Mycorrhizas for Plantation Forestry in Asia

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Contents

Introduction

THE papers presented in this volume resulted from a two-day conference covering the diversity, physiology and ecology of mycorrhizal fungi and associations, and the development of mycorrhizal technologies for use in the nursery and field. This ACIAR-funded conference was jointly organised by the CSIRO Division of Forestry and Murdoch University in Australia, and the Research Institute of Tropical Forestry, Chinese Academy of Forestry, Guangzhou. It was held at Kaiping in Guangdong Province, Peoples Republic of China from November 7–11, 1994. The Crawford Fund generously supported the attendance of some delegates. We gratefully acknowledge the mayor of Kaiping City for hosting the conference and providing facilities.

This conference followed six years of successful research collaboration between the organisations mentioned above. Its purpose was to transfer knowledge gained during research by ACIAR, CSIRO and Murdoch University in Australia, and the Chinese Academy of Forestry and the Chinese Academy of Science, to forest researchers throughout China and neighbouring Asian countries. The conference provided an opportunity to summarise current knowledge of mycorrhizal associations in the region, with emphasis on the development of protocols for practical utilisation of these beneficial fungal associations in plantation forestry. As well, the participants were able to exchange ideas for promoting the use of mycorrhizal technology, and establish contacts with other researchers in the region.

Approximately 100 delegates, including research scientists, forest farm managers and administrators attended the conference. They were from at least 14 provinces in China, as well as Australia, the Philippines, Indonesia, Thailand and Vietnam. Delegates were selected to provide a balance between forest managers and research scientists, and preference was given to personnel with hands-on experience in plantation forestry.

A one-day field trip to the Zhenhai Forest Farm near Kaiping provided an opportunity to visit mycorrhizal field trials with plantation eucalypts conducted by the Australian and Chinese researchers. We gratefully acknowledge the assistance provided by the forestry staff at the forest farm. The field trip was followed by a two-day workshop where methods for fungal identification, nursery inoculation and field implementation were demonstrated. A manual, produced for this workshop, provided methodological information for working with mycorrhizal associations, and a revised version of this will be published separately by ACIAR as *A Practical Guide for Working with Mycorrhizal Fungi in Forestry and Agriculture*.

As the editors of these proceedings, we have tried to assemble a series of papers which present an overview of the conference and are representative of the research interests and skills of the delegates, as well as current world wide trends and objectives in mycorrhizal research. It was possible for only a selection of the mycorrhizal scientists in China to attend this conference and there were few delegates from other countries. Consequently, the research presented in this publication focuses on mycorrhizal research in China and the Australasian region. There is a strong emphasis on the practical utilisation of mycorrhizal associations of tree crops, especially plantation eucalypts, reflecting ACIAR's primary involvement in mycorrhizal research. However, these research topics are also of importance in other regions of the world.

Every attempt has been made to ensure the accuracy of the information presented in the papers in this publication. The manuscripts went through separate stages of writing, translating and editing, and in many cases were substantially rewritten during the editorial process. We apologise to the authors if we have in any way misinterpreted their work during this lengthy process. Detailed information on the research methods used was not always presented in papers, but is available in the separate ACIAR publication, which should be referred to for further information about mycorrhizal fungi and their utilisation.

Mycorrhizal associations are an integral component of natural and managed forests. Mycorrhizas consist of partnerships between fungi and the roots of plants, and their primary role is to augment plant nutrient uptake. These associations are particularly important for plantation forests established on nutritionally impoverished soils. The research presented here concerns the two most common types of mycorrhizal associations: ectomycorrhizas — where Basidiomycete or Ascomycete fungi form a mantle on the surface of roots and a Hartig net between root cells; and vesicular-arbuscular mycorrhizas — where Zygomycete fungi in the order Glomales form associations within root cells.

At the conference papers were presented in three subject categories: the ecology of mycorrhizal fungi and plants; the functioning of mycorrhizal associations; and their practical utilisation in the nursery and field, and this arrangement is retained in these proceedings.

The Editors

M. Brundett, B. Dell, N. Malajczuk and Gong Mingqin

Occurrence and Ecology of Mycorrhizal Fungi and Associated Plants

Diversity of Ectomycorrhizal Fungi Associated with Eucalypts in Australia

N. L. Bougher*

Abstract

The majority of Australian fungi have yet to be discovered and described and the systematic relationships of many known fungi have not been elucidated. Most of the major groups of ectomycorrhizal Ascomycetes, Basidiomycetes and Zygomycetes are well represented in Australia, including about 660 named species. Recent intensive collecting programs and taxonomic studies have indicated that Australia has an extremely diverse set of ectomycorrhizal fungi including many fungi that do not necessarily conform to taxonomic schemes based on northern hemisphere fungi. More than 70% of Australian ectomycorrhizal fungi may be endemic. Within Australia, fungal species generally have a less restricted geographic range than plants. The unique and diverse attributes of Australian ectomycorrhizal fungi may have been the result of many factors including: (1) their co-evolution with Australia's unique indigenous plants during long periods of geographic isolation; (2) the predominance of plants adapted to poor soils, which are widespread in Australia, especially ectomycorrhizal hosts such as *Eucalyptus*; (3) the broad range of environments occurring in Australia, including those that have greatly changed in recent geological time; and (4) both northern and southern geographic origins and affinities of Australian fungi leading to distinct elements in the present-day Australian fungal flora.

Key words: Australia, biodiversity, biogeography, ectomycorrhizal fungi, taxonomy

ECTOMYCORRHIZAL fungi are associated with many forest trees throughout the world, including the important families Betulaceae, Dipterocarpaceae, Fagaceae, Myrtaceae and Pinaceae (Newman and Reddell 1987). One estimate suggests that there are 5000–6000 species of mycorrhizal fungi with the majority being ectomycorrhizal (Molina et al. 1992), although this figure may be exceeded as poorly explored regions receive attention. In Australia most of the predominant forest trees are ectomycorrhizal hosts, including *Eucalyptus* (Myrtaceae), *Allocasuarina* (Casuarinaceae), *Acacia* (Mimosaceae) and *Nothofagus* (Fagaceae or Nothofagaceae). The many unique attributes of the present-day Australian biota as a whole, have been brought about by conditions peculiar to this island continent. These are discussed with reference to ectomycorrhizal fungi below. One outcome has been that the uniqueness and diversity of Australian ectomycorrhizal fungi equals or exceeds that of other parts of the world (Molina et al. 1992; Castellano and Bougher 1994). Another outcome has been that Australian fungi occurring in eucalypt forests are generally not compatible with forest trees from outside Australia such as oaks, pines and dipterocarps (Malajczuk et al. 1982).

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Diversity and Major Characteristics of Australian Fungi

Taxonomic schemes and biodiversity assessments of ectomycorrhizal fungi are based almost exclusively on their sexual fruiting structures, e.g. the mushrooms, toadstools, puffballs, coral and cup fungi, false truffles and truffles often grouped together under the term 'larger fungi'. It is estimated that there may be 250 000 species of fungi in Australia, including about 5000 mushrooms, but less than 5% of these have been named (Pascoe 1991). Another estimate suggests that 10% of Australian fungi have been named and that another 10% are known, but are not named (Office of the Chief Scientist 1992). Although the larger fungi are generally more conspicuous than microscopic fungi, most species are probably unrecorded and yet to be named. For example, a recent survey of larger fungi carried out over two fruiting seasons at Two People's Bay Nature Reserve (4744 hectares) in Western Australia reported 441 species, of which 365 are probably undescribed

(Syme 1992). This included 71 mostly unnamed species of the ectomycorrhizal genus *Amanita*.

There are very few books on larger Australian fungi (e.g. Cleland 1934; Young 1982; Fuhrer 1985; Fuhrer and Robinson 1992) and there is no comprehensive taxonomic treatment of the Australian ectomycorrhizal fungi. The limited literature reflects the general paucity of taxonomic mycology in Australia (Grgurinovic and Hyde 1993). The biodiversity of ectomycorrhizal (and other) fungi remains poorly known in Australia because less attention has been given to them than to vascular plants, and because the fruiting bodies of fungi are ephemeral structures appearing at irregular and unpredictable intervals in response to various climatic stimuli. Sampling of fungi at any one time will inevitably reveal only a fraction of the fungi present at any location.

