schwein.: Fr.) L. E. Petrini apud L. E. Petrini & Muller, Mycol. Helv. 1: 531. 1986.

Hypoxylon durissimum (Schwein.: Fr) Sacc., Syll. Fung. 1, p.378. 1882; no Fr., 1849.

Sphaeria durissima Schwein., Schriften Naturf. Ges. Leipzig 1: 32. 1822; Schwein.: Fr., Syst. Mycol. II, p. 335. 1823.

Sphaeria decorticata Schwein., Trans. Amer. Philos. Soc., n. ser., 4: 191. 1832; non (Pers.: Fr.) Dc.& Lam., 1805; [nom. Rejic., ICBN Arts. 13.1 (d) & 64.1].

Hypoxylon decorticatum (Schwein.) M. A. Curtis, Geol. Nat. Hist. Surv. North Carolina, pt. III, p.140. 1867.

Sphaeria catalpae Schwein., Trans. Amer. Philos. Soc., n. ser., 4; 193. 1832.

Hypoxylon catalpae (Schwein.) Sacc., Syll. Fung.1, p. 392. 1882.

Sphaeria leucostigma Lev., Ann. Sci. Nat. Bot., ser. III, p:142. 1848; no DC. & Lam., 1815; [nom. Rejic., ICBN Art. 64.1].

Hypoxylon leucostigma (Lev.) Sacc., Syll. Fung. 1, p. 367. 1882.

Hypoxylon luridum Nitschke, Pyren. Germ., p. 31. 1867.

Hypoxylon plumbeum Speg., Anales Soc. Ci. Argent. 18: 270. 1884.

Hypoxylon rubiginosum (Per.: Fr) Fr. var. *microcarpum* Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires 17: 120. 1908.

Stromata effused-pulvinate, with inconspicuous to conspicuous perithecial mounds, 5-7 mm long x 2-3 mm broad x 0.5-0.7 mm thick; surface sepia to brown vinaceous; blackish granules immediately beneath surface and between perithecia, with KOH-extractable pigments amber; the tissue below the perthecial layer dark brown to blackish inconspicuous or up to 1 mm thick.

Perithecia spherical, 0.3-0.4 mm diam.

Ostioles lower than the stromatal surface, usually overlain with conspicuous white substance.

Asci cylindrical, eight-spored, 122-150 µm total length x 8-10 µm broad, spore-bearing parts 67-75 µm, the stipes 55-75 µm long, with apical apparatus, discoid, 0.6-1 µm high x 2-2.5 µm broad, blueing in Melzer's iodine reagent.

Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 11.3-13.8 x 5.0-6.3 µm, with straight to slightly sigmoid germ slit spore-length; perispore dehiscent in 10% KOH, with inconspicuous coil-like or smooth ornamentation; epispore smooth.

Culture characteristics and anamorph

Typically produces a *Virgariella-like* anamorph (Ju & Rogers, 1996).

Habitat on wood and bark

Note: This taxon resembles *H. notatum* in most aspects except it is effused not pulvinate. However, Ju & Rogers (pers. comm.) identify this species as *H. perforatum.*

Hypoxlon rubiginosum (Pers.:Fr.) Fr., Summa Veg. Scand. II. p.384.1849. Fig. 28. (a)

Sphaeria rubiginosa Pers., Observ. Mycol. l, p. 69. 1796; Pers.:Fr., Syst. Mycol. II, p. 340. 1823; non Spreng., 1827.

Sphaeria granulosa pers.: Fr., Syn. Meth. Fung., p. 11. 1807, non (Bull.) Sowerby, 1803.

Sphaeria multiformis Fr.: Fr. var. *granulosa* (Pers.: Fr) Fr., Syst. Mycol. II, p. 334.v1823.

Hypoxylon stereoides Fr., Summa Veg. Scand. II, p. 384. 1849.

Hypoxylon botrys Nitschke, Pyren. Germ., p. 34. 1867.

Hypoxylon florideum Berk. & M. A. Curtis apud Berk., Greillea 4: 50. 1875.

Stromata effused-pulvinate, sometimes pulvinate or even hemispherical, plane or with inconspicuous to conspicuous perthecial mounds, 1.0-2.0 cm x 0.5-1.0 cm broad x 0.3-1.3 mm thick; surface dark brick, brown vinaceous, brown granules immediately beneath surface and between perithecia, with KOH-extractable pigments orange; the tissue below the perithecial layer usually inconspicuous.

Perithecia spherical to obovoid, 0.2-0.4 mm diam x 0.3-0.5 mm high.

Figure 28. (a) ***Hypoxylon rubiginosum*** (Pers.:Fr.) Fr.

a Stromal surface (Bar = 1 mm)

Figure 28. (b-c) ***Xylaria culleniae*** **Berk. & Broome**

b Stromata (Bar = 1 cm)

c ascus with ascospores straight germ slit spore-length (Bar = 10 μm)

Figure 28. (d) ***Xylaria badia*** **Pat.**

d Stromata (Bar = 1 cm)

Ostioles lower than the stromatal surface.

Asci 105-165 µm total length x 5.6-8.0 µm broad, the spore-bearing parts 65-85 µm long, the stipes 35-85 µm long, with apical ring lightly blueing to blueing in Melzer's iodine reagent, discoid, 1.3 µm high x 2-3 µm broad.

Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 9.4-11.9 x 3.8-5.0 µm, with straight germ slit spore-length; perispore dehiscent in 10% KOH, smooth or with inconspicuous coil-like ornamentation; epispore smooth.

Colonies on OA covering Petri dish in 4 weeks at first white, becoming luteous, buff to cinnamon, velvety to felty, azonate, sectors, with diffuse margins; reverse uncoloured. Sporulating regions scattered over entire surface of colony, buff. Conidiogenous structure *Nodulisporium-like,* finely roughened, yellowish to pale brown. *Conidiogenous cells* hyaline, smooth, 10-25 x 3-4 µm. *Conidia* hyaline, smooth to finely roughened, 5-6 x 3-4 µm.

Habitat on wood

Hypoxylon subgilvum Berk. & Broome, J. Linn.Soc., Bot.14:120.1873. Fig. 29.

Hypoxylon caaguazu Speg., Anales Soc. Ci. Argent. 18: 275. 1884.

Stromata effused-pulvinate, with inconspicuous to conspicuous perthecial mounds, 0.5-1.5 cm long x 0.5 cm broad x 0.3-0.5 mm thick; surface dark brick; orange red granules immediately beneath surface and between perithecia, with KOH-extractable pigments orange; the tissue below the perithecial layer black, inconspicuous to 0.5 mm thick.

Perithecia obovoid to tubular, 0.1-0.3 mm diam x 0.3-0.8 mm high.

Ostioles lower than the stromatal surface.

Asci 80-90 µm total length x 5.0 µm broad, the spore-bearing parts 45-60 µm long, the stipes 25-30 µm long, with apical apparatus blueing to faintly bluing in Melzer's iodine reagent, discoid, 0.6 µm high x 1.3 µm broad .

Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 7.5-8.8 x 3.8 µm, with straight to slightly sigmoid germ slit spore-length;

Figure 29. *Hypoxylon subgilvum* Berk. & Broome

a Stromata on bark (Bar = 1 cm)

b Stromal surface (Bar = 1 cm)

c ascus (Bar = 10 µm)

d ascospores (Bar = 10 µm)

perispore dehiscent in 10% KOH, with inconspicuous to conspicuous coil-like ornamentation; epispore smooth.

Cultural characteristics and anamorph

Produces a *Nodulisporium-like* anamorph (Ju & Rogers, 1996).

Habitat on wood and bark

Hypoxylon subrutilum Starb., Bih. Kongl. Svenska Vetensk. -Akad. Handl. 27, 3:10. 1901. Fig. 30.

Hypoxylina umbilicata Starbm,. Ark. Bot. 5: 29. 1905.

Hypoxylon glomerulatum Theiss., Ann. Mycol. 6: 345. 1908; non Bull., 1791; [nom. rejic., ICBN Art. 64.1].

Hypoxylon haematites Lev. var. *microspora* Theiss., Ann. Mycol. 6: 345. 1908.

Hypoxylon rubrostromaticum J. H. Miller var. *macrospora* (Theiss.) J. H. Miller, Monogr. of World Species of *Hypoxylon,* p 25. 1961.

Hypoxylon haematites Lev. ex Theiss., Ann. Mycol. 7: 145. 1909; non Lev. ex Cooke, 1883; [nom. rejic., ICBN Art. 64.1].

Hypoxylon rubrostromaticum J. H. Miller, Monogr. of World Species of Hypoxylon, p. 24. 1961.

Hypoxylon haematites Lev. ex Theiss.f *microspora* Theiss., Ann. Mycol. 7: 146. 1909.

Hypoxylon haematites Lev. ex Theiss. var. *microsporum* (Theiss.) Rick, Broteria, ser. Bot., 25: 28. 1931.

Hypoxylon indicum Syd. & P. Syd., Ann. Mycol. 9: 416. 1911.

Hypoxylon congoanum Torrend ex Beeli, Bull. Jard. Bot. Etat 8: 74. 1923.

Illustration: Miller (1961, Figs. 12 & 43) [as *H. rubrostromaticum*]

Stromata glomerate, pulvinate to effused-pulvinate, discrete of confluent, with inconspicuous to conspicuous perithecial mounds, 1.0-6.0 cm long x 1-20 mm broad x 0.5-0.7 mm thick; surface dull brown, sepia; dark reddish brown granules immediately beneath surface and between perithecia, with KOH-extractable pigments amber; the tissue below the perithecial layer inconspicuous.

Perithecia spherical, 0.2-0.5 mm diam.

Figure 30. ***Hypoxylon subrutilum*** **Starb.**

a Stromata on bark (Bar = 1 cm)

b Surface of stroma with conspicuous perithecial mounds (Bar = 1 mm)

c Perithecia with ostiole lower than the stromatal surface (Bar = 0.1 mm)

d Ascospores with straight germ slit spore-length (arrowed) (Bar = 10 μm)

e Ascospore with perispore dehiscent in 10% KOH (Bar = 10 μm)

Ostiole lower than the stromatal surface.

Asci fragmentary, probably short-stipitate 150-160 μm total length, the spore-bearing parts 87-93 μm, the stipes 63 μm, with apical ring blueing in Melzer's iodine reagent, discoid, 1.3 μm high x 3-4 μm broad .

Ascospores dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 15.0-17.5 x 6.3-7.5 μm, with straight germ slit spore-length; perispore dehiscent in 10% KOH, thick, with conspicuous coil-like ornamentation; epispore smooth.

Colonies on OA covering Petri dish in 2 weeks, at first white becoming hazel, velvety to felty, azonate, with diffuse margins; reverse uncoloured. Sporulating regions scattered over entire surface of colony, whitish. Conidiogenous structure *Nodulisporium*-like, pale brown, roughened. Conidiogenous cells hyaline, finely roughened, 8-28 x 3.3.5 μm. Conidia hyaline, smooth. ellipsoid, 5-9 x 3-4 μm.

Habitat endophyte

Hypoxylon sp. 188 **(new sp.)** Fig. 31.

Stromata widely dffused, with conspicuous mounds, 2.5-5 cm long x 1-1.5 cm broad x ca. 0.5 mm thick; surface dark brick; dark brown granules beneath the surface, with KOH-extractable pigments vinaceous purple; the tissue below the perithecial layer black.

Perithecia ovoid to obovoid, 0.1-0.2 mm diam.

Ostioles lower than stromatal surface

Asci 148-171 μm total length x 9.5-11.5 μm broad, the spore bearing part 91-95 μm long, the stipes 53-76 μm long with apical apparatus blueing in Melzer's iodine reagent, discoid, 0.5 μm high x 2.5 μm broad.

Ascospores brown to dark, unicellular, ellipsoid inequilaterally, with broad to narrowly rounded ends, 15.0-21.25 x 7.5-10.0 μm, with straight germ slit slightly less than spore-length; perispore indehiscent in 10% KOH.

Note: This species only occurred as an endophyte and with its distinctive vinaceous purple extract in 10% KOH is unusual. None of the described species of *Hypoxylon* can accommodate this specimen and Ju & Rogers (pers. comm.) have recently confirmed that it represents a new taxon.

Habitat endophyte

Figure 31. ***Hypoxylon sp. 188***

a Mature teleomorphic stromata on bark (Bar = 1 cm)

b Young stromata (Bar = 1 cm)

c Ostiole lower than stromal surface (Bar = 10 µm)

d Young asci with discoid apical apparatus (Bar = 10 µm)

e Ascus with ascospores straight germ slit slightly less than spore-length (arrowed) (Bar = 10 µm)

f Ascospores with perispore indehiscent in 10% KOH (Bar = 10 µm)

Nemania subannulata (Henn. & E. Nyman) Comb. nov. prov. Fig. 32.

Hypoxylon subannulata Henn. & E. Nyman, Monsunia 1: 168(1899).

Stromata erumpent to superficial, small to widely effused, occasionally separating into individual perithecial stromata, 0.5-1 cm long x 2-2.5 broad x 1.2-1.8 mm thick. Externally dark brown to black. Internally dark brown, surface smooth, uneven due to protruding perithecia

Perithecia completely immersed, widely dispersed in an undulating stroma subglobose 1-1.5 mm diam.

Ostioles papillate, each in the centre of a convex annular disc (0.2-0.3 mm diam) surrounded by a wide flattened border, slightly raised above the disc.

Paraphyses filiform, tapering towards the apex, remotely septate, 2 μm diam near the top.

Asci cylindrical, eight-spored, 160-190 μm total length x 4-5 μm broad, spore bearing part 90-110 μm long, the stipe 60-90 μm long, apical apparatus rectangular, 1.8-2.5 μm high x 1.5-2 μm broad, blueing in Melzer's iodine reagent

Ascospores obliquely uniseriate, inequilaterally ellipsoid with rounded ends, medium to dark brown, smooth, (10)11.3-12.5 x (3.8)5-5.6 μm, germ slit straight, on the ventral side of the spore, almost full spore length.

Cultural characteristics and amamorph

Colonies are moderate in growth on malt extract agar appearing white, grey white to brownish with greyish green areas on the surface through conidial production. Reverse becoming dark brown to black. *Conidiophores* typically geniculate as described by Chesters and Greenhalgh for *Geniculosporium serpens* (Chesters & Greenhalgh, 1964)

Habitat endophyte, on bark and wood

Figure 32. ***Nemania subannulata*** **(Henn. & E. Nyman) Comb. nov. prov.**

a Stromata on bark (Bar = 1 cm)

b Stromal surface with protruding perithecia (Bar = 1 cm)

c Ostiole with annular disc (Bar = 10 μm)

d Ascus with ascospores straight germ slit spore-length (arrowed) (Bar = 10 μm)

e Ascospores (Bar = 10 μm)

f Young ascus with rectangular apical apparatus (arrowed) (Bar = 10 μm)

Xylaria allantoidea Fr., Nova Acta Regiae Soc. Sci. Upsal. (Ser. 3) 1: 127 (1851) Fig. 33.

Sphaeria allantoidea Berk., Ann. Mag. Nat. Hist. 3: 397 (1839) [as "allantoidea", corrected by Berk., J. Linn. Soc., Bot. 10 : 380 (1869)]

Sphaeria zeylanica Berk., London J. Bot. 6 : 513 (1847).

Xylaria zeylanica (Berk.) Berk. & Broome, J. Linn. Soc., Bot. 14: 118 (1873).

Hypoxylon domingense Berk., Ann. Mag. Nat. Hist. (ser.2) 9: 202 (1852).

Xylaria domingensis (Berk.) Sacc., Syll. Fung. 1: 315 (1882).

Hypoxylon obtusissimum Berk., Ann. Mag. Nat. Hist (ser. 2) 9: 202 (1852).

Xylaria obtusissima (Berk.) Sacc., Syll. Fung. 1: 318 (1882).

Xylaria regalis Cooke, Grevillea 11 : 86 (1883).

Xylaria emerici Berk. in Cooke, Grevillea 11 : 86 (1883).

Xylaria cynoglossa Cooke, Grevillea 12: 1 (1883).

Xylaria obesa Syd., Ann. Mycol. 5: 400 (1907).

Xylaria composita Lloyd, Mycol. Not. 6: 1055 (1921).

Stromata solitary or in small clusters, not branched or branched near the base, cylindric-fusoid, cylindric-allantoid to clavate, sometimes flattened, occasionally subglobose to penzigioid, with obtusely rounded or tapered fertile apices, usually on short often ill-defined, stout stipe, 2-10 cm total high x 0.5-1 cm diam. Externally light brown, golden brown, becoming black with age. Internally white to beige, becoming hollow with age (in hollow stromata the inner side is usually lined by a dark brown carbonaceous layer becoming vertically striped). Texture hard, surface smooth, sometimes very faintly reticulately cracked around the ostioles, unwrinkled .

Perithecia completely immersed, subglobose, 0.4-0.6 mm diam.

Ostioles punctate, becoming papillate, occasionally annulate.

Paraphyes filiform, tapering towards the apex, remotely septate, 2 μm diam, near the top.

Asci cylindrical, eight-spored, long stalked, 140-190 μm total length x 5-6 μm broad spore-bearing parts 80-100 μm long; apical apparatus quadrate to rectangular, constricted subapically, 2-3 μm high x 1.5- 2.5 μm braod, blueing in Melzer's iodine reagent.

Figure 33. ***Xylaria allantoidea*** **Fr.**

a Stromata (Bar = 1 cm)

b Stroma on wood (Bar = 10 cm)

c Ostiole (Bar = 10 μm)

d Ascus with apical apparatus (arrowed) and ascospores with straight germ slit spore-length (arrowed) (Bar = 10 μm)

Ascospores obliquely uniseriate, inequilaterally ellipsoid, with narrowly rounded ends light to medium brown, smooth, 10.6-13.1 x 3.8-4.4 μm; germ slit conspicuous straight on the ventral side of the spore slightly less than full spore length.

Cultural characteristics and anamorph.

Colonies on oatmeal agar at room temperature, reaching edge of a 9 cm diam. Petri dish in 2 weeks, at first white to light orange-white with plumose margins later becoming velvety, often with faint concentric zones, orange-white to brownish grey towards the centre. Reverse yellowish brown to dark brown towards the centre. Exudate colourless to light orange. Hyphae parallel, sparingly branched, septate, hyaline, smooth, locally becoming dark brown, densely septate and more irregularly branched, and finally drawn into segments.

Stromata developing after 3 weeks, especially abundant at the colony margin, usually cylindrical to a somewhat broadened, uppermost half of the stromata becoming grey due to conidia production, up to 5 mm high x 1-4 mm diam, with the apex remaining sterile and proliferating conidiophores in palisades, repeatedly branched, hyaline to subhyaline, smooth, 2-3 μm diam. *Conidiogenous cells* terminal, cylindrical, hyaline to subhyaline smooth, 30-40 x 2.5-3 μm, bearing in the upper part small circular refractile conidial scars. *Conidia* acropleurogenous, obovoid to ellipsoid, with a flattened circular abscission scar at the base, hyaline, smooth by L.M.,4.5-6.5 x 3-3.5 μm.

Habitat on wood

Xylaria aristata Mont. in Ann. Sci. Nat. Bot (Ser, 4) 3: 106. (1855). Fig. 34.

Stromata globose to some what conical with acute apices, 2-5 mm high x 2-4 mm diam on long, thin finely pubescent stipes up to 3.0 cm high; externally white becoming grey. Stipes dark brown; internally white. Texture becoming fairly hard. Surface roughened with perithecial contours.

Ostioles papillate

Perithecia spherical, 0.7-1.0 mm diam.

Figure 34. ***Xylaria aristata*** **Mont.**

a Culture on PDA

b Mature teleomorphic stromata (Bar = 1 cm)

c Young stromata on twig (Bar = 1 cm)

d Ascospores with straight germ slit spore-length (arrowed) (Bar = 10 μm)

e Ascus with apical apparatus (arrowed) (Bar = 10 μm)

Asci cylindrical, eight-spored, 112-150 μm total length x 6-7 μm broad, the spore-bearing part 60-70 μm long, the stipe 30-40 μm long; with apical apparatus, retangular, quadrate, 2-2.5 μm high x 2-2.5 μm broad, blueing in Melzer's iodine reagent.

Ascospores brown, unicellular, ellipsoid-inequilateral with broadly or narrowly rounded ends, smooth, (8.1)10-11.3 x 5-5.6(6.3) μm, with straight germ slit full spore-length.

Culture characteristics

Habitat Endophyte, on leaves

Xylaria badia Pat., Journ. Bot. (Morot) 5: 319. (1891). Fig. 28. (d)

Type: Vietnam. Tondin, Ke So 436 & 4417, on wood (FH syn-).

Stromata gregarious, not branched, short cylindrical to clavate, with rounded fertile apices, on a broadened discoid pannose base, 0.4-1.2 cm total height x 3.5 mm diam. Externally silvery brown, becoming greyish brown with age. Young stromata covered with a tomentum consisting of brown hyphae. Internally brownish orange, solid. Surface smooth, shining.

Perithecia completely immersed, subglobose, 0.1-0.3 mm diam.

Ostioles finely papillate, black.

Paraphyses filiform, tapering towards the apex remotely septate, 2 μm diam. near the top.

Asci cylindrical, eight-spored, long stalked, 100-110 μm length x 5-6 μm broad, the spore-bearing part 50-55 μm long, the stipe 50-60 μm long; 0.6 μm high x 1.2 μm broad, blueing in Melzer's iodine reagent.

Ascospores uniseriate to obliquely uniserate, ellipsoid, with narrowly rounded ends, light brown, smooth, 8.8-10.0 x 3.8 μm; germ slit inconspicuous, straight, less than full spore length.

Habitat on bamboo

Xylaria cubensis (Mont.) Fr., Nova Acta Regiae Soc. Sci. Upsal. (Ser. 3) 1:126 (1851) Fig. 35.

Type: Cuba (cannot be traced at Paris fide Dennis, 1957)

Hypoxylon cubense Mont., Ann. Sci., Bot. (ser.2) 13: 345 (1840) emend Lloyd, Mycol. not. 5: *Xylaria* notes 4 (1918).

Xylosphaera papyrifera subsp. *cubensis* (Mont.) Dennis, Bull. Jard. Bot. Etat 31: 122 (1961).

Xylaria papyrifera subsp. *cubensis* (Mont.) D. Hawksw., Trans. Brit. Mycol. Soc. 61: 200 (1973).

Xylaria reducta Syd., Ann. Mycol. 5: 339 (1907).

Xylaria subinvolata Hohn., Denkschr. Kaiserl. Akad. Wiss., Math.-Naturwiss. Kl., 83: 27 (1907).

Xylaria fusa Lloyd, Mycol. Not. 5: 770 (1918).

[after Dennis 1961; Roger 1984]

Stromata highly variable, solitary or gregarious, not branched, cylindric-allantoid to clavate, occasionally flattened, sometimes penzigioid, with rounded fertile apices (occasionally apex bifurcate), on short often ill defined stipes, arising from tomentose discoid bases, 1.9-8.5 cm total height x 0.3-1.5 cm diam. Externally bronze to copper coloured, becoming dark, chocolate-brown with age. Internally at first white and solid becoming hollow with age. Texture hard. Surface usually smooth, sometimes very faintly, reticulately cracked around the ostioles, or surface conspicuously cracked into small polygonal surface scales (the latter is usually the case for the smaller stromata). Young stromata covered with tomentum (consisting of brown hyphae)

Perithecia completely immersed, subglobose, 0.5-0.8 mm diam.

Ostioles usually prominent conico-papillate, occasionally finely papillate to annulate

Paraphyses filiform, tapering towards the apex, remotely septate, 2 μm diam. near the top.

Asci cylindrical, eight-spored, long stalked, 119-156 μm length x 5-8 μm broad, the spore-bearing part 56-75 μm long, the stipe 70-82 μm long; apical apparatus cubic to

Figure 35. ***Xylaria cubensis*** **(Mont.) Fr.**

a Young teleomorphic stromata on twig (Bar = 1 cm)

b Mature teleomorphic stromata (Bar = 1 cm)

c Surface of stroma with reticulately cracked around the ostioles (Bar = 1 mm)

d Anamorphic stromata (arrowed) (Bar = 1 cm)

e Ascospores (bar = 1 μm)

f Ascus with apical apparatus (arrowed) (Bar = 10 μm)

g Culture on OA

rectangular, constricted subapically, 1.5-2.0 µm high x 1.5 µm broad, blueing in Melzer's iodine reagent.

Ascospores uniseriate to obliquely uniseriate, inequilaterally ellipsoid, with rounded ends, brown, smooth, (7.5)8.13-10 x 3.8-5 µm; germ slit usually inconspicuous, occasionally visible at the ventral side of the spore, straight, less than full spore length to almost full spore-length.

Anamorph as found in nature

Xylocoremium flabelliforme (Schwein.: Fr.) J.D. Rogers, Mycologia 76: 913-921 (1984).

Type: L. D. Schweinitz, in herb. E. Fries, no further data (Ups-lectotype).

Sphaeria flabelliformis Schwein.: Fr., Elench. Fung. 2: 58 (1828).

Xylaria flabelliformis (Schwein.: Fr.) Berk. & M. A. Curtis, J. Linn. Soc., Bot. 10: 381 (1869).

Isaria flabelliformis (Schwein.: Fr.) Lloyd, Mycol. Not. 4, n° 40: 547 (1916).

Merisma nigripes Schwein., Schriften Naturf. Ges. Leipzig 1: 111 (1822).

Thelephora liliputiana Mont., Ann. Sci. Nat., Bot. (ser. 2) 13; 205 (1840) (as Telephora).

Thelephora rosella Peck, 35 th Rep. New York State Mus. Nat. Hist.: 136 (1884).

[after Rogers 1984]

Anamorph formed on stromata distinct from the teleomorphic stromata.

Anamorphic stromata erect, with black, villose bases and flabellate, foliate or more or less cerebriform, orange white fertile portions, 3-7 mm total height x 2-8 mm diam.

Conidiophores, conidiogenous cells and conidia as described from anamorphic stromata produced in culture.

Cultural characteristics

Colonies on oatmeal agar, under 12 h light and 12 h darkness, reaching edge of a 9 cm diam. Petri dish in 2 weeks, at first white with an orange centre, appressed at the surface, with faintly zonate white margins, often furrowed, later darkening from centre outwards, becoming brownish grey to black and covered by lanose or cottony mycelium (white to tan hyphae). Reverse light orange to greyish orange. Exudate yellowish orange.

Hyphae parallel, sparingly branched, septate, hyaline, smooth, with age becoming brown, densely septate and easily breaking apart. Clusters of dark brown, swollen thick walled cells, remaining in chains are formed in aging colonies.

Conidiogenous structures formed either on small tufts in the centre of the colony, or on cylindrical to flabelliform stromata. Tufts formed after 20 days, subglobose, brownish grey to grey due to conidia production over the whole surface, 1-2 mm diam.

Stromata developing after 3 weeks, throughout the whole colony (at surface and periphery of colony), cylindrical or with a flabelliform conidiogenous upper part, at first white to orange-white with a black base, becoming pink or grey in areas of conidial production, up to 25 mm high x 1-3 diam. Conidium bearing regions usually starting in the somewhat broadened uppermost half of the stromata later covering the entire surface of the stromata. Occasionally the stromata remained sterile. No apparent morphological difference between conidiogenous structures from either location (tufts or stromata).

Conidiophores in dense palisades, repeatedly branched, hyaline to subhyaline, smooth, 2.5-3 µm diam. Palisades sloughing off in dusty flakes to accumulate in small heaps underneath the stromata.

Conidiogenous cells terminal, cylindrical, hyaline to subhyaline, smooth, 9.5-15 x 2-3 µm diam. bearing circular refractile conidial scars apically and laterally.

Conidia acropleurogenous, obovoid to ellipsoid, with a flattened circular abscision scar at the base, hyaline, smooth by L.M. (3)3.5-5(6) x (1)1.5-2(3).

Habitat endophyte, on decaying, usually decorticated, wood of dicotyledonous trees, lying on the forest floor in lowland areas. Often in disturbed, secondary rain forest, and in dry and sun-exposed sites. Very common.

Xylaria culleniae Berk. & Broome, J Linn. Soc. Bot. 14; 119. (1875). Fig. 28. (b-e)

Stromata unbranched or branched, cylindrical, long conical, or flattened the fertile parts bearing more or less naked perithecia, grading into ill-defined stipes. Externally blackish. Internally white, Texture soft. Surface roughened with perithecia and tomentose except the stromatal apices.

Perithecia 0.1-0.3 mm diam.

Ostioles minutely papillate.

Asci cylindrical, eight-spored, 97-120 µm total length x 5.0 µm broad, the spore-bearing parts 67-80 the stipes 30-40 µm long; with apical appatus, more or less rectangular, 1.3 µm x 1.3 µm broad, blueing in Melzer's iodine reagent.

Ascospores brown, unicellular, ellipsoid-inequilateral, smooth, 8.1-9.4 x 3.8-4.4 µm, with straight germ slit slightly less than spore-length.

Habitat on fallen pods

Xylaria feejeensis (Berk.) Fr., Nova Acta Regiae Soc. Sci. Upsal. (ser. 3) 1:128 (1851) Fig. 36.

Sphaeria feejeensis Berk., London J. Bot. 1:456 (1842).

Xylosphaera feejeensis (Berk.) Dennis, Kew Bull. 13:103(1958).

Xylaria rhytidophloea Mont., Ann. Sci. Nat. Bot. (ser. 4)3 ;101(1855).

X. antartica Speg., Bol. Acad.Nac. Ci. 11, Fungi Fuegiani 67 (1887).

X. fuegiensis Speg., Bol. Acad. Nac. Ci.11, Fungi Fuegiani 68 (1887).

X.trivialis Speg., Bol. Acad. Nac. Ci.11, Fungi Puiggariani 135 (1889).

X. obtussissima (Berk.) Sacc. var. *polymorphoides* Rehm, Hedwigia 40:144 (1901).

X. brevipes Sacc. & Fairm., J. Mycol. 12:47 (1906).

X. brevipes var. *africana* Sacc., Ann. Mycol. 4: 75(1906).

Stromata gregarious, not branched or branched, cylindrical, clavate to spathulate, with narrowly rounded usually fertile apices, on short to long, often ill-defined stipes, sometimes with a tomentum (existing of purplish black hyphae) at the base, (1.5)2-7.5(11.5) cm total height x 2-4 mm diam. Externally blackish brown to black. Internally white to yellowish Surface rough, finely reticulately cracked into small, angular, closely spaced scales so as to outline the individual perithecia.

Perithecia completely immersed, subglobose, 0.2-0.4 mm diam

ostioles prominent, finely papillate, in the centre of dome-shaped to hemispherical black discs.

Paraphyses filiform, tapering towards the apex remotely septate, 2-3 µm diam. near the top.

Figure 36. ***Xylaria feejeensis*** **(Berk.) Fr.**

a Young stromata bearing conidia on twig (Bar = 1 cm)

b Mature stromata (Bar = 1 cm)

c Culture on OA

d Stromal surface with distinct perithecial mounds (Bar = 1 mm)

e Ostioles with dome shaped discs (Bar = 0.1 mm)

Asci cylindrical, 8-spored (occasionally with only 4-6 mature spores), 100-125 x 6-7 μm (spore bearing part 51-57 μm long, the stipe 50-68); apical apparatus quadrate to rectangular, 2.0 μm high x 1.3 μm broad, blueing in Melzer's iodine reagent.

Ascospores uniserate to obliquely uniseriate, inequilaterally ellipsoid with rounded ends, brown, smooth by L.M., 8.8-10.0 x 4.4-5.0 μm; germ slit straight, on the ventral side of the spore, almost full spore length.

Cultural characteristics

Colonies on oatmeal agar at ca. 20°C, under 12 h light and 12 h darkness reaching edge of a 9 cm diam. Petri dish in 3 weeks, at first white, very thin with fine texture and a distinct margin, centre becoming faint yellow, later darkening to reddish grey, and becoming furrowed with radiating depressions, floccose. Reverse pale yellow. Exudate colourless or yellow to orange.

Hyphae sparingly branched, septate, highly coiled or straight, at first hyaline, later becoming brown, 2 μm diam.

Stromata formed after 3 weeks at the margin of the colonies, cylindrical, branched, at first white, later covered from base upward with villose black hyphae, 5-15 mm high x 2-3 mm diam. Conidium bearing regions developing on upper part of stromata, which turns pale tan due to conidial production.

Conidiogenous cells terminal, cylindrical, hyaline, smooth, 8-12 x 2-3 μm, bearing circular refractile conidial scars apically and laterally.

Conidia acropleurogenous, obovoid to ellipsoid with a flattened circular abscission scar at the base, hyaline, smooth by L. M., (7)7.5-9(9.5) x 4-4.5 μm. Conidia pale tan in mass, often accumulating in conspicuous heaps beneath stromata.

Habitat endophyte, on wood

Xylaria juruensis var. microspora *sp.* nov. Fig. 37.

Stromata unbranched or branched, cylindrical to irregular, terete to compressed, bearing more or less naked perithecia, with short to long hair-like apiculi, on long thin stipe, 3-16 cm total length x 1-2 mm diam. Externally blackish with grey peeling layer, pubescent

Figure 37. ***Xylaria juruensis var. microspora*** **sp. nov.**

a Culture on PDA

b Young stromata on twig (Bar = 1 cm)

c Mature stromata (Bar = 1 cm)

d Surface of stroma and tip (Bar= 1 mm)

e Ostiole slightly raised (Bar = 10 μm)

f Ascus with rectangular apical apparatus (arrowed) and ascospore with straight germ slit spore-length (arrowed) (Bar = 10 μm)

overall. Internally white. Texture fairly hard. Surface roughened by peeling layer, perithecial contours and pubescence.

Perithecia 0.4-0.6 mm diam.

Ostioles umbilicate to slightly raised

Asci eight-spored, the spore usually arranged in a partly biseriate manner, cylindrical, stipitate, 75 -100 μm total length x 6-7 μm broad, the spore bearing part 60-75 μm, the stipe 25-30 μm long with apical apparatus, rectangular, 1.4-1.8 μm high x 1.4-1.5 μm broad, blueing in Melzer's iodine reagent.

Ascospores brown, unicellular, ellipsoid-inequilateral with rounded to acute ends smooth, (10)11.3-13.75 x (3.8)5-5.6 μm, with straight germ slit slightly less than spore length.

Xylaria multiplex (Kunze) Fr., Nova Acta Regiae Soc. Sci. Upsal. (ser. 3) 1: 127 (1851) sensu Dennis, Kew Bull. 11 (1956): 416 (1957). Fig. 38.

Sphaerea multiplex Kunze, in Fr., Linnaea 5: 536 (1830).

Xylosphaera multiplex (Kunze) Dennis, Kew Bull. 13: 105 (1958).

Xylaria caespitulosa Ces., Myc. It. Born. Becc. Enum.: 15 (1879) .

Xylaria fastigiata Fr., Nova Acta Regiae Soc. Sci. Upsal. (ser. 3) 1: 127 (1851).

Xylosphaera fastigiata (Fr). Dennis, Rev. Biol. 1: 193 (1958)

Xylaria citrina Massee, J. Bot. 30: 163 (1892).

Xylaria cylindrica Ellis & Everh., Bull. Lab. Nat. Hist. lowa State Univ. 2: 414 (1893).

Xylaria teres Sacc. Syll. Fung. 11: 284 (1895).

Xylaria bidentata Pat., Bull. Soc. Mycol. France 11: 87 (1895).

Xylaria subtrachelina Henn., Hedwigia 43: 207 (1904).

[after Dennis 1961]

Stromata gregarious, not branched or occasionally dichotomously branched, cylindrical with an acute sterile apex (up to 1 mm long), on a narrow, smooth stipe (up to 1.2 cm long x 1 mm diam.), arising from an enlarged tomentose base, 1-5.0 cm total length x 1.5-2.5 mm diam. Externally black with dark brown peeling outer layer, splitting longitudenally into

Figure 38. ***Xylaria multiplex*** **(Kuntze) Fr.**

a Stromata (Bar = 10 mm)

b Ascus with apical apparatus (arrowed) and ascospores (Bar = 10 μm)

narrow strips. Internally white, becoming hollow with age, Texture hard. Surface nodulose due to slightly protruding perithecial contours, smooth.

Perithecia partially immersed, subglobose, up to 0.5 mm diam.; ostioles papillate, often surrounded by an annular disc

Paraphyses filiform, tapering towards the apex, remotely septate, 2 μm diam. near the top.

Asci cylindrical, 8-spored, stalked, 110-140 x 5-6 μm (spore bearing part 65-75 μm long); apical apparatus rectangular, 1.3-2.5 μm high x 1.3 μm broad, blueing in Melzer's iodine reagent.

Ascospores uniseriate to obliquely uniseriate, inequilaterally ellipsoid, with narrowly rounded ends, brown, smooth by L.M. 8.8-10.0 x 3.8-4.0 μm; germ slit straight, on the ventral side of the spore, almost full spore length.