Most major groups of ectomycorrhizal Ascomycetes, Basidiomycetes and Zygomycetes are well represented in Australia (Castellano and Bougher 1994). About 660 Australian species of ectomycorrhizal fungi have been named (Tables 1, 2). If it is assumed that about 10% of species have been named (as suggested above), the total number of ectomycorrhizal fungi in Australia could be approximately 6500. Recent intensive collecting programs and taxonomic studies undertaken by the CSIRO and collaborators have confirmed that Australia indeed has an extremely diverse set of ectomycorrhizal fungi — a diversity that has been previously greatly underestimated (Castellano and Bougher 1994).

Truffle-like fungi, which are mostly presumed to be ectomycorrhizal and have subterranean fruiting bodies, may be more abundant and diverse in Australia than anywhere else in the world. During a five-year period of fungal collecting mainly in Queensland and Tasmania (initiated in 1988), about 180 new truffle-like species were recognised amongst the collections examined in detail so far (Castellano and Bougher 1994). Nearly 95% of the described and undescribed Australian truffle-like fungi are novel (Castellano and Bougher 1994). Similarly, many ectomycorrhizal fungi having mushroom-like fruiting bodies have recently been newly discovered in Australia, e.g. seven species of *Rozites* including six novel species (Bougher et al. 1994). That no species of *Rozites*, which produces large and conspicuous fruiting bodies, had been previously confirmed in Australia emphasises the current low level of taxonomic knowledge about Australian fungi.

More floristic information is needed to assess the diversity and characteristics of Australian ectomycorrhizal fungi. To increase the knowledge about Australia's fungal biodiversity, an important priority for taxonomic mycology in Australia is to discover, describe and name new taxa, and to elucidate their relationships. Another major task (to be undertaken concurrently) is to re-examine the relatively few old Australian specimens held in herbaria that may have had names allocated to them many years ago, but often based on northern hemisphere taxonomic schemes. Modern techniques and comparison with recently connected material may shed a different light on their identity and affinities. For example, it is becoming clear that many Australian ectomycorrhizal fungi do not necessarily conform to taxonomic schemes based on northern hemisphere fungi.

The genus *Hymenogaster* is an example where the previously established taxonomy of ectomycorrhizal fungi has proved to be inadequate as a taxonomic framework for Australian fungi. *Hymenogaster* produces underground fruiting bodies and is represented world-wide. It is particularly diverse in association with *Eucalyptus* and other plants in Australia. Since the original concept by its author C. Vittadini in 1831 based on eight European species, the genus had become ill-defined and, until recently, encompassed most truffle-like fungi having a brown non-gelatinised loculate gleba completely enclosed by the peridium. Recent examination and description of Vittadini's type specimens, and comparative studies of fungi attributed to *Hymenogaster* from Australia (Bougher and Castellano 1993) clarified the generic boundaries and identified some new phylogenetic affinities. The prevailing concept had been that *Hymenogaster* is the most reduced member (truffle-like) of the series linking *Cortinarius* (mushroom-like) and *Thaxterogaster* (intermediate fruit body type) in the family Cortinariaceae. However the study demonstrated that *Hymenogaster sensu stricto* is not closely linked to this series. Furthermore, all known Australian fungi formerly placed under the broad concept of *Hymenogaster* were assigned to several different genera, each considered to have different phylogenetic affinities. For example, the genus *Cortinomyces* was proposed to accommodate the many Australian truffle-like species related to *Cortinarius* and *Thaxterogaster*, and the relationships of another new genus *Descomyces* to *Descolea/Setchelliogaster* were outlined (Bougher and Castellano 1993).

The diversity of Australian ectomycorrhizal fungi at intraspecific levels, e.g. variation in subspecies and populations, is as yet largely unmeasured. Data about variation expressed by individuals is also sparse, e.g. little is known about the genetic/molecular basis for physiological characteristics of fungal isolates that vary over time. Some investigations with Australian ectomycorrhizal fungi using molecular tools such as restriction fragment length polymorphism (RFLP) of random/specific regions of DNA and random amplified polymorphic DNA (RAPD)/polymerase chain reaction (PCR) techniques are now being utilised. These studies are revealing that for some fungi, variability within species of ectomycorrhizal fungi is as great as among species (Tommerup and Malajczuk 1993).

Table 1. Main genera of epigeous ectomycorrhizal fungi with the number of named species in Australia considered to be valid by the present author (documented species are included for some genera, e.g. those outlined as distinct species in the literature but not named). Total = 467 species.

Epigeous ectomycorrhizal fungi (**CLASS**/**Order**/Family)	Named species (no.)	Genera
A. BASIDIOMYCETES	422	
Agaricales	334	
Amanitaceae	60	Amanita 60
Boletaceae	93	*Austroboletus* 9, *Boletellus** 15, *Boletochaete* 1, *Boletus* 20, *Buchwaldoboletus** 2 (with conifers), *Chalciporus***, *Fistulinella* 3, *Gyroporus* 5, *Heimielia* 1, *Leccinum* 1 (with indigenous plants) 3 (exotic plants), *Phlebopus* 1, *Phylloporus* 11, *Pulveroboletus* 5, *Rubinoboletus* 1, *Suillus* 5 (with pines), *Tylopilus* 10, *Xanthoconium***
Cortinariaceae	72	*Astrosporina* 4, *Cortinarius* 39, *Cuphocybe***, *Dermocybe** 8, *Descolea* 3, *Hebeloma* 7 (3 with exotic trees), *Inocybe* 4, *Rozites* 7, *Stephanopus***
Entolomataceae	18	*Clitopilus** 3, *Entoloma** 3, *Leptonia** 10, *Rhodocybe** 2
Hygrophoraceae	30	*Bertrandia** 1, *Camarophyllus* 8, *Gliophorus** 3, *Humidicutis** 2, *Hygrocybe* 12, *Hygrophorus** 4
Paxillaceae	9	*Paxillus* 9
Russulaceae	21	*Lactarius* 5, *Russula* 16
Strobilomycetaceae	5	*Strobilomyces* 5
Tricholomataceae	26	*Clitocybe** 7, *Cystoderma** 1, *Laccaria* 6, *Lepista* 3, *Leucopaxillus* 2, *Tricholoma** 6, *Tricholomopsis** 1
Lycoperdales	0	
Sclerodermatales	12	
Pisolithaceae	3	*Pisolithus* 3
Sclerodermataceae	9	*Scleroderma* 9
Aphyllophorales	76	
Bankeraceae	2	*Phellodon** 2
Cantharellaceae	7	*Cantharellula* 1, *Cantharellus* 5, *Craterellus* 1
Clavariaceae	57	*Aphelaria* 1, *Clavaria* 17, *Clavariadelphus* 1, *Clavicorona* 1, *Clavulina* 8, *Clavulinopsis* 2, *Ramaria* 18, *Ramariopsis* 9
Coniophoraceae	1	*Podoserpula** 1
Gomphaceae	1	*Gomphus* 1
Hydnaceae	4	*Hydnum* 4
Thelephoraceae	4	*Boletopsis***, *Thelephora* 4
B. ASCOMYCETES	45	
Helotiales	7	
Geoglossaceae	7	*Geoglossum** 4, *Leotia** 1, *Trichoglossum* 2
Pezizales	38	
Helvellaceae	3	*Gyromitra* 2, *Helvella* 1
Pezizaceae	20	*Aleuria** 3, *Peziza** 12, *Phillipsia** 2, *Pulvinia* 3
Pyrenomycetaceae	10	*Jafneadelphus** 4, *Lamprospora** 5, *Sphaerosporella* 1
Sarcosomataceae	5	*Plectania** 3, *Pseudoplectania** 1, *Sarcocypha** 1

*Taxa for which the mycorrhizal status is uncertain or some species are not mycorrhizal

**Taxa known by present author to occur in Australia, but no species confirmed in the literature. Ectomycorrhizal taxa not yet confirmed for Australia include: Basidiomycetes–*Boletinus*, *Boletopsis*, *Fuscogyroporus*, Gomphidiaceae, and *Leucocortinarius*. Also mycorrhizal taxa of the Astraeaceae, Corticiaceae, Polyporaceae (s.s.), Ascomycetes–Ascobolaceae.