Cultural characterisrics

Colonies on oatmeal agar at air conditioned 20-25°C in the room, reaching edge of a 9 cm diam. Petri dish in 2 weeks, at first white, velvety with distinct prumose margins, later darkening to greenish grey or dull black from centre outwards, finally in the centre brownish orange towards the margins.

Hyphae sparingly branched, septate, hyaline, smooth, 2 μm diam., becoming dark brown, densely septate, more irregularly shaped, branched and finally breaking down into segments Stromata produced within 3 weeks, cylindrical, up to 1 cm long x 1 mm diam., covered from base upwards with villose black hyphae, remaining white at the top. Stromata remaining sterile.

Conidiogenous structures formed on small white tufts, 3-4 mm diam., developing within 4 weeks.

Conidiophores in palisades, sparingly branched, hyaline, smooth, 12 x 2.5 μm, bearing circular refractile conidial scars apically and laterally.

Conidia acropleurogenous, elongated ellipsoid with a small flattened circular abscission scar at the base, and a broadly rounded to flattened apex, hyaline, smooth by L. M., 17.5-18.5 x 2.2-2.5 μm.

Habitat on wood

Xylaria schweinitzii Berk. & M.A. Curtis, J. Acad. Nat. Sci. Philadelphia (Ser.2) 2: 284. (1853). Fig. 39.

Xylaria rugosa Sacc., A. Mycol. 4: 74 (1906).

[after Dennis 1957]

Stromata Solitary or several together on the same log, not branched, cylindrical to clavate to highly irregular, occasionally subglobose with rounded fertile apices, on short to long abruptly narrowed smooth or tomentose stipes 1.0 cm long x 0.1-0.2 cm diam broad or almost sessile, 1.5-3 cm total high x 0.7-1.0 cm diam. Externally dull blackish brown to black. Internally white to beige, becoming hollow with age, splitting longitudinally and becoming inrolled. Surface cracked into minute scales, rugulose.

Perithecia partially immersed, subglobose, 0.3-0.6 mm diam

Ostioles inconspicuous, umbilicate, appearing as small hemispherical black discs in between the dark brown scales.

Paraphyses filiform, tapering towards the apex, remotely septate, 2-3 μm diam. near the top

Asci cylindrical, eight-spored (occasionally only 6-7 mature spores), long stalked, 250-270 μm total length x 9-10 μm broad, the spore-bearing parts, 105-140 μm long, the stipes, 100-135 μm long; apical apparatus rectangular, constricted sub-apically, 4.5-5.0 μm high x 3.8-5.0 μm broad, blueing in Melzer's iodine reagent.

Ascospores uniseriate to obliquely uniseriate, inequilaterally ellipsoid, with narrowly rounded ends, brown to dark brown, smooth, 27.5-32.5 x 6.5-7.5 μm; germ slit straight to slightly spiralling, obliquely oriented to the long axis of the spore, on the ventral side of the spore, short.

Culture characteristics

Appears to be identical to the description by Rogers & Callan (1986)

Habitat on wood

Figure 39. ***Xylaria schweinitzii*** **Berk. & M. A. Curtis.**

a Stromata on wood (Bar = 1 cm)

b Young ascus with rectangular apical apparatus (arrowed) (Bar = 10 μm)

c Ascospore with obliquely oriented (Bar = 10 μm)

Xylaria sp.nov1 (on pod FHL10) Fig. 40.

Stromata solitary, unbranched, arising from fallen seed pods, cylindrical to clavate, with tapered short sterile apices, on long stipe, 2-4.5 cm total height, diam 2-4 mm, stipe 1.5-2.8 cm long, smooth. Externally light brown becoming dull darkish brown. Internally white surface smooth with slight longitudinal cracking.

Perithecia immersed, globose, 0.7 mm diam.

Ostioles appearing as minute black dots surrounded by a small disk.

Asci cylindrical, eight spored spore-bearing part 63-75 µm with stipes 69-100 µm; apical apparatus rectangular, 1.25 x 1.25 µm, blueing in Melzer's iodine reagent.

Ascospores uniserate; inequilaterally ellipsoid with broadly rounded ends, brown to dark brown, smooth, 8-9.4 x 3-3.8 µm; germ slit straight, almost full length of the spore.

Note: This represents a new species occurring in fallen seed pods. According to San Martin & Rogers (1993) the Xylarias growing on fallen seeds, leaves or fruits require much more attention.

Habitat on fallen pods

Xylaria sp. nov2 **(yellow flesh stromata)** Fig. 41.

Stromata solitary and unbranched or several fused together, cylindrical with fertile rounded apices, or long narrow stipes, up to 11 cm total height x 1-3(-8) mm diam. Externally dull dark brown to black. Internally yellow. Surface rugulose.

Perithecia partitially immersed, subglobose, 0.2-0.3 mm diam.

Ostioles conspicuous, papillated, black.

Asci cylindrical, eight-spored, 130-150 µm total length, spore-bearing parts 60-75 µm with stipes 45-75 µm long; apical apparatus rectangular to inverted hat-shaped; 1.25-1.25 µm

Ascospores uniserate, unicellular 9.4-13 x 3.8-4.4 µm, spore-bearing parts 60-75 µm, germ slit sigmoid full length of the spore.

Note: The yellow flesh is highly unusual and this is undoubtedly a new taxon. It has also been recovered as an endophyte as well as from logs in the forest.

Habitat endophyte, on wood

Figure 40. *Xylaria sp. nov1* (on pod FHL 10)

a Stromata on pod (Bar = 1 cm)

b Surface of stroma with slight longitudinal cracking (Bar = 1 mm)

c Ascus with rectangular apical apparatus (Bar = 10 μm)

d Ascospores with straight germ slit almost full length of the spore (Bar = 10 μm)

Xylaria sp. nov 3 **(sp. 29, 32)** Fig. 42.

Stromata solitary, not branched, subglobose to slightly irregular, on short stipe to almost sessile, less than 1.0 cm in total height. Externally dull, dark brown. Internally white. Surface cracked in rectangular scales surrounding the ostioles

Perithecia partially immersed, subglobose, 0.5-0.7 mm diam.

Ostioles minutely papillate, appearing as small conical black dots in between the dark brown scales.

Asci cylindrical, eight-spored, spre-bearing parts 65-70 μm x 10 μm broad, with stipe 90-100 μm long; apical apparatus like a thistle head, 1.25-1.25 μm, blueing in Melzer's iodine reagent.

Ascospores uniseriate to obliquely uniseriate, 10-12.5 x 6.3-7.5 μm, germ slit straight, full length of spores.

Note: This species has only been found as an endophyte and probably represents a new taxon (Ju & Rogers, pers. comm.)

Habitat endophyte

Xylaria sp. nov 4 **(penzigioid *Xylaria*)** Fig. 43.

Stromata globose, subglobose to slightly irregular, single or fused together to form glomerules, sessile, 1-2 mm diam. Externally black. Internally white and solid. Surface slightly roughened.

Perithecia partially immersed, globose, 0.3-0.5 mm diam.

Ostioles prominent conico-papillate.

Asci cylindrical, eight-spored, spore-bearing parts 83-110 μm x 10 μm, with stipes 28-50 μm long; apical apparatus cubic to rectangular, constricted subapically, appearing inverted hat shaped, 2.5 μm high x 3.75 μm broad, blueing in Melzer's iodine reagent.

Ascospores uniseriate to obliquely uniseriate, naricular, dark brown, smooth, 13.2-15.9(17.5) x 8.8-10 μm, germ slit straight, to slightly sigmoid, full length of spore

Note: Only found as an endophyte and appears to be a previously undescribed species. Rogers, (1994) referred to this type of *Xylaria* as *Penzigia* Sacc.

Habitat endophyte

Figure 41. ***Xylaria sp. nov2***

a Young stromata on twig (Bar = 1 cm)

b Mature stromata on wood (Bar = 1 cm)

c Yellow flesh with brown core of stroma with subglobose perithecia (Bar = 1 mm)

d Ascus with apical apparatus (arrowed) (Bar = 10 µm)

e Ascospore with sigmoid germ slit spore-length (Bar = 1 µm)

f Culture on PDA

Figure 42. *Xylaria sp. nov3* (sp. 29)

a Young stromata on twig (Bar = 1 cm)

b Mature stroma on twig (Bar = 1 cm)

c Surface of stroma with cracked in rectangular scales surrounding the ostioles (Bar = 1 cm)

d Ascus with ascospores and apical apparatus (arrowed)

e Culture on PDA

Figure 43. ***Xylaria sp. nov4*** **(penzigioid** ***Xylaria*****)**

a Stromata on twig (Bar = 10 mm)

b Stromal surface slightly roughened (Bar = 1 mm)

c Ascospores with straight germ slit almost full length of the spore (Bar = 10 μm)

d Ascus with rectangular apical apparatus (arrowed) (Bar = 10 μm)

5.2. Taxonomic aspects of non-xylariaceous endophytes

Introduction

A number of representatives from well-known endophytic genera were isolated during the present study. Since a number of these are likely to be undescribed taxa identification has only been made to generic level

5.2.1. Descriptions of the species

Colletotrichum Cda. in Sturm, Deutschlands Flora 3:41 (1831-1832). Fig. 44. (c-d)

Mycelium branched, septate, hyaline, pale brown or dark brown, Conidiomata acervular, separate or confluent, composed of hyaline to dark brown, thin-or thick walled textura angularis; dihiscence irregular. Sclerotia sometimes present in culture, dark brown to black, often confluent, occasionally setose, Setae in conidiomata or sclerotia brown, smooth, septate, tapered to the apices. Conidiophores hyaline to brown, septate, branched only at the base, smooth, formed from the upper cells to the conidiomata. Conidiogenous cells enteroblastic, phialidic, hyaline, smooth, determinate, cylindrical, intrgrated or discrete, channel minute but occasionally collarette and periclinal thickening quite prominent. Conidia hyaline, aseptate (except prior to germination), stright or falcate, smooth, thin-walled, sometimes guttulate, muticate or with the apex prolonged into a simple cellular appendage. Appressoria brown, entire or with crenate to irregular margins, simple or repeatedly germinating to produce complex columns of several closely connected appressoria.

On the basis of cultural and anamorphic characteristics a single species of colletotrichum was recognised. This was not the common pathogenic species known from northern Thailand, *C. gloeosporioides* which has a wide host range.

Figure 44. (a-b) ***Nigrospora***

a Colony on PDA

b Conidia (Bar = 10 µm)

Figure 44. (c-d) ***Colletotrichum***

c Colony on PDA

d Conidia (Bar = 10 µm)

Diplodia Fig 45. (a)

Pycnidia at first immersed becoming erumpent, olivaceous brown, thin-wall, 0.2-0.3 mm diam, darker around the papillate ostiole. Conidia oval or oblong-ellipoid, remaining hyaline along time, eventually becoming pale olivaceous brown and 1-septate, 15-21 x 5-8 µm.

Humicola Traaen, 1914, Nyt. Mag. Naturvid., 52:31-34. Fig. 45. (b-c)

Colonies effuse, cottony, sometimes funiculose, at first white, later pale grey, greyish brown or blackish brown. Mycelium superficial and immersed. Stroma none. Intercalary chlamydospores sometimes formed. Setae and hyphopodia absent. Conidiophores micronematous or semi-macronematous, unbranched or irregularly branched, straight or flexuous, colourless to pale golden brown, smooth. Conidiogenous cells monoblastic, intergrated, terminal, determinate, cylindrical, doliiform, pyriform or infundibuliform. Conidia solitary, dry, acrogenous, simple, typically spherical, occasionally obovoid or pyriform, pale to mid golden brown, ususlly smooth, 0-septate. *Humicola* also has a phialidic state, the phialides being discrete, subulate, colourless, smooth, Phialoconidia catenate or in slimy heads, very small, colourless, smooth. 0-septate. It was 1 spcecies of *Humicola* obtained from teak leaves.

Nigrospora Zimmermann, 1902, Zentbl. Bakt. ParasitKde, Abt. 2, 8: 220. Fig. 44. (a-b)

Colonies at first white small, shining black conidia easily visible under a low-power disecting microscope, later brown or black when sporulation is abundant. Mycelium all immersed or partly superficial. Stroma none. Setae and hyphopodia absent. Conidiophores micronematous or semi-macronematous, branched, flexuous, colourless to brown, smooth. Conidiogenous cells monoblastic, discrere, solitary, deternimate, ampulliform or subspherical, colourless. Conidia solitary, with a violent discharge mechanism , acrogenous, simple, spherical or broadly ellipsoidal, compressed dorsiventrally, black, shining, smooth, 0-septate. It was 1 species of *Nigrospora* obtained from teak leaves.

Fig 45. (a) ***Diplodia***

a Colony on PDA

Fig 45. (b-c) ***Humicola***

b Colony on PDA

c Aleuriospore (Bar = 10 µm)

Phomopsis (Sacc.) Sacc., Annls mycol. 3: 166 (1905) Fig. 46.

Mycelium branched, septate, hyaline to pale brown. Conidiomata eustromatic, immersed, brown to dark brown, separate or aggregated and confluent, globose, ampulliform or applanate, unilocular, multilocular or convoluted, thick-walled; walls of brown, thin-or thick-walled textura angularis, often somewhat darker in the upper region, lined by a layer of smaller- celled tissue. Ostiole single, or several in complex conidiomata, circular, often papillate. Conidiophores branched and septate at the base and above, occasionally short and only 1-2 septate, more frequently multiseptate and filiform, hyaline, formed from the inner cells of the locular walls. Conidiogenous cells enteroblastic,phialidic, determinate, integrate, rarely discrete, hyaline, cylindrical, apertures apical on long or short lateral and main branches of the conidiophores, collarette, channel and periclinal thickening minute. Conidia of two basic types, but in some species with intermediates between the two: a -conidia hyaline, fusiform, straight, ususlly biguttulate (one guttule at each end) but sometines with more guttules, aseptate; b-conidia hyaline, filiform, straight or more often hamate, eguttulate, aseptate. *Phomopsis* isolates obtained from teak leaf isolations were seperated into 4 different groups depending on colony morphology, spore forming and spore size *Phomopsis sp. 1* produce only a-conidia, size 5-7.5(10) µm x 2.5 µm *Phomopsis sp. 2* produce both a-conidia and b-conidia, a-conidia 9.6-10.2 µm x 2.5 µm, b-conodia 27-37.5 x 1.3 µm. *Phomopsis sp. 3.* produce only a-conidia 8.8-12.5 x 1.3 µm *Phomopsis sp. 4.* produce both a-conidia and b-conidia ,a-conidia 8.8-9.6 µm x 1.25 µm, b-conodia 15-21 x 1.3 µm.

Figure 46. ***Phomopsis spp.***

a *Phomopsis sp. 1*

b *Phomopsis sp. 2*

c *Phomopsis sp. 3*

d *Phomopsis sp. 4*

e a-conidia *Phomopsis sp1.*

f a-conidia *Phomopsis sp3.*

CHAPTER 6

GENERAL DISCUSSION

In this investigation of fungal endophytes of leaves of *Tectona grandis* a number of significant findings have been obtained. Since this is, however, the first study of teak endophytes and also one of very few on tropical plants (Petrini & Dreyfuss, 1981; Dreyfuss & Petrini, 1984; Rodrigues & Samuels, 1990,1992; Pereira *et al.*, 1993; Fisher *et al.*, 1994,1995; Rodrigues, 1994) most of the information presented is by default novel! Comparison of the data with those of Rodrigues & Samuels (1990,1992) and Rodrigues (1994) from their studies on the tropical palms *Licuala ramsayi* and *E. oleracea*, data from studies on subtropical hosts (Hyde *et al.*, 1997; Wu, 1997) and temperate hosts (Spurr & Welty, 1975; Bernstein & Carroll, 1977; Carroll, 1979; Petrini & Petrini, 1985; Carroll, 1986; Fisher *et al.*, 1986; Petrini, 1986, 1991; Sherwood-Pike *et al.*, 1986; Fisher & Petrini, 1990; Polishook *et al.*, 1993; Schulz *et al.*, 1993; Fisher *et al.*, 1994; Sieber & Dorworth, 1994; Wu, 1997) revealed a number of interesting features.

In the comparison of taxa isolated both in terms of frequency of isolation and diversity of taxa recovered the most obvious and significant difference in endophyte assemblages concerned those associated with young as opposed to mature leaves. Regardless of site there was a much lower frequency of infection in young leaves, between 27 % and 66.5% depending on year and leaf sampled. In mature leaves an infection frequency of 100% was always obtained i.e endophytic fungi were isolated from all of the samples taken from the leaves regardless of position or geographical location. This major difference in frequency of infection between young and old leaves is in general agreement with the findings from most other endophyte studies (Bernstein & Carroll, 1977; Fisher *et al.*, 1986; Petrini, 1991; Hata & Futai, 1993; Wu, 1997). Furthermore Rodrigues (1994) in her study of *E. oleracea* found a positive correlation between leaf age and overall fungal colonisation. It is therefore apparent that from that study and from the current one that age of the leaf greatly influences the degree of colonisation by endophytic fungi in tropical plants as well as in temperate ones.

Espinosa-Garcia & Langenheim (1990) proposed that the leaf microenvironment and biochemical contents, together with the production of secondary metabolites are possible reasons for an increase in endophytic colonisation of older leaves. As pointed out by Rodrigues (1994) expanded leaves represent a better physical trap for spores than folded leaves and this might explain the higher recovery of endophytic fungi from the palm, *E. oleracea*. Certainly the mature leaves of teak attain a greater size, growing to approximately 40 cm in length and 30 cm at their widest point. The young leaves sampled were around one third the size of the mature leaves. Therefore in teak the mature leaves offer a much greater surface area for inoculum capture. In the current study it was also clear that in the young leaves genera such as *Phomopsis* and *Colletotrichum* were dominant but in mature leaves taxa belonging to the Xylariaceae, in particular *D. eschscholzii*, were dominant. Although it is not possible to demonstrate the reason for this difference in fungal populations it can be speculated that there are major differences in the available fungal inoculum since young leaves were sampled in May and mature leaves in November. It has been suggested that xylariaceous endophytes originate from either ascospores or conidia (Gowan & Vilgalys, 1991) and since the conidia of a number of species of *Xylaria* do not readily germinate (Greenhalgh & Roe, 1984) the production of ascospores in the 'wild' could be highly significant since they are likely to be the originators of the endophyte assemblages. Surveys of the natural forest site at Mae Rim and the plantation at Chiang Dao, which included surrounding forest, resulted in the recording of a substantial number of xylariaceous taxa some of which were also recorded as endophytes. However, the development of these Xylariaceae in natural forest is very dependent on the onset of the rainy season and it is not until late July that teleomorphic material becomes evident and then much of the material does not reach maturity until September. Inoculation experiments with the Xylariaceae demonstrated that at least 12 weeks are required for ascospore production and for *D. eschscholzii* a minimum of 14 weeks (Chapter 4). If ascospores of the Xylariaceae are the source of the endophytes then the absence of xylariaceous taxa in young leaves sampled in May is readily explained. By September there would be mature stromata with discharge of ascospores which could explain the presence of Xylariaceae in mature leaves sampled in November. It is also worth noting that the most frequent xylariaceous endophyte is *D. eschscholzii* which is one of the larger members of the family and a prolific ascospore producer (Ju,

Rogers & San Martin, 1996). The very high incidence of this taxon and other members of the Xylariaceae in mature leaves could easily be related to the external presence of a suitably high inoculum of ascospores. It has also been shown that ascospores of *D. concentrica*, the temperate equivalent of *D. eschscholzii* (Dennis, 1970), are capable of long term survival with spores retaining viability in herbarium packets 20 years after discharge (Gaskell, 1995). Since most ascospores of the Xylariaceae are heavily pigmented and have a thick spore wall, as in *D. concentrica* (Beckett, 1979a,b), it seems likely that many will have good survival ability following discharge and could therefore remain as a potential inoculum for some weeks or even months. Rodrigues *et al.* (1993) provided evidence for a constant arrival of new inoculum units in the leaves of *E. oleraceae* using isoenzyme studies with *Xylaria* species. Vegetative compatibility group tests performed on isolates of *X. cubensis* indicated a high degree of genetic diversity of the isolates (Rodrigues, 1992). The presence of many genetically distinct strains of an endophyte have also been demonstrated for *R. parkeri* in the needles of Douglas fir (McCutcheon, Carroll & Schwab, 1993) and for *Discula umbrinella* (Berk. & Broome) Sutton in beech leaves (Haemmerli *et al.*, 1993) through the application of the random amplified polymorphic DNA technique. The Xylariaceae, especially *Daldinia* species, are well known for their long discharge period (Ingold, 1946; Hodgkiss & Harvey, 1969, 1970; Kramer & Pady, 1970) and this would be compatible with the hypothesis that there is a constant arrival of new inoculum units for some endophytic species. *Phomopsis* and *Colletotrichum* species which dominate in young leaves probably originate from conidia which are produced rapidly in response to the onset of the rainy season. Furthermore species of these 2 genera are well known plant pathogens and host specificity certainly occurs in many host-fungus relationships involving pathogenic fungi. However, their presence as endophytes in teak could be connected with an ability to adapt to an endophytic way of life in the 'wrong host'. Petrini (1991) suggested that genera such as these were probably present as latent colonisers in their hosts. There is some evidence that host-specificity amongst endophytes is not a common feature since in an earlier study of endophytes of the Ericaceae it was found that most species, which included representatives of genera believed to be host specific such as *Phomopsis, Phyllosticta* and *Septoria*, could be isolated from more than a one host plant species (Petrini, 1986). *Colletotrichum* and *Phomopsis* are well represented in the tropics, have a wide host

range and often present their pathogenic traits in young leaves (Holliday, 1980). In Chiang Mai province species of *Colletotrichum* and *Phomopsis* are widespread pathogens of fruit trees, ornamental plants and a wide range of herbaceous plants. They appear as leaf spots in mature leaves early in the rainy season when large quantities of conidia are produced.

Host specificity amongst endophytes is a topic which provokes considerable debate. Petrini & Fisher (1988) and Fisher & Petrini (1990) have used ordination analysis to show that species making up endophyte assemblages for a given host are more or less characteristic. However, in the case of endophytes from teak they appear to be mainly notable for their known occurrence as endophytes of other hosts. Therefore, in this particular situation host specificity appears to be low. It is not however, possible to be certain if some of the taxa recovered are host specific or not since identification to species was not possible. A number of these might represent new taxa and only time and further investigations of tropical plant endophytes will show if these particular taxa are host specific or not. Examination of the species lists for the endophytic isolates of Xylariaceae from teak so far identified indicates that most are widely occurring species of which a number e.g. *X. cubensis* are already known to be endophytes (Rodrigues, 1994). The endophytes from *E. euterpe* were dominated by species of the Xylariaceae with a total of 18 taxa being recognised although confident identification to species was only possible in 10 of these and was based on cultural characteristics (Rodrigues, 1994). As discussed earlier (Chapter 4) it is not entirely satisfactory to base identification of cultural characteristics alone. A number of common xylariaceous taxa recorded as endophytes by Rodrigues (1994) e.g *H. stygium* (Lev.) Sacc., *X. adscendens* (Fr.) Fr. and *X. curta* Fr. although known from Thailand (Thienhirun, 1997) were not recorded as endophytes from teak. However, on the basis of induced teleomorphs a number of *Xylaria* isolates and at least one *Hypoxylon* isolate which were recovered represent undescribed taxa (Rogers, pers. comm.). A number of the xylariaceous endophytic species identified have also been found to be present as mature stromata close to the sampling sites. Thus, a total of 24 taxa, comprising 2 species of *Daldinia,* 11 species of *Hypoxylon,* 1 species of *Nemania* and 10 species of *Xylaria* were recorded (Chapter 5). Most of these represent well known and widespread species which have already been reported from Thailand (Thienhirun, 1997). However *D. bambusicola* Y.-M. Ju, J. D. Rogers & San Martin and

X. badia Pat, which are both bamboo species, appear to be genuinely rare (Thienhirun, 1997; Rogers pers. comm.) and *X. juruensis var. microspora* is, following Thienhirun (1997), recognised as a new small spored variety. Both of the bamboo species are likely to be host specific (Rogers pers. comm.) and were not recovered as endophyte from teak.

The Xylariaceae isolated and identified at this time consist of the following 9 species, *D. eschscholzii, H. haematostroma, H. subrutilum, H. cf. anthochroum, N. subannulata, X. cubensis, X. juruensis var. microspora, X. aristata and X. feejeensis.* It is found that at least one new species of *Hypoxylon* (*Hypoxylon sp. 188*) and 3 new species of *Xylaria* (*Xylaria sp. nov.* 2, 3 and 4) have been isolated as endophytes and mature stromata produced (Table 34. and chapter 5). These identifications are on the basis of teleomorph development using the inoculation and selective incubation method detailed in Chapter 4. *Xylaria grammica* was also identified on the basis of its matched secondary metabolite profile. The frequently isolated xylariaceous endophytes, *D. eschscholzii, H. haematostroma, N. subannulata, X. cubensis* and *X. feejeensis* all occurred in mature teleomorph form in the near vicinity of the sampled trees. Further searches of natural and surrounding forest may add to this list in the future. It is surprising to observe that teak branches and logs occurring under natural field conditions do not appear to be a regular substratum for xylariaceaous fungi.

It is interesting to note that many species of *Xylaria* inhabit fallen leaves, seeds or fruits where they develop their stromata. These species are well represented in Thailand (Thienhirun, 1997) but surprisingly only one of these species, *X. aristata*, has been recognised as an endophyte during the present study. This may provide additional support for the view that many of the seed and fruit inhabiting species are in fact litter inhabitants and that the fallen seeds or fruits act as baits (Rogers, 1979; Whalley, 1996). Bayman *et al.*,(1998) in their study of transmission of *Xylaria* endophytes of *Causarina equisetifolia* L. and *Manilkara bidentata* (A. DC.) A. Chev. demonstrated a high recovery of *Xylaria spp.* from shoots of *Casuarina* and from leaves of *Manilkara* respectively. *Xylaria* was recovered from 8% of seeds of *Casuarina* but none from *Manilkara* seeds. They concluded that vertical transmission of *Xylaria* may be possible but not necessary for infection in *Casuarina* (Bayman *et al.*, 1998).

It has been shown that many endophytic species are biochemically very active and

that they possess a greater ability to produce pectinase and esterase enzymes (Schulz *et al.*, 1995). This might provide a means of recognising which *Xylaria* species inhabiting leaves, fruits or seeds are endophytes and which are litter inhabiting fungi.

The ability to fruit and produce mature stromata on flimsy leaves or on inhospitable seeds might also be linked with the speed of development of the fungus in attaining maturity. However, comparison of development times for those species which attained maturity following inoculation onto woody substrata does not provide any clear indication. *Xylaria aristata* matured in 13-15 weeks and was therefore one of the most rapidly developing species but other species also exhibiting similar rates of development were *X. cubensis, D. eschscholzii* and *N. subannulata.* It is worth noting that the most frequently isolated species of Xylariaceae also exhibited the greatest rate of maturity. This in turn may be related to their ability to mature rapidly and produce ascospores which then infect the leaves and develop as endophytes.

The Xylariaceae are now recognised to be regular and numerically important components of endophytic assemblages of tropical plants. However, virtually nothing is known about their activities as endophytes or indeed why they are so common as endophytes in the tropics when in temperate areas although present they only occupy a small percentage of the total endophyte populations. It has been suggested that many Xylariaceae are latent invaders which become aggressive to the host plant once the host is stressed. This usually occurs through water stress caused by drought, fire, mechanical or insect damage or prior infection by other fungi causing wilt conditions (Whalley, 1996). There seems little doubt that this is the case with many species of *Biscogniauxia* where canker disease in *Quercus suber* L. (cork oak), *Fagus sylvatica* L. (beech) and *Nothofagus cunninghamii* Oerst. (southern beech) is caused by *B. mediterranea* (De Not.) Kuntze, *B. nummularia* (Bull.:Fr.) Kuntze and *B. nothofagi* Whalley, Laessøe & Kile respectively (Macara, 1974, 1975; Whalley, Laessøe & Kile, 1990; Granata & Whalley, 1994). *Biscogniauxia* is a common endophyte in temperate hosts (Vannini & Scarascia Mugnozza, 1991; Petrini *et al.*, 1995; Wu, 1997) but was not isolated from teak during this investigation. It is concluded that canker diseases in trees caused by *Biscogniauxia* species is not a usual phenomenon in tropical situations. Whether any of the identified xylariaceous endophytes act as latent pathogens or have any adverse affect on teak is not known.

It is also difficult to explain the extremely high occurrence of members of the Xylariaceae as endophytes of teak leaves. Their significant presence as endophytes in tropical plants has been shown by Rodrigues & Samuels (1990; 1992), Rodrigues (1994) and is reviewed by Petrini *et al.* (1995). As stated by Rodrigues & Petrini (1997) "Xylariaceae, rare or detectable only after using selective isolation methods (Chapela & Boddy, 1988b) in hosts collected in the temperate zones, were frequently and commonly isolated from tissues of tropical plants using standard isolation techniques." However, in the current study isolation frequencies of over 60% are exceptional. It is possible that the presence of a high inoculum through their ascospores coupled with their ability to withstand desiccation contribute to their ability to infect the leaves and to adapt to an endophytic lifestyle. The isolation of taxa belonging to the genera *Daldinia, Hypoxylon, Nemania* and *Xylaria* is in agreement with the findings of Rodrigues (1994) and the general views of Petrini *et al.*, (1995). However, it is also clear that representatives of other xylariaceous genera such as *Anthostomella* and *Kretzschmaria* recorded by Rodrigues (1994) are notably absent during the present study.

Comparison of the endophytes from the two sites, one natural the other plantation, indicates that overall there are very few differences. The application of the Sorenson index demonstrates a high species similarity between the two sites. Other studies of endophyte assemblages from the same plant species growing in different geographical locations often show them to be completely different (Petrini, 1986, 1991; Fisher & Petrini, 1990; Fisher *et al.*, 1993, 1994). This has been explained to be the result of differences in the surrounding vegetation, temperature, rainfall, humidity and perhaps even pollution (Petrini, 1991). However, in the current investigation the sites were only 80 km apart and the surrounding vegetation is similar although less diverse or extensive in the plantation area. Investigation of the Xylariaceae fruiting on logs, branches and other woody substrata in both areas again showed them to be similar. Sieber-Canavesi & Sieber (1988) found marked differences in species richness and in the distribution of certain individual fungal species between populations of the same plant growing in different sites. This they regarded as site-specificity of endophytes (Sieber-Canavesi & Sieber, 1988). In the current investigation this was not the case. Greater variation in endophyte assemblages was observed between years than between sites. In 1995 *Phomopsis* species were dominant in the Chiang Dao samples and in Mae Rim a

greater diversity of species was recorded. It is most probable that variations in frequency of individual species and overall species richness is a reflection on the climatic conditions that year. The higher percentage of *Phomopsis* in mature leaves in Chiang Dao in 1995 could be associated with a later than usual rainy season and a lack of mature Xylariaceae to provide and ascospore inoculum at the appropriate time. In a comparison of leaf endophytes from *Leptosperum scoparium* Forst (manka), a member of the Myrtaceae, their diversity and frequency occurring in naturally regenerating and planted stands was undertaken (Johnston, 1998). Unlike the current study Johnston (1998) found that a *Phyllosticta* species dominated the leaves in natural stands but was mainly absent from the planted stands. It was found, that members of the Xylariaceae were frequently recovered from many of the stands and at maximum were recovered at a level of 28%. However no attempt was made to identify these further although diversity amongst the Xylariaceae was found to be high overall (Johnston, 1998). The study of fungal endophytes of teak however differs in that the natural forest site investigated was chosen because it is unlogged whereas the natural stand sites investigated by Johnston (1998) were non-virgin being regenerated forest. It is also clear that in the case of teak leaves there is no definite tissue specificity with regards to frequency of isolation or specific species of fungi. Tissue and organ specificity appears to be common for endophytic fungi. Organ specificity was demonstrated for wheat endophytes (Sieber, 1985) and later for other plants (Sieber, 1988; Sieber *et al.*, 1991) and earlier Carroll, *et al.*, (1977) had found indications of tissue specificity for endophytes from European conifers. Numerous studies since then have demonstrated the widespread occurrence of tissue specificity amongst endophytic fungi. In teak leaves isolations from leaf lamina, midrib or vein were in most of the samples not significantly different and there was little indication that there were differences in frequency or in distribution of individual species by area of a leaf. Rodrigues (1994) obtained conflicting results for endophytes of *E. oleracea* with evidence to support the existence of tissue specificity of the endophytic fungi recorded. For example, xylariaceous fungi were found predominantly in the veins whilst *Letendraeopsis palmarum* K. F. Rodrigues & Samuels and *Idriella euterpes* K. F. Rodrigues & Samuels were more frequent in the intervein.

Future studies of teak endophytes could include the use of molecular techniques for the identification of non-sporulating isolates, investigation of the internal leaf tissue

for the location of endophytic fungi by application of light, electron and confocal microscopy. Examination of leaves at different stages of maturity to determine when members of the Xylariaceae become dominant and an extension of metabolite profiling would be expected to provide useful data.

Table 34. New species of xylariaceous fungi have been isolated as endophytes.

Hypoxylon	*Xylaria*
Hypoxylon sp. 188	*Xylaria sp. nov 2*
	Xylaria sp. nov 3
	Xylaria sp. nov 4

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APPENDICES

Appendix 1.

Melzer's reagent

Chloral hydrate	100 g.
Potassium iodine	5 g.
Iodine	5 g.
Distilled water	100 g.

Appendix 2.

Media

Potato Dextrose Agar (Difco)
Malt Extract Agar (Difco)
Oatmeal Agar (Difco)

Prepared following manufacturers' instructions.

Table 1. Number of taxa identified from each area of mature leaves at Mae Rim forest (1995).

Leaf number	Midrib		Veins		Leaf lamina						Max.taxa
	1	2	3	4	5	6	7	8	9	10	
1	2	3	3	4	3	2	5	4	4	5	5
2	3	4	3	4	6	3	4	4	3	3	6
3	3	3	3	3	3	4	4	3	2	3	4
4	5	3	2	2	4	2	1	4	4	4	5
5	2	4	5	5	3	3	4	4	3	3	5
Max. taxa	5	4	3	4	6	4	5	4	4	5	
Min. taxa	2	3	2	2	3	2	1	3	2	3	
Mean	3	3.4	3.2	3.6	3.8	2.8	3.6	3.8	3.2	3.6	

Table 2. Number of taxa found from each area of mature leaves at Mae Rim forest (1996).

Leaf number	Midrib		Veins		Leaf lamina						Max.taxa
	1	2	3	4	5	6	7	8	9	10	
1	6	5	3	5	5	5	5	3	4	2	6
2	1	4	4	6	6	5	5	4	5	6	6
3	3	3	4	5	3	4	6	4	5	4	6
4	3	3	4	5	3	3	4	2	3	5	5
5	3	4	5	3	4	4	3	5	5	5	5
Max. taxa	6	5	5	6	6	5	6	5	5	6	
Min. taxa	1	3	3	3	3	3	3	2	3	2	
Mean	3.2	3.8	4	4.8	4.2	4.2	4.6	3.6	4.4	4.4	

Table 3. Number of taxa identified from each area of mature leaves at Mae Rim forest (1997).

Leaf number	Midrib		Veins		Leaf lamina						Max.taxa
	1	2	3	4	5	6	7	8	9	10	
1	4	4	8	4	6	5	5	4	5	4	8
2	7	3	3	5	3	5	4	4	3	5	7
3	4	5	5	5	3	4	3	6	5	6	6
4	4	6	2	5	4	4	5	3	4	5	6
5	3	6	3	7	5	4	5	5	7	3	7
Max. taxa	7	6	8	7	6	5	5	6	7	6	
Min. taxa	3	3	2	4	3	4	3	3	3	3	
Mean	4.4	4.8	4.2	5.2	4.2	4.4	4.4	4.4	4.8	4.6	

Table 4. Total isolates found on each area of mature leaves at Mae Rim forest (1995).

Leaf number	Midrib		Veins		Leaf lamina						Total isolates
	1	2	3	4	5	6	7	8	9	10	
1	4	5	6	5	5	3	5	4	4	7	48
2	5	4	5	5	8	4	5	5	3	4	48
3	6	6	5	3	4	5	6	4	3	4	46
4	5	7	5	5	4	3	1	5	4	5	44
5	4	5	6	6	4	3	4	4	4	5	45
Max.	6	7	6	6	8	5	6	5	4	7	48
Min.	4	4	5	3	4	3	1	4	3	4	44
Mean	4.8	5.4	5.4	4.8	5	3.6	4.2	4.4	3.6	5	46.2
Total isolates	24	27	27	24	25	18	21	22	18	25	231

Table 5. Total isolates found on each area of mature leaves at Mae Rim forest (1996).