Table 2. Main genera of hypogeous ectomycorrhizal fungi, with the number of named Australian species (see criteria as for Table 1). Total = 196 species.

Hypogeous ectomycorrhizal fungi (**CLASS/Order/**Family)	Named species (no.)	Genera
BASIDIOMYCETES	137	
Agaricales	86	
Amanitaceae	1	*Torrendia* 1
Boletaceae	I0	*Alpova or Alpova*-like 3, *Boughera* 0 (present in Aust.), *Chamonixia* 3, *Gastroboletus***, *Rhizopogon* 3 (with pines), *Rhizopogon*- like**, *Royoungia* 1
Cortinariaceae	19	*Cortinarius (hypogeous)* 1, *Cortinomyces* 6, *Descomyces* 2, *Hymenogaster* ** (mostly doubtful), *Quadrispora* 1, *Setchelliogaster* 2, *Thaxterogaster* 7
Cribbiaceae	4	*Cribbea* 4
Entolomataceae	2	*Rhodogaster***, *Richoniella* 2
Octavianinaceae	13	*Octavianina* 12, *Sclerogaster* 1
Russulaceae	21	*Archangelielia* 3, *Cystangium* 4, *Elasmomyces* 1, *Gymnomyces* 4, *Macowanites* **, *Martellia* 3, *Zelleromyces* 6
Strobilomycetaceae	10	*Austrogautieria* 4, *Chamonixia* 3, *Gautieria* 3
Tricholomataceae	6	*Gigasperma* 1, *Hydnangium* 4, *Podohydnangium* 1
Aphyllophorales	1	
Stephanosporaceae	1	*Stephanospora* 1
Chondrogastrales**		
Chondrogastraceae**		*Chondrogaster* **
Lycoperdales	30	
Astraeaceae**		*Radiigera* **
Mesophelliaceae	29	*Castoreum 2, Diploderma 9, Gummiglobus* **, *Malajczukia* 7, *Mesophellia* 10, *Nothocastoreum* 1
Geastraceae	1	*Cycloderma* 1
Phallales	17	
Gelopellidaceae	1	*Gelopellis* 1
Hysterangiaceae	15	*Hysterangium* 15, *Pseudohysterangium* **
Phallaceae	1	*Protubera* 1
Podaxales	1	*Chainoderma* 1
Sclerodermatales	2	
Sclerodermataceae	2	*Horakiella* 1, *Scleroderma* (hypogeous) 1
Leucogastrales	1	
Leucogastraceae	1	*Leucogaster* 1, *Leucophleps***
Melanogastrales**		
Melanogastraceae**		*Melanogaster***
ASCOMYCETES	37	
Elaphomycetales	1	
Elaphomycetaceae	1	*Elaphomyces* 1

Table 2. (Continued) Main genera of hypogeous ectomycorrhizal fungi, with the number of named Australian species (see criteria as for Table 1). Total = 196 species.

Hypogeous ectomycorrhizal fungi (CLASS/Order/Family)	Named species (no.)	Genera
Pezizales	36	
Ascobolaceae	3	*Sphaerosoma* 3
Balsamiaceae	1	*Balsamia* 1
Geneaceae	3	*Genea* 2, *Geneaba* 1
Helvellaceae	5	*Gymnohydnotrya* 2, *Hydnotrya* 1, *Mycoclelandia* 2
Pezizaceae	6	*Amylascus* 1, *Hydnotryopsis***, *Muciturbo* 3, *Pachyphloeus* 0, *Peziza* 1, *Ruhlandiella* 1
Pyronemataceae	15	*Dingleya* 6, *Elderia* 1, *Hydnocystis* 1, *Labyrinthomyces* 1, *Paurocotylis* 1, *Reddellomyces* 4, *Sphaerozone* 1
Terfeziaceae	1	*Choiromyces* 1
Tuberaceae	2	*Mukagomyces* 1, *Tuber* 1
ZYGOMYCETES	8	
Endogonales	8	
Endogonaceae	8	*Endogone* 7, *Sclerogone* 1
TAXA OF UNCERTAIN AFFINITIES	13	*Gymnogaster* 1, *Hysterogaster* 3, *Mycoamaranthus* 1, *Noahmyces***, *Potoromyces* 1, *Secotium* 3, *Timgrovea* 4

*, ** See Table 1.

Notes: Ectomycorrhizal taxa not yet confirmed for Australia include: Basidiomycetes — *Destunzia*, Gomphidiaceae, *Mycolevis*, *Pyrenogaster*, *Trappea*, *Truncocolumella*, *Wakefieldia* Ascomycetes — *Fischerula*, *Geopora*, *Hydnobolites*, *Hydnoplicata*, *Paradoxa*, *Picoa*, *Stephensia*, and *Terfezia*.

Endemism of Australian Fungi and Geographic Distribution within Australia

It has been estimated that 70% of all Australian fungi may be endemic, compared with 85% of plants (Office of the Chief Scientist 1992). However, more comprehensive floristic studies need to be undertaken in Australia and neighbouring regions before the true extent of endemism among Australian fungi can be made. Many species of ectomycorrhizal fungi are not currently known to occur outside Australia. For example, at least 22 genera and 3 families of truffle-like fungi discovered in Australia since 1988 are endemic (Castellano and Bougher 1994). Endemism is also high with some Australian ectomycorrhizal mushroom taxa. For example, six out of seven species of *Rozites* recently discovered in Australia are endemic — only *R. fusipes* also occurs in New Zealand (Bougher et al. 1994). With some notable exceptions (see discussion on biogeographic origins below), Australia has a unique bolete flora (Watling and Gregory 1988). Most species of *Amanita* in Australia are probably endemic (e.g. see Miller 1992). Many fungi associated with *Eucalyptus* are not compatible with other host plant genera (Malajczuk et al. 1982), and it seems probable that Australian ectomycorrhizal fungi may exceed 70% endemism.

Some ectomycorrhizal fungi with narrow host compatibility occur within forested regions of Australia other than those dominated by eucalypts, and these fungi often have a restricted geographical distribution, e.g. the *Nothofagus* forests of eastern Australia harbour many endemic fungal species (Fuhrer and Robinson 1992). Many ectomycorrhizal fungi associated with *Eucalyptus* favour different broad climatic zones within Australia, e.g. tropical and temperate regions (see discussion below on biogeographic origins and affinities). However, species of larger fungi of Australia occurring in forests dominated by eucalypts are generally more widely distributed throughout Australia than species of plants (Bougher and Tommerup 1995). For example, southwest Australia has a high level of plant diversity (Marchant 1973) and endemism (Beard 1969) compared with eastern Australia, due to factors such as geographic isolation and climatic fluctuations from the late Tertiary onwards (Hopper 1992). Many discontinuous plant species and presumed vicarious species pairs on either side of the arid Nullarbor Plain

have been recognised (Green 1964). A few examples of vicarious ectomycorrhizal fungal species pairs have been presumed, e.g. *Descolea maculata* in the west and *D. recedens* in the east (Bougher and Malajczuk 1985). However in contrast to the plants, probably most ectomycorrhizal fungal species (as well as many saprophytic and parasitic fungi) associated with *Eucalyptus* are present in both western and eastern Australia. And the comparatively high level of plant species diversity in the southwest compared with eastern Australia (Marchant 1973) does not appear to be so for the fungi.

There is experimental evidence that individual isolates of ectomycorrhizal fungi compatible with eucalypts have a greater tolerance for functioning in a wider range of conditions than have many plants (Harley and Smith 1983; I. Tommerup and B. Bougher, pers. comm.). This could be one factor accounting for their generally wider geographic distribution than plant species in Australia. Another factor may be that compatibility between Australian plants and ectomycorrhizal fungi does not often appear to be expressed at the plant species level or even at the genus level. For example, there is little evidence with Australian ectomycorrhizal fungi of host plant specificity within the genus *Eucalyptus* (Malajczuk et al. 1982).

Factors Shaping the Unique and Diverse Attributes of Australian Fungi

The unique and diverse attributes of Australian ectomycorrhizal fungi may have been brought about by many factors (Bougher and Tommerup 1995). One factor may have been co-evolution with Australia's unique indigenous plants during long periods of geographic isolation. Many Australian fungi have developed highly specific associations with Australian plants and are not compatible with non-Australian plants (Malajczuk et al. 1982). Another factor may be the predominance of plants adapted to poor soils which are widespread in Australia. Mycorrhizal associations are one of the common strategies used by Australian plants to obtain nutrients from poor soils (Pate 1994).

Ectomycorrhizal arborescent plants such as *Eucalyptus* dominate forests and many other ecosystems in Australia. Australia has a broad range of environments ranging from dry to temperate to tropical. Many Australian environments have greatly changed in recent geological time, providing opportunities for speciation, niches, and other pressures such as localised extinction of favoured host plants. For example, ectomycorrhizal fungi were undoubtedly well established in Australia before the late Tertiary when major climatic and geological changes began to determine the present-day characteristics of the plant flora, such as high diversity and endemism of south Western Australia. Another factor contributing to the unique and diverse attributes of Australian ectomycorrhizal fungi is the geographic origins of Australian fungi which has resulted in some distinct elements in the present-day Australian fungal flora.

Biogeographic Origins of Australian Ectomycorrhizal Fungi and Present-day Floristic Elements

Recent data on ectomycorrhizal fungi in Australia has begun to shed light on the question posed by Horak (1983) as to the origin of mycorrhizal fungi associated with myrtaceous plants in the southern hemisphere (e.g. Wolfe and Bougher 1993, Bougher et al. 1994). Generally, the biogeographical patterns of Australian ectomycorrhizal fungi support the idea (Truswell 1993) of long-term bi-directional interchange of biota between Australia and the north since the late Cretaceous. Data about some fungi conforms with the general notion (Malloch et al. 1980) that the mycorrhizal biota associated with *Eucalyptus* and other Myrtaceae in the tropics and Southern Hemisphere spread there with *Nothofagus* or its ancestors (fungi of southern affinity). Australian species of ectomycorrhizal fungi group into three broad biogeographical elements, each with different origins. These are (1) fungi with northern affinity, (2) fungi with southern affinity, and (3) recent introductions.

Fungi with northern affinities

Many ectomycorrhizal fungi that occur in the subtropical and tropical forests of northern Australia also occur in Asia, e.g. *Heimiella* and *Pulveroboletus ravenelii* (Watling 1993). The present day disjunct distribution of *Tylopilus* subg. *Roseoscabra* (northern Australia, east Asia, eastern North America and Central America) indicates a Laurasian origin for the group (see Wolfe and Bougher 1993). Some Asian fungal species also extend into temperate southern Australia, e.g. *Boletellus obscure-coccineus.*

Fungi with southern affinities

The origin of some Australian ectomycorrhizal fungi can be traced to a putative association with Gondwanan plants such as *Nothofagus* (southern beeches) and their close ancestors. Examples include the genera *Rozites* and *Descolea*, most species of which occur within a disjunct distribution in the southern hemisphere and are associated with *Nothofagus* (Horak 1983; Bougher et al. 1994). Several representatives of these genera also occur in the northern hemisphere associated with hosts other than

Nothofagus. For *Rozites* it is suggested by Bougher et al. (1994) that an ancient stock arose somewhere within the geographical range of a generalised fagalean complex of plants that occurred in Asia during the Cretaceous before the origin of *Nothofagus* (Hill 1992, Truswell 1993). The postulation that a northern portion of the ancestral *Rozites* stock gave rise to extant northern hemisphere *Rozites* species and a southern portion speciated as *Nothofagus* itself speciated (Bougher et al. 1994) is also applicable to *Descolea*.

Numerous other examples of disjunct distributions in the southern hemisphere suggest that at least some Australian ectomycorrhizal fungi originated in Gondwana before its break-up. For example, *Cortinarius phalarus* from Western Australia, and several undescribed similar species in eastern Australia, are members of an unusual group of volvate cortinarii (section *Fulvi* subsection *Coleopodes*) also found in South America (Bougher and Hilton 1989). In contrast to these examples, Australian boletes appear to have minimal similarity to those of South America (which has a relatively low diversity of boletes) (Watling and Gregory 1988). Australian fungi of southern origin include species presently occurring only in *Nothofagus* forests e.g. *Descolea phlebophora* and *Rozites metallica*. Fungi presently associated with *Nothofagus* and *Eucalyptus* e.g. *Descolea recedens*, and putative relict Gondwanan ectomycorrhizal fungi that have survived local extinction of *Nothofagus* and much of its associated vegetation presently occur in eucalypt forests where *Nothofagus* formerly existed, e.g. *Descolea maculata* and *Rozites symea* in southwest Australia — a region with many relict plant taxa (Hopper 1992).

Recent introductions

Many fungi have been introduced with exotic trees into Australia in the past two centuries (Cleland 1934). Poor establishment and growth of early *Pinus* plantations in Australia was alleviated in the 1920s when soil and leaf litter was introduced from pine forests overseas. Ectomycorrhizal fungi such as *Suillus granulatus*, *Lactarius deliciosus* and *Rhizopogon luteolus* are now frequent in Australian pine plantations. Less desirable and/or accidental introductions of fungi into Australia include *Amanita phalloides*, a deadly species which now occurs with deciduous trees such as oaks in southeastern Australia and is of considerable medical concern (Cole 1993). Most fungi introduced into Australia are incompatible with indigenous Australian plants and are therefore restricted to gardens and plantations of exotic trees. However, a major ecological concern has arisen with some exotic fungal species invading native Australian forests as these have the potential to compete with, and exclude, indigenous Australian fungi. For example, *Amanita muscaria* which normally associates with exotic trees such as pines and birches has invaded *Nothofagus* forests in Australia (Fuhrer and Robinson 1992) and may be capable of invading eucalypt forests as is the case in some *Eucalyptus globulus* plantations outside Australia (N. Bougher, pers. comm.).

Conclusion

There is a large diversity of ectomycorrhizal fungi in Australia, each potentially possessing unique biological properties and roles in ecosystems. There are many potential benefits of Australian fungal diversity including economic benefits to commercial forestry and long-term ecological costs and benefits. However, there is a need to discover and characterise the majority of Australian fungi. Characterisation of fungi at the specific and intraspecific levels will enable more consistent, accurate and reproducible research experiments, by allowing results obtained with various fungi at different times and locations to be directly compared. New technologies for quantifying taxon-level and genetic-level diversity, such as networked information databases linking taxonomic, biogeographic and habitat data, provide the means to standardise and widely disseminate research data. There is some degree of urgency to characterise Australian fungal biodiversity because habitat destruction is threatening the existence of many species and therefore the genetic resource base is rapidly diminishing.

Characterisation of Australia's fungal resource base can proceed alongside a continued and expanded program to utilise a wider range of fungi in future forestry operations. The low diversity of fungi currently being used in Australasian eucalypt plantations may give minimal benefit to tree production because the fungi may not necessarily be well suited to the local site characteristics (climate, soil type, host plants, etc.). In the longer term, maintenance of soil structure, fertility and general ecosystem stability in the face of environmental changes and disturbances may be enhanced by the presence of a broader diversity of fungi.

Acknowledgments

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Ectomycorrhizal Fungus Communities in Southern China

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Abstract

A variety of ectomycorrhizal fungus communities occur in parts of southwestern China which have different climatic conditions and plant communities. Information is presented on the distribution of some ectomycorrhizal fungi and their associated host trees in the eastern Himalayas, Hengduan Mountain, Yunnan Plateau, Lingnan Hill, Taiwan and Hainan Island regions of China.