Leaf number	Midrib		Veins		Leaf lamina						Total isolates
	1	2	3	4	5	6	7	8	9	10	
1	8	6	4	7	5	6	6	4	4	5	55
2	4	5	7	9	8	9	9	8	9	7	75
3	6	5	5	6	5	5	6	5	5	6	54
4	6	5	6	7	3	4	6	6	5	7	55
5	4	5	6	3	6	6	4	5	5	5	49
Max.	8	6	7	9	8	9	9	8	9	7	75
Min.	4	5	4	3	3	4	4	4	4	5	49
Mean	5.6	5.2	5.6	6.4	5.4	6	6.2	5.6	5.6	6	57.6
Total isolates	28	26	28	32	27	30	31	28	28	30	288

Table 6. Number of isolates found from each area of mature leaves at Mae Rim forest (1997).

Leaf number	Midrib		Veins		Leaf lamina						Total isolates
	1	2	3	4	5	6	7	8	9	10	
1	6	6	10	7	9	5	6	5	5	4	63
2	10	6	6	6	5	7	5	6	6	6	63
3	6	8	5	7	5	5	5	6	6	7	60
4	5	9	5	8	7	6	6	4	5	9	64
5	5	9	5	8	7	6	6	4	5	9	64
Max.	10	9	10	8	9	7	6	6	6	9	60
Min.	5	6	5	6	5	5	5	4	5	4	64
Mean	6.4	7.6	6.2	7.2	6.6	5.8	5.6	5	5.4	7	62.8
Total isolates	32	38	31	36	33	29	28	25	27	35	314

Table 7. Number of taxa identified from each area of mature leaves at Chiang Dao plantation (1995).

Leaf number	Midrib		Veins		Leaf lamina						Max.taxa
	1	2	3	4	5	6	7	8	9	10	
1	2	2	3	3	5	6	3	3	4	4	6
2	4	4	2	4	3	4	3	4	5	4	5
3	1	3	7	4	4	2	4	6	4	5	7
4	1	2	3	2	3	4	7	3	3	7	7
5	1	4	2	3	5	1	4	7	2	5	7
Max. taxa	4	4	7	4	5	6	7	6	5	7	
Min. taxa	1	2	2	2	3	1	3	3	2	4	
Mean	1.8	3	3.4	3.2	4	3.4	4.2	4.6	3.6	5	

Table 8. Number of taxa identified from each area of mature leaves at Chiang Dao plantation (1996).

Leaf number	Midrib		Veins		Leaf lamina						Max.taxa
	1	2	3	4	5	6	7	8	9	10	
1	2	3	4	4	5	3	5	6	5	8	8
2	4	2	3	3	5	5	3	6	4	5	6
3	4	5	4	5	6	5	4	4	4	6	6
4	2	3	4	3	2	6	5	4	5	4	6
5	4	5	5	5	7	4	5	5	5	7	7
Max. taxa	4	5	5	5	6	6	5	6	5	8	
Min. taxa	2	2	3	3	2	4	3	3	4	4	
Mean	3.2	3.6	4	4	5	4.6	4.4	5	4.6	6	

Table 9. Number of taxa identified from each area of mature leaves at Chiang Dao plantation (1997).

Leaf number	Midrib		Veins		Leaf lamina						Max.taxa
	1	2	3	4	5	6	7	8	9	10	
1	3	4	4	6	5	3	3	3	5	4	6
2	2	4	5	6	5	5	5	4	4	5	6
3	1	2	5	6	6	6	10	7	7	7	10
4	2	3	2	7	2	5	4	6	3	6	7
5	1	4	2	4	7	4	5	6	4	4	7
Max. taxa	3	4	5	7	7	6	10	7	7	7	
Min. taxa	1	2	2	4	2	4	3	3	3	4	
Mean	1.8	3.4	3.6	5.8	5	4.6	5.4	5.2	4.6	5.2	

Table 10. Total isolates found from each area of mature leaves at Chiang Dao plantation (1995).

Leaf number	Midrib		Veins		Leaf lamina						Total
	1	2	3	4	5	6	7	8	9	10	isolates
1	4	5	4	6	6	9	4	4	7	6	55
2	4	5	4	5	7	5	6	5	6	5	52
3	4	7	8	5	6	4	5	10	7	7	63
4	4	4	5	4	4	5	7	5	5	9	52
5	4	5	4	4	5	4	4	8	4	7	49
Max.	4	7	8	7	6	9	7	10	8	9	63
Min.	4	4	4	4	4	4	4	4	4	5	49
Mean	4	5.2	5	4.8	5.6	5.4	5.2	6.4	5.8	6.8	54.2
Total isolates	20	26	25	24	28	27	26	32	29	34	271

Table 11. Total isolates found from each area of mature leaves at Chiang Dao plantation (1996).

Leaf number	Midrib		Veins		Leaf lamina						Total
	1	2	3	4	5	6	7	8	9	10	isolates
1	5	4	5	5	6	4	5	7	6	8	55
2	5	3	5	4	9	7	4	7	4	6	54
3	6	10	4	8	7	6	4	6	4	9	64
4	4	5	4	6	4	7	7	4	5	6	52
5	6	6	8	5	9	6	6	6	7	8	67
Max.	6	10	8	8	9	7	7	7	7	9	67
Min.	4	3	4	4	4	4	4	4	4	6	52
Mean	5.2	5.6	5.2	5.6	7	6	5.2	6	5.2	7.4	58.4
Total isolates	26	28	26	28	35	30	26	30	26	37	292

Table 12. Total isolates found from each area of mature leaves at Chiang Dao plantation (1997).

Leaf number	Midrib		Veins		Leaf lamina						Total
	1	2	3	4	5	6	7	8	9	10	isolates
1	9	6	5	10	5	5	3	6	5	5	59
2	4	5	6	9	7	6	7	4	5	7	60
3	4	4	6	6	6	7	10	7	7	7	64
4	4	5	4	8	5	5	5	7	5	8	56
5	4	6	4	5	8	5	6	8	5	5	56
Max.	9	6	6	10	8	7	10	8	7	8	56
Min.	4	4	4	5	5	5	3	4	5	5	64
Mean	5	5.2	5	7.6	6.2	5.6	6.2	6.4	5.4	6.4	59
Total isolates	25	26	25	38	31	28	31	32	27	32	295

Table 13. Total isolates found from each area of young leaves at Mae Rim forest (1996).

Leaf number	Midrib		Veins		Leaf lamina						Total isolates
	1	2	3	4	5	6	7	8	9	10	
1	4	3	1	2	0	2	0	0	0	3	15
2	2	0	0	0	0	0	0	0	0	1	3
3	2	0	1	3	1	0	0	0	0	1	8
4	4	0	1	2	2	3	3	1	0	1	17
5	2	2	1	0	2	0	1	1	1	1	11
Max.	4	3	1	3	2	3	3	1	1	3	17
Min.	2	0	0	0	0	0	0	0	0	1	3
Mean	2.8	1	0.8	1.4	1	1	0.8	0.4	0.2	1.4	10.8
Total isolates	14	5	4	7	5	5	4	2	1	7	54

Table 14. Total taxa found from each area of young leaves at Mae Rim forest (1996).

Leaf number	Midrib		Veins		Leaf lamina						Max.taxa
	1	2	3	4	5	6	7	8	9	10	
1	2	2	1	2	0	1	0	0	0	2	2
2	1	0	0	0	0	0	0	0	0	1	1
3	2	0	1	2	1	0	0	0	0	1	2
4	1	0	1	1	2	2	2	1	0	1	2
5	1	2	1	0	1	0	1	1	1	1	2
Max. taxa	2	2	1	2	2	2	2	1	1	2	
Min. taxa	1	0	0	0	0	0	0	0	0	1	

Table 15. Total isolates found from each area of young leaves at Mae Rim forest (1997).

Leaf number	Midrib		Veins		Leaf lamina						Total isolates
	1	2	3	4	5	6	7	8	9	10	
1	3	1	3	3	3	1	1	2	1	2	20
2	2	1	2	1	0	0	0	1	1	0	8
3	1	2	3	1	2	4	4	2	2	3	24
4	4	3	1	2	4	2	1	1	1	2	21
5	0	3	0	5	0	0	1	3	3	2	17
Max.	4	3	3	5	4	4	4	3	3	3	24
Min.	0	1	1	1	0	0	0	1	1	0	8
Mean	2	2	1.8	2.4	1.8	1.4	1.4	1.8	1.6	1.8	10.8
Total isolates	10	10	9	12	9	7	7	9	8	9	90

Table 16. Total species found from each area of young leaves at Mae Rim forest (1997).

Leaf number	Midrib		Veins		Leaf lamina						Max.taxa
	1	2	3	4	5	6	7	8	9	10	
1	2	1	2	2	2	1	1	1	1	2	2
2	1	1	1	1	0	0	0	1	1	0	1
3	1	1	3	1	1	1	1	1	1	1	3
4	2	1	1	1	2	2	2	1	1	2	2
5	0	2	0	2	0	0	1	2	1	2	2
Max. taxa	2	2	3	2	2	2	2	2	1	2	
Min. taxa	0	1	0	1	0	0	0	1	1	0	

Table 17. Total isolates found from each area of young leaves at Chiang Dao plantation (1996).

Leaf number	Midrib		Veins		Leaf lamina						Total isolates
	1	2	3	4	5	6	7	8	9	10	
1	3	0	0	0	0	0	0	1	0	0	4
2	4	3	4	2	2	4	0	2	1	1	23
3	4	3	2	4	1	0	0	0	0	0	14
4	2	2	2	4	1	0	2	4	2	1	20
5	2	2	4	3	0	0	2	0	4	1	18
Max.	4	3	4	4	2	4	2	4	4	1	23
Min.	2	2	2	2	0	0	0	0	0	0	4
Mean	3	2	2.4	2.6	0.8	0.8	0.8	1.4	1.4	0.6	15.8
Total isolates	15	10	12	13	4	4	4	7	7	3	79

Table 18. Total taxa found from each area of young leaves at Chiang Dao plantation (1996).

Leaf number	Midrib		Veins		Leaf lamina						Max.taxa
	1	2	3	4	5	6	7	8	9	10	
1	2	0	0	0	0	0	0	1	0	0	2
2	1	2	1	2	1	0	1	1	1	1	2
3	3	1	1	2	1	0	0	0	0	0	3
4	1	1	1	2	1	0	1	1	1	1	2
5	1	1	1	2	0	0	1	0	1	1	2
Max. taxa	3	2	1	2	1	0	1	1	1	1	
Min. taxa	1	1	0	0	0	0	0	0	0	0	

Table 19. Total isolates found from each area of young leaves at Chiang Dao (1997).

Leaf number	Midrib		Veins		Leaf lamina						Total isolated
	1	2	3	4	5	6	7	8	9	10	
1	2	3	3	4	2	2	1	3	2	4	26
2	4	2	2	3	3	5	2	1	3	0	25
3	4	4	2	2	2	1	4	4	4	2	29
4	4	5	6	3	2	1	2	1	3	2	29
5	2	4	4	4	2	2	1	4	4	3	30
Max.	4	5	6	4	3	5	4	4	4	4	30
Min.	2	2	2	2	2	1	1	1	1	0	25
Mean	3.2	3.6	3.4	3.2	2.2	2.2	2.0	2.6	3.2	2.2	27.8
Total isolates	16	18	17	16	11	11	10	13	16	11	139

Table 20. Total taxa found from each area of young leaves at Chiang Dao (1997).

Leaf number	Midrib		Veins		Leaf lamina						Max.taxa
	1	2	3	4	5	6	7	8	9	10	
1	1	3	2	3	1	1	1	3	2	1	3
2	1	1	1	2	1	3	1	1	1	0	3
3	3	2	1	2	1	1	1	1	1	2	3
4	1	2	2	2	1	1	1	1	1	1	2
5	1	3	1	2	1	2	1	2	2	2	3
Max. taxa	3	3	2	3	1	3	1	3	2	2	
Min. taxa	1	1	1	2	1	1	1	1	1	0	

Table 21. Percentage of isolation on young leaves at Mae Rim forest in 1996.

Leaf number	Midrib		Veins		Leaf lamina						Average
	1	2	3	4	5	6	7	8	9	10	
1	100	75	25	50	0	50	0	0	0	75	37.5
2	50	0	0	0	0	0	0	0	0	25	7.5
3	50	0	25	75	25	0	0	0	0	25	20
4	100	0	25	50	50	75	75	25	0	25	42.5
5	50	50	25	0	50	0	25	25	25	25	27.5
% of isolation	70	25	20	35	25	25	20	10	5	35	27

Table 22. Percentage of isolation on young leaves at Mae Rim forest in 1997.

Leaf number	Midrib		Veins		Leaf lamina						Average
	1	2	3	4	5	6	7	8	9	10	
1	75	25	75	75	75	25	25	50	25	50	50
2	50	25	50	25	0	0	0	25	25	0	20
3	25	50	75	25	50	100	100	50	50	75	60
4	100	75	25	50	100	50	25	25	25	50	52.5
5	0	75	0	100	0	0	25	75	75	50	40
% of isolation	50	50	45	55	45	35	35	45	40	45	44.5

Table 23. Percentage of isolation on young leaves at Mae Rim forest in 1997.

Leaf number	Midrib		Veins		Leaf lamina						Average
	1	2	3	4	5	6	7	8	9	10	
1	75	0	0	0	0	0	0	25	0	0	10
2	100	50	100	50	50	100	0	50	25	25	55
3	100	75	50	100	25	0	0	0	0	0	35
4	50	50	50	100	25	0	50	100	50	25	50
5	50	50	100	75	0	0	50	0	100	25	45
% of isolation	75	45	60	65	20	20	20	35	35	15	39

Table 24. Percentage of isolation on young leaves at Mae Rim forest in 1997.

Leaf number	Midrib		Veins		Leaf lamina						Average
	1	2	3	4	5	6	7	8	9	10	
1	50	75	75	100	50	50	25	75	50	100	65
2	100	50	50	75	75	100	50	25	75	0	60
3	100	100	50	50	50	25	100	100	100	50	72.5
4	100	100	100	75	50	25	50	25	25	50	60
5	50	100	100	100	50	50	25	100	100	75	75
% of isolation	80	85	75	80	55	50	50	65	70	55	66.5

Table 25. Species identified from mature leaf no.1 at Mae Rim forest (1995).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	0	0	2	0	1	1	1	1	6	5
Daldinia eschscholzii	0	2	0	1	0	2	1	1	0	1	8	6
Diplodia sp.	0	1	0	0	0	0	0	0	0	0	1	1
Fusarium sp.	3	0	0	0	0	0	0	0	0	0	3	1
Humicola sp.	0	0	0	0	0	0	0	0	0	1	1	1
Hypoxylon cf. subrutilum	0	0	1	0	0	0	0	0	0	0	1	2
H. subrutilum	0	0	1	1	1	1	0	1	1	0	6	6
H. haematostroma	0	1	0	2	2	0	1	1	1	1	9	7
Nigrospora sp.	0	0	0	0	0	0	1	0	0	0	1	1
Phoma sp.	0	0	3	0	0	0	0	0	0	0	3	1
Phomopsis sp.1	0	1	0	0	0	0	0	0	0	0	1	1
Phomopsis sp.2	1	0	0	0	0	0	0	0	0	0	1	1
Phomopsis sp.3	0	0	0	0	0	0	0	0	0	1	1	1
Xylaria cubensis	0	0	0	0	0	0	0	0	0	2	2	1
X. feejeensis	0	0	0	0	0	0	1	0	0	0	1	1
Sterile sp.1	0	0	1	1	0	0	0	0	1	0	3	3
Number of isolates	4	5	6	5	5	3	5	4	4	7	48	
Number of taxa	2	3	3	4	3	2	5	4	4	5		
Total species	16											

Table 26. Species identified from mature leaf no.2 at Mae Rim forest (1995).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	1	0	0	0	0	0	1	0	2	2
Daldinia eschscholzii	1	0	0	2	2	0	0	0	1	1	7	5
Fusarium sp.	0	0	0	1	0	0	0	0	0	0	1	1
Humicola sp.	0	0	0	0	0	0	1	0	0	0	1	1
Hypoxylon cf. subrutilum	0	1	0	1	0	1	1	1	0	0	5	5
H. subrutilum	1	1	0	0	0	1	1	1	0	1	6	6
Hypoxylon sp. 188	0	0	0	0	1	1	1	1	0	0	4	4
H. haematostroma	0	0	1	0	1	0	0	0	0	0	2	2
Nemania subannulata	0	0	0	0	1	1	1	0	0	0	3	3
Phomopsis sp.1	0	1	0	0	0	0	0	1	0	0	2	2
Phomopsis sp.2	0	0	0	1	0	0	0	0	1	0	2	2
Phomopsis sp.3	3	1	0	0	2	0	0	1	0	2	9	4
X. juruensis var. microspora	0	0	3	0	0	0	0	0	0	0	3	1
Sterile sp. 3	0	0	0	0	1	0	0	0	0	0	1	1
Number of isolates	5	4	5	5	8	4	5	5	3	4	48	
Number of taxa	3	4	3	4	6	3	4	4	3	3		
Total species	14											

Table 27. Species identified from mature leaf no.3 at Mae Rim forest (1995).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Daldinia eschscholzii	0	2	0	1	2	2	2	1	2	1	13	8
Hypoxylon cf. subrutilum	0	0	0	0	1	0	0	0	0	1	2	10
H. subrutilum	0	2	1	1	0	0	0	2	0	0	6	4
H. haematostroma	0	0	2	0	0	0	1	0	0	0	3	2
Nemania subannulata	0	0	0	1	1	1	1	0	1	2	7	6
Phomopsis sp.1	1	0	0	0	0	0	0	0	0	0	1	1
Phomopsis sp.2	2	0	0	0	0	0	0	0	0	0	2	1
Phomopsis sp.3	3	0	0	0	0	0	0	0	0	0	3	1
Phomopsis sp.4	0	0	2	0	0	0	0	0	0	0	2	1
Xylaria sp. 271	0	0	0	0	0	0	0	1	0	0	1	1
Xylaria sp. 342	0	0	0	0	0	1	0	0	0	0	1	1
X. grammica	0	0	0	0	0	1	2	0	0	0	3	2
X. juruensis var. microspora	0	2	0	0	0	0	0	0	0	0	2	1
Number of isolates	6	6	5	3	4	5	6	4	3	4	46	
Number of taxa	3	3	3	3	3	4	4	3	2	3		
Total species	13											