Key words: ectomycorrhizal fungi, fungal communities, geographic regions, China

IN recent years, it has been found that symbiotic associations between fungi and plants play a crucial role in the mineral nutrition of most plant species. Many trees, including members of the families Pinaceae, Fagaceae, Betulaceae and Myrtaceae form ectomycorrhizal associations, and these associations are widely distributed. The host plants and mycorrhizal fungi form associations that increase the growth and survival of the host plant (Harley and Smith 1983). Metabolites are supplied by the root to these heterotrophic fungi in exchange for mineral nutrients obtained by the fungus. These mutualistic associations are essential for both partners. Mycorrhizal associations are found in almost all terrestrial habitats, including alpine, lowlands, subtropical and tropical ecosystems (Brundrett 1991).

China can be classified into physical regions (Shao 1984) and this classification has been used to characterise fungal habitats in southern and southwestern China (Fig. 1). In southern China, the topography of the land varies, from the highest peak of Mount Qomolangma (Mount Everest) at 8848 m, in the northwest, to the China–Vietnam border, which is only 76.4 m above sea-level, and the Pacific coast in the southeast. The main provinces in the geographic regions cited in this paper are Tibet, Yunnan, Sichuan, Guizhou, Guangxi, Guangdong, Hainan and Taiwan, listed in order from west to east. The purpose of this paper is to provide a summary of information about mycorrhizal fungus habitats in southern China, which includes some examples of fungi which occur in different regions.

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Southeastern Himalayas Uplands — Tropical and Subtropical Montane Forests

There is a rich flora of forest plants and fungi in the southern and southeastern slopes of the Tibetan plateau. This is the highest plateau in the world, where large-scale geological uplift has resulted in a mean elevation of 4000 m. In this region, the ectomycorrhizal fungi *Suillus spraguei* (Berk. et Curt.) Kuntze (Fig. 2) and *S. flavus* (Withering) Singer are very common in alpine coniferous forests, growing in association with five needle pines (*Pinus griffithii* Clelland, and *P. armandli* Franch).

Suillus granulatus (Fr.) Kuntze and *S. flavidus* (Fr.) Singer are also commonly associated with *Pinus* species and other hosts (*Abies squamata* Mast., *A. georgei* Orr., *Picea purpurea* Mast., etc.) in these forests. *Suillus grevillei* (Klotzsch.) Singer and *S. plorans* (Rolland) Kuntze commonly occur in eastern Tibet, Yunnan and western Sichuan coniferous forests with host trees such as *Larix speciosa* Cheng et Law. and *L. potaninii* Batalin.

A large and highly valuable species of delicious edible fungus, *Tricholoma quercicola* Zang (Fig. 2) occurs in some dry slopes of the eastern Himalayas and Hengduan Mountains, where alpine evergreen *Quercus* species are dominant in mesophytic forests. It also occurs in eastern Tibet and western Sichuan, associated with *Quercus semicarpifolia* Smith, *Q. aquifolioides* Rehd. et Wils and *Q. pannosa* Hand.-Mazz.

Hengduan Mountains and Yunnan Plateau — Coniferous, Deciduous and Evergreen Broad-Leaved Forests

The Hengduan mountain range is located in the west of Tibet and includes Kunming, the Ailao Mountains and parts of Qujing and Sichuan Provinces. Vegetation in this region includes coniferous forests with *Pinus* spp. and deciduous broad-leaved trees in temperate areas, and evergreen broad-leaved forests in subtropical areas. Here among parallel rows of craggy mountains, the Nujiang (Salween), Lancangjing (Mekong), and Jinshajing (Upper Yangtse River) have cut steep canyons, up to 2000–3000 meters deep. The Hengduan mountains in this plateau extend from north to south. The monsoon from the Indian Ocean advances north along the river valleys during seasonal warm wet weather. This wide diversity in climate and topography would be expected to support a rich and varied diversity of plants and fungi. Typical ectomycorrhizal fungi in this region include *Boletus kauffmanii* Lohway and *Tylopilus chlorinosmus* Wolfe et Bougher, *T. chromoreticulatus* Wolfe et Bougher, in Lijiang, under *Pinus densata* Mast. (Wolfe and Bougher 1993).

There are some fungi, such as members of the genus *Sinoboletus,* that have a limited distribution which is limited to this area. *Sinoboletus* species include *S. duplicatoporus* Zang (Fig. 2) found in the Ailao and south terminal Hengduan mountains, *S. magniporus* Zang from Western Yunnan, *S. guizhouensis* Zang et Wu, a mycorrhizal associate of fagaceous species from eastern Guizhou, and *S. duplicatoporus* Zang, which is associated with *Lithocarpus chingtungensis* Hsu et Qian in this region.

Tricholoma matsutake (Ito et Imai) Singer, which is known as Song Rong, Matsutake or pine mushroom, is the most sought after edible mycorrhizal mushroom in eastern Asia. This fungus occurs in pine forests in northeastern and southwestern China, as well as in Japan and Korea. Taxa in the matsutake group of the genus *Tricholoma* often form ectomycorrhizal associations with conifers, but several species associate with *Quercus, Castanopsis* etc. *Tricholoma bakamatsutake* Hongo (Fig. 2) is associated with *Quercus serrata* Sieb. et Zucc., *Q. variable* Bl. etc. from western China (Zang 1990) and Japan (Hongo 1974).

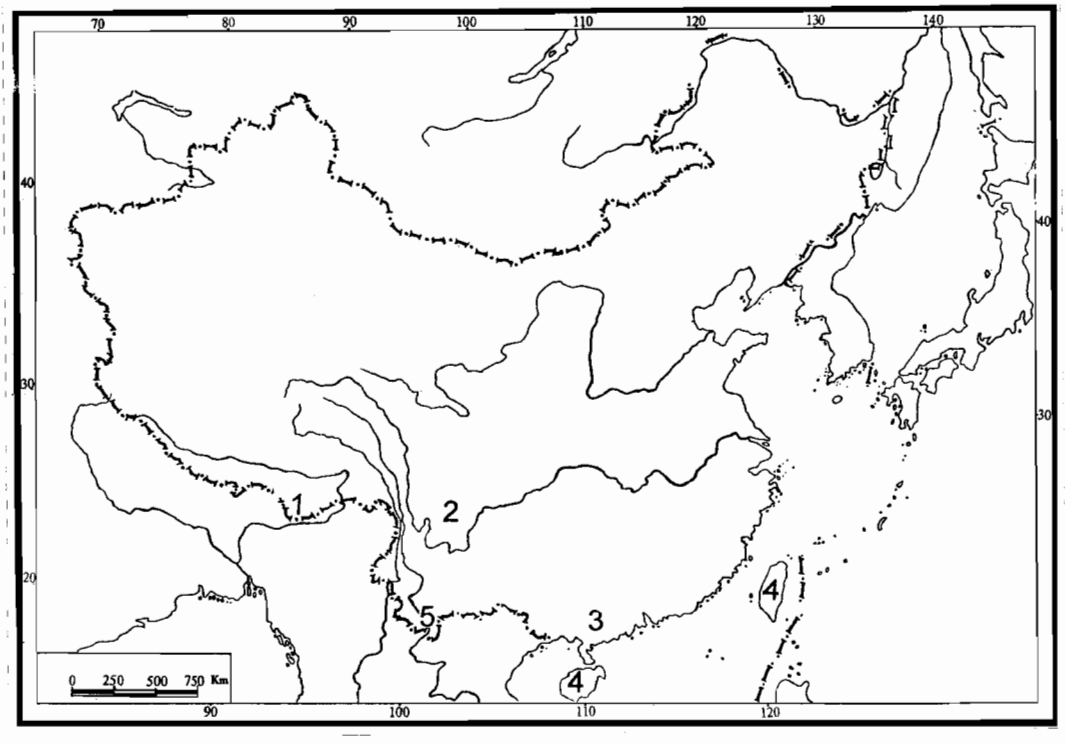

Figure 1. Physical regions of southern China. Region numbers are as follows: (1) Southeastern Himalayas uplands — tropical and subtropical montane forests; (2) Hengduan Mountains and Yunnan Plateau — coniferous, deciduous and evergreen broad-leaved forests; (3) Lingnan hills — evergreen monsoon forest; (4) Taiwan and Hainan Island — evergreen broad-leaved forest and monsoon forest; (5) South Yunnan — monsoon forest.

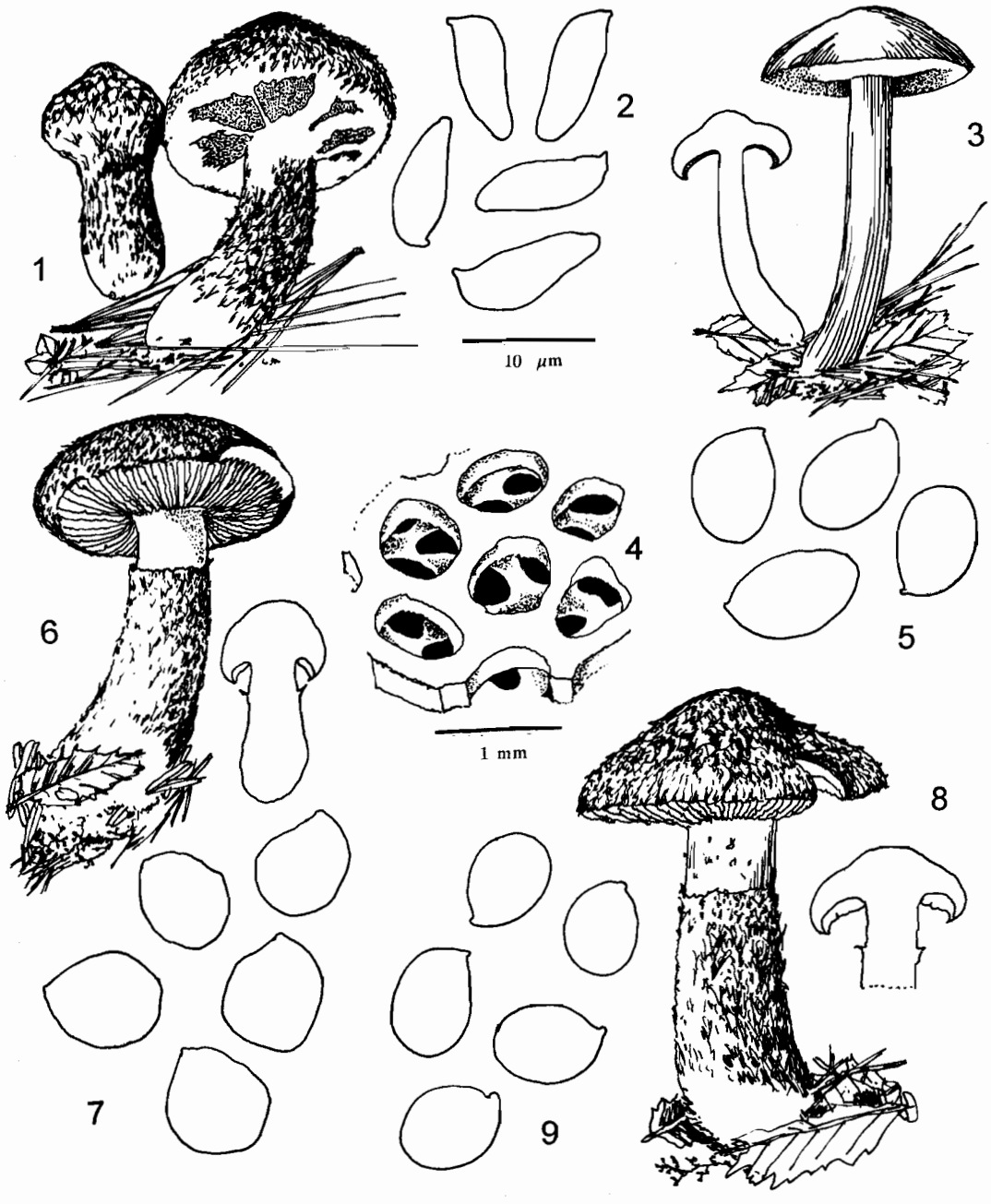

Figure 2. Some mycorrhizal fungi from southern China.

Suillus spraguei — 1. Basidiocarps, 2. Basidiospores
Sinoboletus duplicatoporus — 3. Basidiocarps, 4. A part of the hymenium, 5. Basidiospores
Tricholoma quercicola — 6. Basidiocarps, 7. Basidiospores
Tricholoma bakamatsutake — 8. Basidiocarps, 9. Basidiospores.

Lingnan Hills — Evergreen Monsoon Forest

This area in eastern monsoonal China is influenced by strong summer maritime monsoonal rainfall, resulting in sharp seasonal variation both in wind direction and in precipitation. Climate in this region is humid with evergreen monsoon forests as the predominant vegetation. *Pinus kwangtungensis* Chun ex Tsiang, occurs above 1000 m in this area, where it is associated with *Suillus spraguei*. A number of tropical taxa, such as *Boletus borneensis* Corner, which has also been recorded in Borneo (Corner 1972) and even Suixi County, Guangdong, occurring under *Eucalyptus* forest (Zang et al. 1993).

Eucalyptus forests in this area have ectomycorrhizal communities in which the fungi *Pisolithus tinctorius* (Pers.) Coker et Couch, *Scleroderma cepa* Pers., *S. polyrhizum* Pers., are abundant. Ectomycorrhizal fungi in the genera *Pisolithus* and *Scleroderma* are considered to have great potential for use in exotic plantations of *Eucalyptus* species to increase the growth and survival of trees after planting (Gong et al. 1992). Inoculation of eucalypt seedlings resulting in extensive development of ectomycorrhiza have been shown to improve tree growth (Gong Mingqin et al., Ji Dagan et al., Xu Daping et al. this volume).

Casuarina equisetifolia Forst is an important species in eastern areas near the sea, which has roots containing *Frankia*, an actinomycete which forms symbiotic nitrogen fixing associations. *Frankia* spp. also form associations with *Alnus nepalensis* D. Don which occurs in the Yunnan plateau.

Taiwan and Hainan Island — Evergreen Broad-Leaved Forest and Monsoon Forest

Massive and high mountain ranges occupy most of the central portion of Taiwan, and have a diverse alpine-montane flora. These plants show distinctively close relationships with those of Hengduan and eastern Himalayan mountains. For example, the coniferous tree genus *Taiwania*, has only two species; *T. cryptomerioides* Hayata — distributed in Taiwan, *T. flousiarta* Gaussen — which occurs in Taiwan, Guizhou and Yunnan. Most trees in these forests are associated with vesicular-arbuscular mycorrhizal fungi.

The Song Rong fungus (Matsutake group, as discussed above), a variety of *Tricholoma matsutake* (Ito et Imai) Singer var. *formosana* Sawada, is replaced by a closely related variety in Taiwan under *Pinus densiflora* Sieb. et Zucc. and *P. taiwanensis* Hayata (Chen 1983). In contrast to the south and eastward migration of temperate floristic elements, the northward movement of tropical species from Philippines to Taiwan is limited practically to the lowlands, favoured by the gradual rising of temperature. Thus tropical plants and fungi that find their way to Taiwan (Li 1953) and Hainan Island are able to establish themselves there. For example, the shrub *Acacia confusa* Herr. occurs widely in the Philippines, but also occurs in Taiwan and Hainan. Fungi occurring in this region include *Laccaria amethystea* (Bull. ex Gray.) Murr., and *Boletus brevitubus* Zang. which occur under *Acacia* forest and with *Delonix regia* (Bojer) Rafin. A number of tropical *Boletus* species, such as *B. nigerrimus* Helm, *B. portentosus* Berk. et Bri. and *Boletellus longlcollis* (Ces.) Pegler et Young, occur in Hainan Island, under evergreen broad-leaved forests dominated by *Eucalyptus* or fagaceous species.

South Yunnan Monsoon Forest

South Yunnan is strongly influenced by the tropical monsoon, with distinct alternations between dry and rainy seasons. The main vegetation in this region is seasonal rain forest communities. Common fungi include *Gyroporus atroviolacius* (Hohn.) Gilbert. which grows with *Toona* sp. (Meliaceae) and also occurs in Hainan province under *Pinus* species. Additional mycorrhizal fungi include *Boletus squamulistipes* Zang, *B. rufo-aureus* Mass. and *B. reayi* Heim. The latter is considered to be hallucinogenic by some people and also occurs in New Guinea (Corner 1972). However, subtropical and tropical fungi often occur in *Lithocarpus* and *Castanopsis* forests in South Yunnan and in neighbouring South Guanxi provinces.