Table 28. Species identified from mature leaf no.4 at Mae Rim forest (1995).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Cladosporium sp.	1	0	0	0	1	0	0	0	0	0	2	2
Colletotrichum sp.	0	0	0	1	0	0	0	0	0	0	1	1
Daldinia eschscholzii	1	1	0	0	0	2	0	2	0	2	8	5
Hypoxylon cf. subrutilum	0	0	0	0	0	0	0	1	0	1	2	2
H. subrutilum	0	0	0	0	1	0	0	0	1	0	2	2
H. haematostroma	1	0	0	0	1	0	0	1	0	1	4	4
Nemania subannulata	0	0	0	0	0	1	1	0	1	1	4	4
Nigrospora sp.	0	0	0	0	0	0	0	0	1	0	1	1
Phomopsis sp.2	1	0	0	0	0	0	0	0	0	0	1	1
Phomopsis sp.3	0	2	0	0	1	0	0	1	0	0	4	3
Septonema sp.	1	0	1	0	0	0	0	0	0	0	2	2
X.juruensis var. microspora	0	4	4	4	0	0	0	0	0	0	12	3
Sterile sp.6	0	0	0	0	0	0	0	0	1	0	1	1
Number of isolates	5	7	5	5	4	3	1	5	4	5	44	
Number of taxa	5	3	2	2	4	2	1	4	4	4		
Total species	13											

Table 29. Species identified from mature leaf no.5 at Mae Rim forest (1995).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Cladosporium sp.	0	0	0	1	0	0	0	0	0	0	1	1
Curvularia sp.	0	1	0	1	0	0	0	0	0	0	2	2
Daldinia eschscholzii	0	0	2	1	0	0	0	0	2	3	8	4
Fusarium sp.	0	0	0	0	0	0	0	0	1	0	1	1
Humicola sp.	0	0	1	0	1	1	1	0	1	0	5	5
Hypoxylon cf. subrutilum	0	0	0	1	1	1	0	0	0	0	3	3
H. haematostroma	0	0	0	0	0	1	1	1	0	0	3	3
Phomopsis sp.1	2	1	0	2	0	0	1	0	0	0	6	4
Phomopsis sp.2	2	2	0	0	2	0	0	1	0	1	8	5
Phomopsis sp.4	0	0	1	0	0	0	0	0	0	1	2	2
Nigrospora sp.	0	1	0	0	0	0	0	0	0	0	1	1
Xylaria sp.347	0	0	1	0	0	0	0	0	0	0	1	1
Xylaria sp.565	0	0	0	0	0	0	1	0	0	0	1	1
Trichoderma sp.	0	0	0	0	0	0	0	1	0	0	1	1
Sterile sp.1	0	0	1	0	0	0	0	1	0	0	2	2
Number of isolates	4	5	6	6	4	3	4	4	4	5	45	
Number of taxa	2	4	5	5	3	3	4	4	3	3		
Total species	15											

Table 30. Species identified from mature leaf no.1 at Chiang Dao plantation (1995).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Curvularia sp.	0	0	0	0	1	0	0	0	0	0	1	1
Daldinia eschscholzii	0	0	0	1	1	0	1	0	1	0	4	3
Diplodia sp.	0	0	1	0	0	1	0	0	0	0	2	2
Fusarium sp.	0	0	0	0	0	1	0	0	0	1	2	2
Humicola sp.	0	0	0	0	0	0	0	1	0	0	1	1
Hypoxylon subrutilum	0	1	0	0	1	0	0	0	0	0	2	2
H. haematostroma	0	0	0	0	0	0	0	0	1	1	2	2
Nigrospora sp.	0	0	1	1	0	0	0	0	0	0	2	2
Pestalotia sp.	0	0	0	0	0	1	0	0	0	1	2	2
Phomopsis sp.1	2	4	2	3	2	4	1	1	4	3	26	10
Phomosis sp.2	0	0	0	0	0	1	2	0	0	0	3	2
Phomosis sp.3	2	0	0	0	1	0	0	2	0	0	5	3
Sterile sp. 1	0	0	0	1	0	1	0	0	1	0	3	3
Number of isolates	4	5	4	6	6	9	4	4	7	6	55	
Number of taxa	2	2	3	3	5	6	3	3	4	4		
Total species	13											

Table 31. Species identified from mature leaf no.2 at Chiang Dao plantation (1995).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Cladosporium sp.	0	0	0	1	0	0	0	0	0	0	1	1
Colletotrichum sp.	0	0	0	0	0	0	0	1	0	0	1	1
Daldinia eschscholzii	0	1	0	0	0	0	0	1	0	0	2	2
Diplodia sp.	1	0	0	0	0	1	0	0	0	0	2	2
Fusarium sp.	0	0	0	0	0	0	1	0	0	0	1	1
Humicola sp.	0	0	0	0	2	0	0	0	1	0	3	2
Hypoxylon cf. subrutilum	0	0	0	0	0	0	0	0	1	1	2	2
H. haematostroma	0	0	0	1	0	0	1	0	0	0	2	2
Nigrospora sp.	1	1	0	0	0	1	0	0	0	0	3	3
Phomopsis sp.1	1	0	2	2	4	2	4	2	2	2	21	9
Phomopsis sp.2	0	1	0	0	0	0	0	0	1	1	3	3
Phomopsis sp. 3	1	2	2	1	0	0	0	0	0	1	7	5
Phomopsis sp.4	0	0	0	0	0	1	0	0	0	0	1	1
Sterile sp. 6	0	0	0	0	1	0	0	1	1	0	3	3
Number of isolates	4	5	4	5	7	5	6	5	6	5	52	
Number of taxa	4	4	2	4	3	4	3	4	5	4		
Total species	14											

Table 32. Species identified from mature leaf no.3 at Chiang Dao plantation (1995).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Cladosporium sp.	0	0	0	0	0	0	0	2	0	0	2	1
Colletotrichum sp.	0	0	0	0	1	0	0	1	0	0	2	2
Curvularia sp.	0	0	1	0	0	0	0	0	0	0	1	1
Daldinia eschscholzii	0	0	1	0	0	0	0	0	0	2	3	2
Diplodia sp.	0	1	0	0	0	0	0	0	0	0	1	1
Humicola sp.	0	0	2	0	0	0	0	0	0	0	2	1
Hypoxylon cf. subrutilum	0	0	0	0	0	0	0	0	0	1	1	1
Nigrospora sp.	0	0	0	0	0	0	0	1	1	0	2	2
Phomopsis sp.1	4	2	1	3	3	3	1	3	3	2	25	10
Phomopsis sp.2	0	4	1	0	1	0	2	2	2	0	12	6
Phomopsis sp. 3	0	0	1	0	1	0	1	0	0	1	4	4
Phomopsis sp.4	0	0	0	1	0	1	0	0	1	0	3	3
X. juruensis var. microspora	0	0	0	1	0	0	0	0	0	0	1	1
X. cubensis	0	0	1	0	0	0	0	0	0	1	2	2
Sterile sp. 3	0	0	0	0	0	0	1	1	0	0	2	2
Number of isolates	4	7	8	5	6	4	5	10	7	7	63	
Number of taxa	1	3	7	4	4	2	4	6	4	5		
Total species	16											

Table 33. Species identified from mature leaf no.4 at Chiang Dao plantation (1995).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Cladosporium sp.	0	0	0	0	0	0	1	0	0	0	1	1
Daldinia eschscholzii	0	0	0	0	0	0	1	0	0	1	2	2
Diplodia sp.	4	0	0	0	0	0	0	0	0	0	4	1
Humicola sp.	0	0	0	0	1	0	0	0	2	0	3	2
Nemania subannulata	0	0	0	0	0	1	0	0	1	0	2	2
Nigrospora sp.	0	0	0	0	0	0	1	0	0	1	2	2
Phoma sp.	0	0	0	1	0	0	0	0	0	0	1	1
Phomopsis sp.1	0	2	3	3	2	2	0	3	2	3	20	9
Phomopsis sp.2	0	2	0	0	1	1	1	1	0	1	7	6
Phomopsis sp.3	0	0	1	0	0	1	0	0	0	1	3	3
Phomopsis sp.4	0	0	0	0	0	0	1	0	0	1	2	2
Xylaria sp.564	0	0	0	0	0	0	1	0	0	0	1	1
Xylaria sp.294	0	0	1	0	0	0	0	0	0	0	1	1
X. feejeensis	0	0	0	0	0	0	0	0	0	1	1	1
Trichoderma sp.	0	0	0	0	0	0	0	1	0	0	1	1
Sterile sp.6	0	0	0	0	0	0	1	0	0	0	1	1
Number of isolates	4	4	5	4	4	5	7	5	5	9	52	
Number of taxa	1	2	3	2	3	4	7	3	3	7		
Total species	16											

Table 34. Species identified from mature leaf no.5 at Chiang Dao plantation (1995).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Cladosporium sp.	0	0	0	0	0	0	1	0	0	0	1	1
Daldinia eschscholzii	0	0	0	0	1	0	0	0	0	1	2	2
Diplodia sp.	0	1	0	0	1	0	0	1	0	0	3	3
Fusarium sp.	0	0	0	0	0	0	1	1	0	0	2	2
Humicola sp.	0	0	0	0	1	0	1	0	0	1	3	3
Nigrospora sp.	0	1	0	0	1	0	0	1	0	3	6	4
Phialophora sp.	0	0	0	0	0	0	0	1	0	0	1	1
Phomopsis sp.1	4	1	3	0	0	4	0	0	0	0	12	4
Phomopsis sp.2	0	0	0	2	0	0	0	1	2	1	6	4
Phomopsis sp. 3	0	0	0	1	0	0	0	1	0	0	2	2
Phomopsis sp.4	0	0	1	1	1	0	0	0	2	0	5	4
Septonema sp.	0	0	0	0	0	0	1	0	0	0	1	1
Sterile sp.3	0	2	0	0	0	0	0	2	0	1	5	3
Number of isolates	4	5	4	4	5	4	4	8	4	7	49	
Number of taxa	1	4	2	3	5	1	4	7	2	5		
Total species	13											

Table 35. Endophytic fungi isolated from mature leaf no. 1 at Mae Rim forest (1996).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	0	0	0	0	1	0	0	0	4	7	3
Daldinia eschscholzii	1	1	0	1	1	2	2	1	1	0	10	8
Humicola sp.	0	0	0	2	0	0	0	0	0	0	2	1
Hypoxylon subrutilum	0	0	0	1	0	1	0	0	1	0	3	3
Hypoxylon sp.188	0	0	0	0	0	0	1	0	0	0	1	1
H. haematostroma	0	0	1	1	1	0	1	0	1	1	6	5
Nemania subannulata	0	0	0	0	0	0	0	1	0	0	1	1
Penzigia sp.	0	0	0	0	0	1	0	0	0	0	1	1
Phomopsis sp.1	2	2	2	0	0	0	0	0	0	0	6	3
Phomopsis sp. 2	1	1	0	0	0	0	0	0	0	0	2	2
Phomopsis sp. 4	0	0	0	2	0	0	0	0	1	0	3	2
Septonema sp.	1	0	0	0	0	0	0	0	0	0	1	1
X. cf. allantoidea	1	0	0	0	0	0	0	0	0	0	1	1
Xylaria sp. 69	0	1	0	0	0	0	0	0	0	0	1	1
X. cubensis	0	0	1	0	1	1	1	2	0	0	6	5
X. juruensis var. microspora	0	0	0	0	1	0	1	0	0	0	2	2
Sterile sp.6	0	1	0	0	1	0	0	0	0	0	2	1
Number of isolates	8	6	4	7	5	6	6	4	4	5	55	
Number of taxa	6	5	3	5	5	5	5	3	4	2		
Total species	17											

Table 36. Endophytic fungi isolated from mature leaf no. 2 at Mae Rim forest (1996).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Cladosporium sp.	0	0	0	0	0	0	1	0	0	0	1	1
Daldinia eschscholzii	0	1	2	1	2	3	3	3	3	2	20	9
Humicola sp.	0	0	0	1	0	0	0	0	0	0	1	1
Hypoxylon haematostroma	0	0	0	0	0	0	0	0	1	0	1	1
H. subrutilum	0	0	1	0	0	1	1	1	0	0	4	4
H. cf. subrutilum	0	0	0	2	1	0	0	0	0	1	4	3
Nemania subannulata	0	1	1	1	0	0	0	2	0	0	5	4
Phomopsis sp.1	4	1	0	0	0	2	0	0	0	0	7	3
Phomopsis sp.2	0	2	0	2	2	0	1	0	0	1	8	5
Phomopsis sp.3	0	0	3	0	0	0	0	0	2	0	5	2
X. aristata	0	0	0	0	0	0	0	0	0	1	1	1
X. cubensis	0	0	0	2	0	2	3	0	0	0	7	3
X. feejeensis	0	0	0	0	0	0	0	0	0	1	1	1
X. juruensis var. microspora	0	0	0	0	0	1	0	2	2	0	5	3
Xylaria sp.5	0	0	0	0	1	0	0	0	0	0	1	1
Xylaria sp.7	0	0	0	0	1	0	0	0	0	0	1	1
Xylaria sp.15	0	0	0	0	0	0	0	0	0	1	1	1
Xylaria sp.24	0	0	0	0	1	0	0	0	0	0	1	1
Xylaria sp.40	0	0	0	0	0	0	0	0	1	0	1	1
Number of isolates	4	5	7	9	8	9	9	8	9	7	75	
Number of taxa	1	4	4	6	6	5	5	4	5	6		
Total species	19											

Table 37. Endophytic fungi isolated from mature leaf no.3 at Mae Rim forest (1996).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	0	2	0	0	0	0	0	0	2	6	3
Daldinia eschscholzii	0	1	1	1	3	1	1	1	1	0	10	8
Hypoxylon cf. subrutilum	0	0	0	0	0	1	0	0	0	0	1	1
H. subrutilum	0	0	0	1	0	1	1	0	0	0	3	3
Hypoxylon sp. 188	0	0	0	0	1	0	0	0	1	0	2	2
H. haematostroma	0	0	0	0	0	0	1	1	0	0	2	2
Nemania subannulata	0	0	0	1	0	0	0	0	1	0	2	2
Phomopsis sp.1	0	0	1	0	0	0	1	0	0	0	2	2
Phomopsis sp. 2	0	2	0	2	0	0	0	0	0	2	6	3
Phomopsis sp.3	3	2	0	0	1	2	0	2	0	0	10	5
Septonema sp.	1	0	0	0	0	0	0	0	0	0	1	1
X. grammica	0	0	1	0	0	0	0	0	0	1	2	2
X. cubensis	0	0	0	0	0	0	1	1	1	0	3	3
X. aristata	0	0	0	1	0	0	0	0	0	1	2	2
X. juruensis var. microspora	0	0	0	0	0	0	0	0	1	0	1	1
Sterile sp.1	0	0	0	0	0	0	1	0	0	0	1	1
Number of isolates	6	5	5	6	5	5	6	5	5	6	54	
Number of taxa	3	3	4	5	3	4	6	4	5	4		
Total species	16											

Table 38. Endophytic fungi isolated from mature leaf no.4 at Mae Rim forest (1996).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	0	1	0	0	0	0	0	0	1	4	3
Daldinia eschscholzii	0	0	0	0	1	2	0	2	1	2	8	5
Hypoxylon cf. anthochroum	0	0	0	0	0	0	0	0	0	1	1	1
H. haematotroma	0	0	0	2	0	0	0	0	0	0	2	1
H. cf. subrutilum	0	0	0	1	1	0	0	0	0	0	2	2
H. subrutilum	0	2	0	1	0	0	0	0	0	0	3	2
Nemania subannulata	0	1	0	0	0	0	1	0	0	0	2	2
Phomopsis sp.1	0	0	3	0	0	0	0	0	0	0	3	1
Phomopsis sp.2	2	2	1	0	1	1	0	0	0	1	8	6
Phomopsis sp.3	2	0	0	2	0	0	2	0	3	0	9	4
Phomopsis sp.4	0	0	0	0	0	0	0	4	0	0	4	1
Xylaria sp. 9	0	0	0	0	0	0	0	0	1	0	1	1
X. aristata	0	0	0	0	0	0	0	0	0	2	2	3
X. cubensis	0	0	1	0	0	1	2	0	0	0	4	1
X. feejeensis	0	0	0	1	0	0	0	0	0	0	1	1
X. juruensis var. microspora	0	0	0	0	0	0	1	0	0	0	1	1
Number of isolates	6	5	6	7	3	4	6	6	5	7	55	
Number of taxa	3	3	4	5	3	3	4	2	3	5		
Number of species	16											

Table 39. Endophytic fungi isolated from mature leaf no.5 at Mae Rim forest (1996).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	0	0	0	0	0	0	0	1	1	1
Daldinia eschscholzii	0	2	2	0	2	0	0	1	0	0	7	4
Hypoxylon cf. anthochroum	0	0	0	1	0	0	0	0	0	1	2	2
Humicola sp.	0	0	0	0	0	0	0	0	0	1	1	1
H. cf. subrutilum	0	0	0	0	0	1	0	1	1	1	4	4
H. subrutilum	0	0	0	0	0	1	1	1	1	0	4	4
Hypoxylon sp. 188	0	0	1	1	0	0	0	0	0	0	2	2
H. haematostroma	0	0	0	0	2	2	0	1	0	0	5	3
Nemania subannulata	0	0	0	1	0	2	1	0	0	0	4	3
Phomopsis sp.1	2	1	0	0	0	0	0	0	0	0	3	2
Phomopsis sp.3	0	0	0	0	0	0	0	0	1	0	1	1
X. feejeensis	0	0	0	0	0	0	0	1	1	0	2	2
X. juruensis var. microspora	0	1	1	0	0	0	0	0	0	0	2	2
Xylaria sp.64	0	0	0	0	0	0	0	0	1	0	1	1
Xylaria sp.69	0	0	0	0	1	0	0	0	0	1	2	2
Xylaria sp.73	0	0	0	0	1	0	0	0	0	0	1	1
Xylaria sp.76	0	1	0	0	0	0	0	0	0	0	1	1
X. aristata	1	0	1	0	0	0	0	0	0	0	2	2
Sterile sp.1	1	0	0	0	0	0	0	0	0	0	1	1
Sterile sp.6	0	0	1	0	0	0	2	0	0	0	3	2
Number of isolates	4	5	6	3	6	6	4	5	5	5	49	
Number of taxa	3	4	5	3	4	4	3	5	5	5		
Total species	20											