Conclusions

There are many plant habitats in southern China, which support distinctive communities of ectomycorrhizal fungi. The fungus flora of this region is diverse and contains unique taxa, as well as widespread species. Further collecting and taxonomic research is required to catalogue this fungus diversity. This information will be of great value because ectomycorrhizal fungi in southern China include species that are important sources of food and medicine and others that have great potential for future use in forestry, as is explained elsewhere in this volume.

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Ectomycorrhizal Fungi Associated with Forest Trees in Guizhou Province, China

He Shaochang, Lian Bing and Zhang Lin*

Abstract

This paper lists 159 species of ectomycorrhizal fungi associated with forest trees in Guizhou Province of China. These fungi belong to the Basidiomycotina in 2 classes, 7 orders, 13 families and 29 genera. These fungi are considered to be symbiotic associates of 27 species of forest trees. The growth habitat, geographical distribution and the human use of these fungi are described. Ectomycorrhizal fungi are valuable biological resources and issues relating to their potential for utilisation are discussed.

Key words: ectomycorrhizal fungi, forest trees, basidiomycetes, biodiversity

GUIZHOU Province is situated in the eastern part of the Yunnan–Guizhou plateau in the southwest of China. It covers eastern longitude of 103° 36' – 109° 39' and north latitude of 24° 37' – 29° 13'. This is a mountainous region with a complex topography, situated at 1000 metres above sea level, with an annual average temperature of 15°C, annual precipitation of 1100 mm and average relative air humidity above 80%. The climate is subtropical and humid, with seasonal winds. Vegetation is diverse, with more than 7000 species of seed plants. As a result of natural environmental factors, Guizhou Province has a diverse natural fungus flora, including many fungi which form symbiotic mycorrhizal root associations with forest trees. The purpose of this paper is to summarise information about the fungus flora in Guizhou Province and provide information about the distribution, habits, associated trees and value of these fungi.

Results and Discussion

The present paper lists 159 species of ectomycorrhizal fungi associated with forest trees in Guizhou Province (Table 1). According to the taxonomy system of Ainsworth and Bisby (Hawksworth et al. 1983), all these fungi belong to the Basidiomycotina. These ectomycorrhizal fungi can be further divided into 2 subclasses, 7 orders, 13 families and 29 genera (Table 1). There are 27 species of forest trees that are considered to be ectomycorrhizal associates of these fungi (Table 1). However, this list may contain some species which are saprophytic or associate with smaller plants that occur in the same habits as these trees.

Ectomycorrhizal roots were observed in soil in the habitats where fungi were collected. The morphology of mycorrhizal roots could be grouped into 4 types with: clavate, coralloid, dichotomous-branched, or pinnate mycorrhizal morphology. The structure of the Hartig net of ectomycorrhizal roots also varied with different species of fungi and trees. In different ecological environments, the types and quantity of each type of ectomycorrhizal fungi differ greatly, and may be correlating with *in situ* conditions. Symbiotic characteristics of ectomycorrhizal fungi, such as their habitat and host tree preferences, that would directly effect their successful use in forestry are listed in Table 1.

During 1986–90, 25 species of fungi out of 61 species of purely isolated fungi were tested for tree inoculation at several locations of Guizhou Province, including Guiyang, Zhunyi, Zhenning, Dujun and Zhenyuan. *Pinus massoniana* seedlings were inoculated with *Russula delica*, resulting in an increase of 79.7% in seedling height, 87.2% in the diameter of end-butt, 47.3% in root length, 319% in fresh weight, and 320% in dry weight.

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Table 1. Ectomycorrhizal basidiomycete fungi associated with forest trees in Guizhou Province showing their habitat, utilisation, associated trees and geographic range.

Species	Habitat	Use	Host tree	Range
Aphyllophorales				
Hydnaceae				
Hydnum repandum L.:Fr.	forests	E	Pinus massoniana	Gu, An, Zh, Xi, Zu, Hb, Jl, Sa, Js, Tw, Sc, Yn, Sa, Qh, AS, EU
Hydnum repandum L.: Fr. var. *album* (Quel.) Rea.	forests	E	Pinus massoniana	Gu, Xi, Ky, Hu, Js, Jx, Gd, Gg, Sh, AS, AF, EU, AU
Cantharellales				
Cantharellaceae				
Cantharellus cibarius Fr.	forests	M	*Pinus massoniana*, broad leaf trees	Gu, Rn, Gi, An, Pb, Qg, Ky, Xi, Xg, Jl, Hb, Js, Zj, Ah, Fu, Hl, Hn, Gd, Ha, Gg, Sh, Yn, Tb, Sa, Gs, AS, EU, NA
Cantharellus cinnabarinus Schw.	mixed forest		*Pinus yunnensis*	Xi, Bj, Js, Ah, Gd, Gg, Sh, Tb, Yn, NA
Cantharellus clavatus Fr.	pine forest		*Pinus* spp.	Gu, Sh, Yn, Tb, Gs, EU, NA
Cantharellus floccosus Schw.	mixed forest		*Pinus yunnensis*, *Quercus* spp.	Xi, Bj, Gu, Sd, Ah, Fu, Hb, Hn, Sh, Yn, Tb, Sa, JA, EU, NA
Cantharellus minor PK.	mixed forest	M	*Pinus massoniana* & *P. yunnensis*	Gu, Rn, An, Xu, Zh, Zh, Xi, Tz, Su, Js, Fu, Jx, Hn, Gd, Ha, Sh, Sa, JA, NA
Gleocantharellus purpuracens (Hesler) Sing.	*Quercus* forest		*Quercus* spp.	Gu, Rn, Ar, JA
Tricholomatales				
Hygrophoraceae				
Hygrophorus eburneus (Bull.: Fr.) Fr.	mixed forest		*Quercus* spp.	Su, Gu, Jp, Lp, Hl, Jl, Li, Hb, Sh, Yn, Qh, AS, EU, NA
Hygrophorus lucorum Kalchbr.	mixed forest		*Pinus* spp.	Tu, Ji, Li, Hn, AS, EU
Hygrophorus pudorinus (Fr.) Fr.	mixed forest		?	Zh, Tu, Hl, Jl, Sh, Tb, EU
Amanitaceae				
Amanita caesarea (Scop.: Fr.) Pers. ex Schw.	broad-leaf forest	M	*Quercus* spp.	Gu, Rn, Xg, Ka, Zu, He, Jl, Li, Hb, Im, Js, Ah, Fu, Ha, Qu, Sh, Yn, Tb, R, EU, AF, NA.
Amanita caesarea var. *alba* Gill.	forests		*Quercus* spp.	Gu, Rn, Hl, Js, Ah, Ha, Sh, Yn, EU
Amanita farinosa Schw.	forests	P	*Pinus massoniana*, *P. yunnensis*, *P. taeda*, *P. pinaster*	?
Amanita flava Imai.	forests		*Populus devidiana*, *Betula luminifera*, *Pinus yunnensis*	Bj, Tb, JA
Amanita fulva (Schaeff:Fr.) Pers. ex Sing.	mixed forests		*Fagus lucida*, *Castanea sequinii*, *Pinus massoniana*	Gu, An, Xi, Ar, Tu, Bj, Hl, Jl, Li, He, Js, Ah, Fu, Gd, Ha, Gg, JA, R, EU, NA
Amanita inaurata Secr.	mixed forests	M	*Pinus* spp.	Gu, Xi, Ar, Zu, Tu, Ch, Zh, Bj, Pt, Hl, Jl, Li, Hb, Js, Ah, Fu, Gd, Ha, Sh, Yn, JA, R, EU, NA
Amanita kwangsiensis Wang	broad-leaf forests	P	*Quercus* spp.	Gu, Ha, Gg
Amanita manginiana Pat. et Har.	pine forests		Pinus yunnensis	Xi, Js, Fu, Sh, Yn, EU, NA

Table 1. (Continued) Ectomycorrhizal basidiomycete fungi associated with forest trees in Guizhou Province showing their habitat, utilisation, associated trees and geographic range.