Table 40. Endophytic fungi isolated from mature leaf no.1 at Chiang Dao plantation (1996).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	0	1	0	0	0	0	0	0	1	1
Daldinia eschscholzii	0	2	1	0	2	1	1	2	1	1	11	8
Hypoxylon cf. subrutilum	0	0	0	0	0	0	1	1	1	1	4	4
H. cf. anthochroum	0	0	0	2	0	0	0	0	0	0	2	1
H. subrutilum	0	0	0	0	0	0	1	1	1	1	4	4
H. haematostroma	0	0	0	0	0	2	1	0	0	0	3	2
Nemania subannulata	1	0	1	0	1	0	0	1	1	0	5	5
Nigrospora sp.	0	0	0	0	0	0	0	0	0	1	1	1
Phomopsis sp.1	4	1	2	1	0	0	0	1	0	0	9	4
Phomopsis sp.2	0	0	0	1	0	0	0	0	0	0	1	1
Phomopsis sp.3	0	0	1	0	0	0	0	0	0	0	1	1
Xylaria sp.32	0	0	0	0	0	0	0	0	0	1	1	1
Xylaria sp.45	0	0	0	0	1	0	0	0	0	0	1	1
X. aristata	0	0	0	0	0	0	1	0	0	1	2	2
X. cubensis	0	1	0	0	0	1	0	0	2	1	5	4
X. juruensis var. microspora	0	0	0	0	1	0	0	1	0	0	2	2
Sterile sp.3	0	0	0	0	1	0	0	0	0	1	2	2
Number of isolates	5	4	5	5	6	4	5	7	6	8	**55**	
Number of taxa	2	3	4	4	5	3	5	6	5	8		
Number of species	**17**											

Table 41. Endophytic fungi isolated from mature leaf no.2 at Chiang Dao plantation (1996).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	0	0	0	0	1	0	0	1	0	4	3
Daldinia eschscholzii	0	2	2	2	2	2	2	2	0	2	16	8
Hypoxylon cf. subrutilum	1	0	0	1	0	0	1	1	0	0	4	4
H. cf. anthochroum	0	0	0	0	0	0	0	0	0	1	1	1
H. subrutilum	0	0	1	1	0	1	0	1	0	0	4	4
H. haematostroma	0	0	0	0	3	1	0	0	1	0	5	3
Nemania subannulata	0	0	0	0	2	2	0	1	0	0	5	3
Phomopsis sp.1	1	0	0	0	1	0	0	0	0	0	2	2
Phomopsis sp.2	0	0	2	0	0	0	1	0	0	1	4	2
Phomopsis sp.3	0	0	0	0	0	0	0	0	1	0	1	1
Phomopsis sp.4	1	0	0	0	0	0	0	0	0	0	1	1
Xylaria sp.7	0	0	0	0	0	0	0	0	0	1	1	1
X. aristata	0	0	0	0	0	0	0	0	1	0	1	1
X. juruensis var. microspora	0	0	0	0	0	0	0	1	0	0	1	1
Sterile sp. 3	0	1	0	0	1	0	0	0	0	0	2	2
Sterile sp.6	0	0	0	0	0	0	0	1	0	1	2	2
Number of isolates	5	3	5	4	9	7	4	7	4	6	54	
Number of taxa	4	2	3	3	5	5	3	6	4	5		
Number of species	16											

Table 42. Endophytic fungi isolated from mature leaf no.3 at Chiang Dao plantation (1996).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	1	0	0	0	0	0	1	1	0	3	3
Daldinia eschscholzii	0	2	1	1	2	2	1	1	0	2	12	8
Humicola sp.	0	0	0	0	1	0	0	0	0	0	1	1
Hypoxylon cf. subrutilum	0	0	1	0	1	1	0	0	0	1	4	4
H. cf. anthochroum	0	2	0	1	0	0	0	0	0	0	3	2
H. subrutilum	0	0	1	1	0	1	0	0	0	1	4	4
H. haematostroma	0	1	0	0	0	1	0	0	0	2	4	3
Nemania subannulata	2	0	0	0	1	0	0	0	0	2	5	3
Phomopsis sp.1	1	0	1	0	1	0	0	3	0	0	6	4
Phomopsis sp.2	2	0	0	3	0	0	0	0	0	0	5	2
Phomopsis sp.3	1	4	0	0	0	0	0	0	0	0	5	2
Phomopsis sp.4	0	0	0	0	0	0	0	0	1	0	1	1
X. cubensis	0	0	0	2	1	0	1	1	0	1	6	5
X. aristata	0	0	0	0	0	0	0	0	1	0	1	1
X. juruensis var. microspora	0	0	0	0	0	0	1	0	1	0	2	2
Sterile sp.6	0	0	0	0	0	1	1	0	0	0	2	2
Number of isolates	6	10	4	8	7	6	4	6	4	9	64	
Number of taxa	4	5	4	5	6	5	4	4	4	6		
Number of species	16											

Table 43. Endophytic fungi isolated from mature leaf no.4 at Chiang Dao plantation (1996).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	0	0	0	1	0	0	0	0	1	1
Daldinia eschscholzii	0	0	1	1	0	1	0	1	1	1	6	6
Hypoxylon cf. subrutilum	0	0	0	0	0	0	1	0	1	0	2	2
Hypoxylon cf. anthochroum	0	0	1	0	0	0	0	0	0	1	2	2
Hypoxylon sp.188	0	0	0	0	0	2	0	0	0	0	2	1
H. subrutilum	0	0	1	0	0	1	0	0	1	0	3	3
H. haematostroma	0	0	0	0	1	0	0	0	1	0	2	2
Phomopsis sp.1	0	2	1	4	0	1	2	1	0	0	11	6
Phomopsis sp.2	2	0	0	0	0	0	1	1	0	0	4	3
Phomopsis sp.3	2	2	0	0	3	0	1	1	0	3	12	6
X. aristata	0	0	0	1	0	1	0	0	1	0	3	3
X. cubensis	0	1	0	0	0	0	2	0	0	0	3	2
Sterile sp. 3	0	0	0	0	0	0	0	0	0	1	1	1
Number of isolates	4	5	4	6	4	7	7	4	5	6	52	
Number of taxa	2	3	4	3	2	6	5	4	5	4		
Number of species	13											

Table 44. Endophytic fungi isolated from mature leaf no.5 at Chiang Dao plantation (1996).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	0	0	1	0	0	0	0	0	1	1
Daldinia eschscholzii	1	2	1	0	2	3	2	2	1	0	14	8
Humicola sp.	0	0	2	0	0	0	0	0	0	0	2	1
Hypoxylon cf. subrutilum	0	0	0	0	1	0	0	1	1	1	4	4
Hypoxylon sp.188	0	1	0	0	0	0	0	0	1	0	2	2
H. subrutilum	0	0	0	0	2	0	0	1	0	1	4	3
H. haematostroma	0	0	2	0	0	1	0	1	0	0	4	3
Nemania subannulata	0	1	0	0	1	0	1	0	0	1	4	4
Phomopsis sp.1	0	0	1	1	0	0	1	1	0	0	4	4
Phomopsis sp.2	2	0	0	1	0	1	0	0	2	0	6	4
Phomopsis sp.3	2	0	0	0	1	0	0	0	0	0	3	2
Septonema sp.	1	0	0	0	0	0	0	0	0	0	1	1
Xylaria sp.32	0	0	0	0	0	0	1	0	0	1	2	2
Xylaria sp.36	0	0	0	0	0	0	1	0	0	0	1	1
X. grammica	0	0	0	1	0	0	0	0	0	0	1	1
X. aristata	0	0	0	1	0	0	0	0	0	1	2	2
X. cubensis	0	1	2	0	0	0	0	0	0	2	5	3
X. juruensis var. microspora	0	0	0	0	1	1	0	0	0	1	3	2
Sterile sp.3	0	1	0	0	0	0	0	0	0	0	1	1
Sterile sp.6	0	0	0	1	0	0	0	0	2	0	3	2
Number of isolates	6	6	8	4	9	6	6	6	5	8	64	
Number of taxa	4	5	5	5	7	4	5	5	5	7		
Number of species	19											

Table 45. Endophytic fungi isolated from mature leaf no.1at at Mae Rim forest (1997).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	0	2	2	1	1	1	0	1	8	6
Daldinia eschscholzii	2	0	2	0	0	1	2	0	0	0	7	4
Diplodia sp.	0	0	0	0	0	0	0	0	1	0	1	1
H. haematostroma	0	0	0	0	0	0	1	0	0	0	1	1
Hypoxylon subrutilum	1	0	0	0	0	1	0	0	1	1	4	4
Nigrospora sp.	0	0	0	0	0	0	0	0	1	0	1	1
Phomopsis sp.2	0	2	1	0	0	1	0	0	1	0	5	4
Phomopsis sp.3	1	0	1	0	1	0	0	0	0	0	3	3
Phomopsis sp.4	0	1	0	0	0	0	1	2	0	0	4	3
Phomopsis sp.1	0	0	0	1	0	0	0	0	0	1	2	2
Pycnidia	0	2	2	0	2	0	0	0	1	1	8	5
Septonema sp.	0	0	1	0	0	0	0	0	0	0	1	1
Xylaria sp.	0	1	1	0	0	0	0	0	0	0	2	2
X. juruensis var. microspora	0	0	0	0	1	0	0	0	0	0	1	1
X. aristata	0	0	0	2	2	0	1	1	0	0	6	4
X. cubensis	0	0	0	2	0	1	0	1	0	0	4	3
Sterile sp.1	0	0	1	0	1	0	0	0	0	0	2	2
Sterile sp.3	0	0	1	0	0	0	0	0	0	0	1	1
Sterile sp.6	2	0	0	0	0	0	0	0	0	0	2	1
Number of isolates	6	6	10	7	9	5	6	5	5	4	63	
Number of taxa	4	4	8	4	6	5	5	4	5	4		
Total species	19											

Table 46. Endophytic fungi isolated from mature leaf no.2 at Mae Rim forest (1997).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Cladosporium sp.	1	0	0	0	0	0	0	0	0	0	1	1
Colletotrichum sp.	0	4	2	1	0	0	0	0	0	1	8	4
Daldinia eschscholzii	0	0	0	2	3	0	0	0	4	2	11	4
Diplodia sp.	1	0	0	1	0	0	0	0	0	0	2	2
Hypoxylon cf. subrutilum	0	0	1	0	0	2	1	0	0	0	4	3
H. subrutilum	0	0	0	1	1	0	1	0	0	0	3	3
H. haematostroma	0	0	0	0	0	1	0	1	0	0	2	2
H. cf. anthochroum	1	0	0	0	0	1	0	0	0	0	2	2
Humicola sp.	0	0	0	0	0	0	0	0	1	0	1	1
Nemania subannulata	2	0	0	0	0	0	2	3	0	0	7	3
Phomopsis sp.1	3	0	0	1	0	0	0	0	0	0	4	2
Xylaria sp. 69	0	1	1	0	0	0	0	0	0	0	2	2
Xylaria sp. 294	0	1	0	0	0	0	0	1	0	0	2	2
X. aristata	0	0	0	0	0	2	1	0	1	0	4	2
X. juruensis var. microspora	0	0	0	0	0	1	0	0	0	1	2	2
X. cubensis	0	0	2	0	0	0	0	0	0	1	3	2
Sterile sp. 1	1	0	0	0	0	0	0	0	0	0	1	1
Sterile sp.2	0	0	0	0	0	0	0	1	0	0	1	1
Sterile sp. 3	0	0	0	0	1	0	0	0	0	0	1	2
Sterile sp. 6	1	0	0	0	0	0	0	0	0	1	2	1
Number of isolates	10	6	6	6	5	7	5	6	6	6	63	
Number of taxa	7	3	3	5	3	5	4	4	3	5		
Total species	20											

Table 47. Endophytic fungi isolated from mature leaf no.3 at Mae Rim forest (1997).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	1	0	3	0	1	1	0	0	6	4
Daldinia eschscholzii	0	0	0	0	0	0	0	1	1	0	2	2
Hypoxylon subrutilum	0	0	0	0	0	0	0	1	1	0	2	2
H. cf. subrutilum	0	1	0	0	0	1	1	0	0	0	3	3
Hypoxylon sp. 188	0	0	0	0	0	1	0	0	1	0	2	2
H. haematostroma	0	0	0	0	0	1	0	0	0	0	1	1
Humicola sp.	0	0	0	0	0	0	0	0	0	1	1	1
Nemania subannulata	0	0	1	0	0	0	2	0	0	0	3	2
Phomopsis sp.1	0	0	0	0	0	0	1	0	0	0	1	1
Pycnidia	1	0	0	2	0	0	0	0	0	0	3	2
Xylaria sp. 36	1	2	0	0	0	0	0	1	0	0	4	3
Xylaria sp. 69	0	0	1	1	0	2	0	0	0	0	4	3
X. aristata	1	1	0	0	0	0	0	0	1	1	4	4
X. aristata	2	2	1	0	0	0	0	1	0	1	7	5
X. juruensis var. microspora	0	0	0	1	1	0	0	0	2	0	4	3
X. cubensis	0	0	0	2	1	0	0	0	0	1	4	3
Sterile sp.1	0	0	0	1	0	0	0	0	0	0	1	1
Sterile sp.3	0	2	0	0	0	0	0	0	0	1	3	2
Sterile sp. 6	1	0	1	0	0	0	0	1	0	2	5	4
Number of isolates	6	8	5	7	5	5	5	6	6	7	60	
Number of taxa	4	5	5	5	3	4	3	6	5	6		
Total species	19											

Table 48. Endophytic fungi isolated from mature leaf no.4 at Mae Rim forest (1997).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	3	1	2	0	0	0	1	0	2	9	5
Daldinia eschscholzii	1	1	0	1	1	0	0	1	2	0	7	6
Humicola sp.	0	0	0	0	0	0	1	0	0	0	1	1
Hypoxylon sp.188	0	0	0	0	0	0	0	2	0	0	2	1
H. haematostroma	0	0	0	0	3	0	0	0	0	0	3	1
H. subrutilum	0	0	0	0	0	2	0	0	0	2	4	2
H. cf. subrutilum	0	0	0	1	0	0	1	0	0	1	3	3
Nemania subannulata	0	0	0	0	0	2	0	0	1	2	5	3
Phomopsis sp.1	0	0	0	0	0	1	1	0	0	0	2	2
Phomopsis sp.3	2	1	4	2	0	0	0	0	0	0	9	4
Phomopsis sp.4	1	2	0	0	0	0	0	0	0	0	3	2
Pycnidia	1	0	0	0	0	0	0	0	0	0	1	1
Septonema sp.	0	0	0	0	0	0	2	0	0	0	2	1
Xylaria sp.69	0	0	0	0	0	0	0	0	1	0	1	1
X. juruensis var. microspora	0	0	0	0	2	0	0	0	0	0	2	1
X. aristata	0	1	0	0	0	1	0	0	1	2	5	4
X. cubensis	0	0	0	2	0	0	0	0	0	0	2	1
Sterile sp.1	0	1	0	0	1	0	1	0	0	0	3	3
Number of isolates	5	9	5	8	7	6	6	4	5	9	64	
Number of taxa	4	6	2	5	4	4	5	3	4	5		
Total species	18											

Table 49. Endophytic fungi isolated from mature leaf no.5 at Mae Rim forest (1997).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	2	1	1	1	1	1	1	1	1	12	10
Daldinia eschscholzii	0	0	0	1	0	2	0	1	1	1	6	5
Diplodia sp.	0	1	0	1	0	0	1	0	0	0	3	3
Hypoxylon subrutilum	0	0	0	1	0	0	1	2	0	0	4	3
H. cf. subrutilum	0	0	0	0	0	0	0	0	1	0	1	3
Nemania subannulata	0	0	0	1	1	1	0	2	1	0	6	1
Phomopsis sp.1	1	0	2	0	0	0	0	0	0	1	4	5
Phomopsis sp.2	0	4	0	2	0	0	0	0	0	0	6	3
Phomopsis sp.3	2	0	2	0	0	0	0	0	0	0	4	2
Phomopsis sp.4	0	1	0	0	0	0	0	0	0	0	1	2
Pycnidia	0	1	0	0	1	0	0	0	0	0	2	1
Xylaria sp.69	0	0	0	0	0	0	0	0	2	0	2	2
Xylaria sp.36	0	0	0	0	0	0	1	0	0	0	1	1
X. grammica	0	0	0	0	0	0	0	0	2	0	2	1
X. juruensis var. microspora	0	0	0	0	0	0	0	0	1	0	1	1
X. aristata	0	1	0	0	1	0	0	1	0	0	3	1
X. cubensis	0	0	0	1	0	2	1	0	0	0	4	3
Sterile sp.1	0	0	0	0	2	0	0	0	0	0	2	1
Number of isolates	5	10	5	8	6	6	5	7	9	3	64	
Number of taxa	3	6	3	7	5	4	5	5	7	3		
Total species	18											

Table 50. Endophytic fungi isolated from mature leaf no.1 at Chiang Dao plantation (1997).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Daldinia eschscholzii	0	1	1	1	1	0	0	1	1	1	7	7
Hypoxylon subrutilum	0	0	1	1	1	0	0	0	0	0	3	3
Hypoxylon sp.188	0	0	0	0	1	0	0	0	0	2	3	2
H. subrutilum	0	0	1	0	1	0	0	0	1	1	4	4
H. haematostroma	1	0	0	0	0	0	0	1	0	1	3	3
Nemania subannulata	0	2	0	1	1	2	1	0	1	0	8	6
Phomopsis sp.1	4	1	0	0	0	0	0	0	0	0	5	2
Phomopsis sp.3	0	0	0	2	0	0	0	0	0	0	2	1
Phomopsis sp.4	4	2	2	0	0	0	0	0	0	0	8	3
Xylaria sp.69	0	0	0	3	0	1	1	0	1	0	6	4
X. aristata	0	0	0	0	0	0	0	0	1	0	1	1
X. cubensis	0	0	0	2	0	0	0	4	0	0	6	2
Sterile sp. 1	0	0	0	0	0	2	1	0	0	0	3	2
Number of isolates	9	6	5	10	5	3	2	6	5	5	56	
Number of taxa	3	4	4	6	5	3	3	3	5	4		
Total species	13											

Table 51. Endophytic fungi isolated from mature leaf no.2 at Chiang Dao plantation (1997).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Daldinia eschscholzii	0	0	1	0	1	1	1	0	1	0	5	5
Humicola sp.	0	0	0	0	0	0	0	1	0	0	1	1
Hypoxylon cf. subrutilum	0	1	0	0	2	0	0	0	1	0	4	3
H. subrutilum	0	0	0	0	0	1	0	1	1	1	4	4
Nemania subannulata	0	1	1	1	1	1	0	0	0	0	5	5
Phomopsis sp.1	0	0	0	3	2	0	3	1	0	1	10	5
Phomopsis sp.2	0	0	1	0	0	2	0	0	0	0	3	2
Phomopsis sp.3	0	2	2	2	0	1	1	0	0	1	9	6
Phomopsis sp.4	3	0	0	0	0	0	0	0	0	3	6	2
Pycnidia	0	0	0	1	0	0	0	0	0	0	1	1
Septonema sp.	0	1	0	0	0	0	0	0	0	0	1	1
Xylaria sp.69	0	0	1	1	1	0	1	0	0	0	4	4
X. aristata	0	0	0	1	0	0	0	0	0	0	1	2
X. cubensis	1	0	0	0	0	0	1	1	0	1	4	4
Sterile sp.1	0	0	0	0	0	0	0	0	2	0	2	1
Number of isolates	4	5	6	9	7	6	7	4	5	7	60	
Number of taxa	2	4	5	6	5	5	5	4	4	5		
Total species	15											

Table 52. Endophytic fungi isolated from mature leaf no.3 at Chiang Dao plantation (1997).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	1	0	0	0	1	0	0	0	2	2
Daldinia eschscholzii	0	1	1	1	1	1	1	1	1	1	9	9
Diplodia sp.	0	0	0	0	0	0	0	0	0	1	1	1
Hypoxylon cf. subrutilum	0	0	1	1	0	0	1	0	1	0	4	4
H. subrutilum	0	0	1	0	1	0	0	1	1	0	4	4
Humicola sp.	0	0	0	1	1	1	1	1	1	1	7	7
Nemania subannulata	0	0	0	0	1	1	1	0	1	1	5	5
Phomopsis sp.1	0	0	0	0	0	1	0	1	0	0	2	2
Phomopsis sp.2	0	0	0	0	0	0	1	0	0	1	2	2
Phomopsis sp.3	0	0	2	0	0	0	0	0	0	0	2	1
Phomopsis sp.4	4	3	0	1	0	0	0	0	0	1	9	4
H. haematostroma	0	0	0	0	0	0	1	0	0	0	1	1
Pycnidia	0	0	0	0	1	0	1	0	1	0	3	3
Xylaria sp. 29	0	0	0	1	0	0	0	0	0	0	1	1
Xylaria sp.69	0	0	0	0	1	0	1	1	0	0	3	3
Xylaria sp 36	0	0	0	0	0	0	0	0	1	1	2	2
X. cubensis	0	0	0	1	0	2	1	1	0	0	5	4
Sterile sp.1	0	0	0	0	0	1	0	1	0	0	2	2
Number of isolates	4	4	6	6	6	6	10	6	7	7	62	
Number of taxa	1	2	5	6	6	6	10	7	7	7		
Total species	18											

Table 53. Endophytic fungi isolated from mature leaf no.4 at Chiang Dao plantation (1997).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	0	0	0	0	1	0	0	2	3	2
Daldinia eschscholzii	0	0	0	1	1	0	0	1	0	0	3	3
Humicola sp.	0	0	0	1	0	0	2	2	0	1	6	4
Hypoxylon sp.188	0	0	0	0	0	0	0	1	0	0	1	1
Hypoxylon cf. subrutilum	0	0	0	1	0	0	1	0	1	0	3	3
H. subrutilum	0	0	0	2	0	0	1	0	0	0	3	2
H. haematostroma	1	0	1	0	0	1	0	0	0	1	4	4
Nemania subannulata	0	1	0	0	0	1	0	1	2	1	6	5
Phomopsis sp.1	0	0	0	1	0	1	0	0	0	0	2	2
Phomopsis sp.2	0	2	0	0	0	0	0	0	0	0	2	1
Phomopsis sp.3	0	0	3	0	0	0	0	0	0	0	3	1
Phomopsis sp.4	3	0	0	1	0	0	0	0	0	0	4	2
Pycnidia	0	0	0	1	0	0	0	0	0	1	2	2
Xylaria sp.69	0	2	0	0	0	0	0	1	2	0	5	3
X. aristata	0	0	0	0	0	0	0	0	0	2	2	1
X.cubensis	0	0	0	0	4	1	0	0	0	0	5	2
X. juruensis var.microspora	0	0	0	0	0	1	0	0	0	0	1	1
Sterile sp.3	0	0	0	0	0	0	0	1	0	0	1	1
Number of isolates	4	5	4	8	5	5	5	7	5	8	56	
Number of taxa	2	3	2	7	2	5	4	6	3	6		
Total species	18											

Table 54. Endophytic fungi isolated from mature leaf no.5 at Chiang Dao plantation (1997).

Taxa	Midrib		Veins		Leaf lamina						Number of isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	0	0	1	0	0	1	0	0	2	2
Daldinia eschscholzii	0	0	0	0	1	1	2	2	1	1	8	6
Diplodia sp.	0	0	1	0	0	0	0	0	0	0	1	1
Hypoxylon sp.1	0	1	0	0	0	1	1	0	0	0	3	3
Hypoxylon cf. subrutilum	0	0	0	0	1	0	0	1	0	1	3	3
H. subrutilum	0	0	0	0	1	0	1	1	1	1	5	5
H. haematostroma	0	0	0	1	1	1	1	0	0	0	4	4
Humicola sp.	0	0	0	0	0	0	0	2	0	0	2	1
Nemania subannulata	0	1	0	1	2	0	0	0	0	0	4	3
Phomopsis sp.1	0	0	3	1	1	0	0	0	0	0	5	2
Phomopsis sp.3	4	2	0	0	0	0	0	0	0	0	6	2
Phomopsis sp.4	0	0	0	0	0	0	0	0	2	0	2	1
Phomopsis sp.2	0	2	0	2	0	0	0	0	0	0	4	2
Xylaria cubensis	0	0	0	0	0	2	1	1	1	2	7	5
Number of isolates	4	6	4	5	8	5	6	8	5	5	56	
Number of taxa	1	4	2	4	7	4	5	6	4	4		
Total species	14											

Table 55. Species identified from young leaf no.1 at Mae Rim forest (1996).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	1	1	1	0	2	0	0	0	2	9	6
Phomopsis sp.2	0	0	0	0	0	0	0	0	0	1	1	1
Sterile	2	2	0	1	0	0	0	0	0	0	5	3
Number of isolates	4	3	1	2	0	2	0	0	0	3	15	
Number of taxa	2	2	1	2	0	1	0	0	0	2		
Total species	3											

Table 56. Species identified from young leaf no.2 at Mae Rim (1996).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Pycnidia	2	0	0	0	0	0	0	0	0	1	3	2
Number of isolates	2	0	0	0	0	0	0	0	0	1	3	
Number of taxa	1	0	0	0	0	0	0	0	0	1		
Total species	1											

Table 57. Species identified from young leaf no.3 at Mae Rim forest (1996).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	1	0	1	1	0	0	0	0	0	0	3	3
Pycnidia	1	0	0	2	1	0	0	0	0	1	5	4
Number of isolates	2	0	1	3	1	0	0	0	0	1	8	
Number of taxa	2	0	1	2	1	0	0	0	0	1		
Total species	2											

Table 58. Species identified from young leaf no.4 at Mae Rim forest (1996).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	4	0	1	2	1	1	0	0	0	0	9	5
Phomopsis no.1	0	0	0	0	1	0	1	0	0	1	3	3
Phomopsis no.2	0	0	0	0	0	0	0	1	0	0	1	1
Sterile	0	0	0	0	0	2	2	0	0	0	4	2
Number of isolates	4	0	1	2	2	3	3	1	0	1	17	
Number of taxa	1	0	1	1	2	1	2	1	0	1		
Total species	4											

Table 59. Species identified from young leaf no.5 at Mae Rim forest (1996).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	1	0	0	2	0	0	1	1	0	7	5
Nigrospora sp.	0	0	1	0	0	0	0	0	0	0	1	1
Phomopsis no.1	0	0	0	0	0	0	1	0	0	0	1	1
Sterile	0	1	0	0	0	0	0	0	0	1	2	2
Number of isolates	2	2	1	0	2	0	1	1	1	1	11	
Number of taxa	1	2	1	0	1	0	1	1	1	1		
Total species	4											

Table 60. Species identified from young leaf no. 1 at Chiang Dao plantationn (1996).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	1	0	0	0	0	0	0	1	0	0	2	2
Phomopsis sp.1	2	0	0	0	0	0	0	0	0	0	2	1
Number of isolates	3	0	0	0	0	0	0	1	0	0	4	
Number of taxa	2	0	0	0	0	0	0	1	0	0		
Total species	2											

Table 61. Species identified from young leaf no.2 at Chiang Dao plantation (1996).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	4	1	4	2	1	4	0	2	1	1	20	9
Phomopsis sp.1	0	2	0	0	0	0	0	0	0	0	2	1
N. subannulata	0	0	0	0	1	0	0	0	0	0	1	1
Number of isolates	4	3	4	2	2	4	0	2	1	1	23	
Number of taxa	1	2	1	1	2	1	0	1	1	1		
Number of species	**2**											

Table 62. Species identified from young leaf no.3 at Chiang Dao plantion (1996).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	1	3	2	0	1	0	0	0	0	0	7	4
Phomopsis no.1	2	0	0	2	0	0	0	0	0	0	4	2
Phomopsis no.2	1	0	0	2	0	0	0	0	0	0	3	2
Number of isolates	4	3	2	4	1	0	0	0	0	0	14	
Number of taxa	3	1	1	2	1	0	0	0	0	0		
Number of species	3											

Table 63. Species identified from young leaf no.4 at Chiang Dao plantation (1996).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	2	2	3	0	0	2	0	0	1	12	6
D. eschscholzii	0	0	0	0	1	0	0	0	0	0	1	1
Phomopsis sp.1	0	0	0	1	0	0	0	0	2	0	3	2
Sterile	0	0	0	0	0	0	0	4	0	0	4	1
Number of isolates	2	2	2	4	1	0	2	4	2	1	20	
Number of taxa	1	1	1	2	1	0	1	1	1	1		
Total species	4											

Table 64. Species identified from young leaf no.5 at Chiang Dao plantation (1996).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	2	0	2	0	0	2	0	0	0	8	4
Phomopsis sp.2	0	0	0	0	0	0	0	0	4	1	5	2
Sterile	0	0	4	1	0	0	0	0	0	0	5	2
Number of isolates	2	2	4	3	0	0	2	0	4	1	18	
Number of taxa	1	1	1	2	0	0	1	0	1	1		
Total species	**3**											

Table 65. Species identified from young leaf no. 1 at Mae Rim forest (1997).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	0	0	2	1	0	0	2	0	0	7	4
Phomopsis sp.1	1	1	0	0	0	1	0	0	0	1	4	4
Phomopsis sp.2	0	0	2	0	0	0	1	0	1	1	5	4
Phomopsis sp.3	0	0	1	1	0	0	0	0	0	0	2	2
Phomopsis sp.4	0	0	0	0	2	0	0	0	0	0	2	1
Number of isolates	3	1	3	3	3	1	1	2	1	2	20	
Number of taxa	2	1	2	2	2	1	1	1	1	2		
Total species	5											

Table 66. Species identified from young leaf no. 2 at Mae Rim forest (1997).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	1	2	0	0	0	0	1	1	0	7	4
Daldinia eschscholzii	0	0	0	1	0	0	0	0	0	0	1	1
Number of isolates	2	1	2	1	0	0	0	1	1	0	8	
Number of taxa	1	1	1	1	0	0	0	1	1	0		
Total species	2											

Table 67. Species identified from young leaf no. 3 at Mae Rim forest (1997).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	1	2	1	0	2	4	4	2	2	3	21	9
Daldinia eschscholzii	0	0	1	0	0	0	0	0	0	0	1	1
Phomopsis sp.1	0	0	1	1	0	0	0	0	0	0	2	2
Number of isolates	1	2	3	1	2	4	4	2	2	3	24	
Number of taxa	1	1	3	1	1	1	1	1	1	1		
Total species	3											

Table 68. Species identified from young leaf no. 4 at Mae Rim forest (1997).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	3	1	2	4	2	0	0	0	0	14	6
Nigrospora sp.	0	0	0	0	0	0	0	0	0	1	1	1
Phomopsis sp.4	2	0	0	0	0	0	1	0	0	0	3	2
Pycnidia	0	0	0	0	0	0	0	1	1	1	3	
Number of isolates	4	3	1	2	4	2	1	1	1	2	21	
Number of taxa	2	1	1	1	1	1	1	1	1	2		
Total species	4											

Table 69. Species identified from young leaf no. 5 at Mae Rim forest (1997).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	1	0	4	0	0	0	2	3	1	11	5
Phomopsis sp.1	0	2	0	1	0	0	0	0	0	0	3	2
Sterile	0	0	0	0	0	0	1	1	0	1	3	3
Number of isolates	0	3	0	5	0	0	1	3	3	2	17	
Number of taxa	0	2	0	2	0	0	1	2	1	2		
Total species	3											

Table 70. Species identified on young leaf no. 1 at Chiang Dao plantation (1997).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	1	1	2	2	0	1	0	0	0	7	5
Diplodia sp.	0	0	0	0	0	0	0	0	1	0	1	1
Phomopsis sp.1	2	1	2	0	0	0	0	0	0	0	5	3
Phomopsis sp.2	0	0	0	0	0	0	0	0	1	4	5	2
Phomopsis sp.3	0	0	0	1	0	2	0	0	0	0	3	2
Phomopsis sp.4	0	1	0	1	0	0	0	3	0	0	5	3
Number of isolates	2	3	3	4	2	2	1	3	2	4	26	
Number of taxa	1	3	2	3	1	1	1	3	2	1		
Total species	6											

Table 71. Species identified from young leaf no. 2 at Chiang Dao (1997).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	0	0	3	2	2	0	3	0	10	4
Phomopsis sp.1	4	2	0	1	0	0	0	0	0	0	7	3
Phomopsis sp.2	0	0	2	2	0	2	0	1	0	0	7	4
Phomopsis sp.3	0	0	0	0	0	1	0	0	0	0	1	1
Number of isolates	4	2	2	3	3	5	2	1	3	0	25	
Number of taxa	1	1	1	2	1	3	1	1	1	0		
Total species	4											

Table 72. Species identified from young leaf no. 3 at Chiang Dao (1997).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	2	0	0	1	0	0	4	4	2	1	14	6
Phomopsis sp.1	1	2	2	0	0	1	0	0	0	0	6	4
Phomopsis sp.2	1	2	0	0	2	0	0	0	0	0	5	3
Phomopsis sp.3	0	0	0	1	0	0	0	0	0	0	1	1
Phomopsis sp.4	0	0	0	0	0	0	0	0	2	1	3	2
Number of isolates	4	4	2	2	2	1	4	4	4	2	29	
Number of taxa	3	2	1	2	1	1	1	1	1	2		
Total species	5											

Table 73. Species identified from young leaf no. 4 at Chiang Dao (1997).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	0	4	1	2	0	2	1	1	2	13	7
Phomopsis sp.1	4	4	0	0	0	1	0	0	0	0	9	3
Phomopsis sp.2	0	1	0	0	0	0	0	0	0	0	1	1
Phomopsis sp.3	0	0	2	0	0	0	0	0	0	0	2	1
Phomopsis sp.4	0	0	0	2	0	0	0	0	0	0	2	1
Sterile	0	0	0	0	0	0	0	0	2	0	2	1
Number of isolates	4	5	6	3	2	1	2	1	1	2	27	
Number of taxa	1	2	2	2	1	1	1	1	1	1		
Total species	6											

Table 74. Species identified from young leaf no. 5 at Chiang Dao (1997).

Taxa	Midrib		Veins		Leaf lamina						Total isolates	Infected areas
	1	2	3	4	5	6	7	8	9	10		
Colletotrichum sp.	0	1	4	0	0	1	0	3	1	1	11	6
Phomopsis sp.1	0	2	0	1	2	0	1	0	0	0	6	4
Phomopsis sp.2	2	0	0	0	0	1	0	0	0	2	5	3
Phomopsis sp.3	0	1	0	3	0	0	0	0	0	0	4	2
Phomopsis sp.4	0	0	0	0	0	0	0	1	3	0	4	2
Number of isolates	2	4	4	4	2	2	1	4	4	3	30	
Number of taxa	1	3	1	2	1	2	1	2	2	2		
Total species	5											

Appendix 5.

Examples for statistic analysis

1. ID: TOTAL ISOLATES FOUND FROM AREAS ON LEAVES AT CHIANG DAO (1997)

KRUSKAL-WALLIS ONEWAY NONPARAMETRIC AOV FOR ISOLATES = AREAS

AREAS	MEAN RANK	SAMPLE SIZE
1	30.7	5
2	34.9	5
3	30.3	5
4	31.0	5
5	18.6	5
6	17.9	5
7	16.3	5
8	23.8	5
9	31.0	5
10	20.5	5
TOTAL	25.5	50

KRUSKAL-WALLIS STATISTIC	10.5295
P-VALUE, USING CHI-SQUARED APPROXIMATION	0.3093

PARAMETRIC AOV APPLIED TO RANKS

SOURCE	DF	SS	MS	F	P
BETWEEN	9	2.084E+03	231.6	1.22	0.3124
WITHIN	40	7.615E+03	190.4		
TOTAL	49	9.699E+03			

TOTAL NUMBER OF VALUES WHICH WERE TIED 48

MAX. DIFF. ALLOWED BETWEEN TIES 1.0E-0005

CASES INCLUDED 50 MISSING CASES 0

2. ID: COMPARISON OF TAXA FOUND FROM AREA 3 & AREA 6 ON MATURE LEAVES AT CHING DAO (1997)

VIEW DATA

CASE	AREA 3	AREA 6
1	4.0	3.0
2	5.0	5.0
3	5.0	6.0
4	2.0	5.0
5	2.0	4.0

WILCOXON SIGNED RANK TEST FOR AREA 3 & AREA 6

SUM OF NEGATIVE RANKS	-8.50
SUM OF POSITIVE RANKS	1.50

EXACT PROBABILITY OF A RESULT AS OR MORE EXTREME THAN THE OBSERVED RANKS (1 TAILED P VALVE) 0.1250

NORMAL APPROXIMATION WITH CONTINUITY CORRECTION	1.095
TWO TAILED P VALUE FOR NORMAL APPROXIMATION	0.2733

TOTAL NUMBER OF VALUES WHICH WERE TIED	2
NUMBER OF ZERO DIFFERENCES DROPPED	1
MAX. DIFF. ALLOWED BETWEEN TIES	1.0E-0005

CASES INCLUDED 4 MISSING CASES 1

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