Species	Habitat	Use	Host tree	Range
Amanita muscaria (L.) Pers. ex Hook.	forests	P, M	*Pinus, Quercus, Betula* spp. other conifers	Gu, An, Qg, Ky, Xf, Zh, Gl, Xi, Jp, Lp, Tn, Sj, Bj, Sh, He, Jl, Li, Hb, Ah, Fu, Gd, Yn, Tb, R, AS, EU, AF, NA
Amanita phalloides (Fr.) Secr.	forests	P, M	*Pinus, Quercus* spp.	Gu, An, Qg, Ky, Xf, Gs, Xg, Xi, Tn, Wo, Hl, Jl, Li, Js, Ah, Fu, Gd, Ha, Gg, JA, R, EU, NA
Amanita porphyria (Alb. et Schw.: Fr.) Secr.	pine & mixed forests	P	Pinus yunnensis	Bj, Ha, EU
Amanita rubescens (Pers.) Gray	forests		Pinus massoniana	Gu, An, Jl, Js, Ah, Sh, Tb, EU
Amanita rufoferruginea Hongo	forests		*Pinus* spp.	Gu, Xi, Su, Gg, JA
Amanita solitaris (Bull.) Darst.	forests		Pinus massoniana	Gu, Rn, Jl, Js, Ah, Gd, Ha, Gg, Sh, Yn, EU, NA
Amanita spissacea Imai	pine and broad-leaf forests	P	Pinus massoniana	An, Ky, Xf, Qg, Xi, Hb, Js, Ah, Jx, He, Gd, Ha, Qu, Sh, Yn, Tb, JA, EU, NA
Amanita vaginata (Bull.: Fr.) Vitt.	forests	M	*Pinus, Quercus* spp., etc.	Gu, An, Zh, Qg, Ky, Xf, Pb, Xg, Xi, Bj, Zu, Tu, Hl, Jl, Li, Js, Zj, Fu, Hb, Sa, Gd, Ha, Sh, Yn, Tb, Gs, Qh, R, JA, EU, AF, NA
Amanita vaginata var. *crassivolvata* PK.	forests	E	Pinus massoniana	Gu, EU
Amanita verna (Lam.: Fr.) Pers. ex Vitt.	forests	P	*Pinus* spp.	Rn, Zh, Hl, Jl, Li, Js, Ah, Jx, Hn, Gd, Ha, Gg, Sh, JA, R, EU, NA
Amanita virosa Lam. ex Secr.	broad-leaf forests	P	*Pinus* spp.	Gu, Rn, Hl, Jl, Li, Hb, Gd, Ha, Sh, R, JA, EU, NA
Tricholomataceae				
Laccaria amethystea (Bull.: ex Gray) Murr.	forests	M	*Pinus massoniana*	Gu, An, Zu, Xi, Bj, Zu, Dj, Ka, Tn, Hl, Jl, Li, Hb, Js, Zj, Ah, Fu, Gg, Yn, Tb, JA, R, EU, NA
Laccaria laccata (Scop.: Fr.) Berk. et Br.	forests	M	*Pinus. Quercus* spp.	Gu, Gi, An, Xg, Bj, Zu, Dj, Hl, Jl, Li, Hb, Js, Zj, Ah, Fu, Jx, Hn, Gd, Ha, Gg, Sh, Yn, Tb, Xj, JA, EU, AF, NA
Laccaria proxima (Boud.) Pat.	forests	M	*Pinus, Quercus* spp.	Gu, Zh, Hl, Jl, Hb, Js, Zj, Jx, Gd, Ha, Gg, Sh, Yn
Laccaria tortilis Bolt.: Fr.) Pat.,	forests		*Pinus, Quercus* spp.	Zh, Xi, Sh, Yn, JA, EU, NA
Lepista nuda (Bull.: Fr.) W.G.Smith	broad-leaf & conifer forests	M	*Pinus* spp.	Gu, Ds, Hl, Jl, Li, Sa, Fu, Qh, Xj, AS, EU, NA
Lyophyllum cinerascens (Bull. ex Konr.) Konr. et Maubl.	mixed forests		*Pinus, Quercus* spp.	Xi, Zu, Li, Yn, Qh, AS, EU, NA
Lyophyllum decastes (Fr.) Sing.	broad-leaf & conifer forests		Pinus massoniana	Zh, Xi, Hl, Jl, Li, Js, Sh, Yn, Qh, Xj, JA, R, EU, NA
Marasmius oreades (Bolt.: Fr.) Fr.	forests	M	*Pinus massoniana*	Gu, An, Zh, Zu, Jl, Hb, Sa, Ah, Jx, Sd, Gd, Sh, Yn, Tb, JA, EU, AS, NA
Melanoleuca melanoleuca (Pers.:Fr.) Murr.	forests		*Populus* spp.	Fm, Gu, Hl, Jl, Li, Sh, Tb, Gd, AS, EU, AF, NA
Tricholoma album (Schaeff.: Fr.) Quel.	pine and *Quercus* spp. forests	P, M	Quercus spp.	Fm, Rn, Jl, Sh, JA, R, EU, NA

Table 1. (Continued) Ectomycorrhizal basidiomycete fungi associated with forest trees in Guizhou Province showing their habitat, utilisation, associated trees and geographic range.

Species	Habitat	Use	Host tree	Range
Tricholoma equestre (L.: Fr.) Quel.,	pine forests		*Pinus* spp.	Gu, An, Zu, Xi, Sh, We, Hl, Jl, Li, Js, Hn, Sh, Yn, Qh, JA, EU, NA
Tricholoma imbricatum (Fr.) Quel.	forests		Pinus yunnensis	Xi, Jl, Hn, Yn, Tb, EU, NA
Tricholoma matsutake (S. Ito et Imai) Sing.	forests	E, M	Pinus yunnensis	Xn, Px, He, Jl, Li, Ah, Tw, Sh, Yn, Tb, JA, K
Tricholoma pessundatum (Fr.) Quel.	forests		Pinus massoniana	Gu, Zu, Xn, Jl, Sd, He, Hn, Gd, Sh, Yn, EU, AS, NA
Tricholoma portentosum (Fr.) Quel.	mixed forests		Pinus massoniana	Ky, Li, Jl, JA, EU, NA
Tricholoma sulphureum (Bull.: Fr.) Kumm.	pine forests		Pinus massoniana	Ky, EU
Tricholoma terreum (Schaeff.: Fr.) Quel.	pine forests		Pinus massoniana, P. yunnensis	Gu, Xi, Rn, Dj, Ds, An, Zh, Hl, Jl, Li, Hb, Js, Hn, Gd, Yn, Tb, Qs, JA, R, EU, NA
Agaricales				
Entolomataceae				
Entoloma clypeatum (L.: Fr.) Quel.	orchards	M	**Malus, *Prunus* spp.	Zh, Hj, Hl, Jl, Hb, Hn, Tb, JA, R, EU, NA
Entoloma rhodopolium (Fr.) Quel.	forests	P	*Pinus* spp.	An, Fm, Jl, Fu, Yn, JA, Rn, AS, EU, NA
Entoloma sinuatum (Bull.: r.) Quel.	mixed forests	P	*Pinus* spp.	An, Hl, Jl, Hb, Js, Ah, Fu, Hj, Tw, Hn, Yn, JA, EU, NA
Cortinariaceae				
Inocybe asterospora Quel.	forests	P	Pinus massoniana, *Sassafras tzumu	Gu, An, Tu, Xi, Bj, Hl, Jl, Li, Sa, Zj, Hn, Sh, JA, R, EU, AS, NA
Inocybe caesariata Fr.	forests		*Pinus massoniana, Quercus* spp.	Fm, Jl, Hb, Hn, Yn, Xj, EU, NA
Inocybe fastigiata (Schaeff.: Fr.) Quel.	mixed forests	P	*Quercus, Salix* spp.	Gu, An, Zh, Zh, Ky, Xi, Tn, Sq, Hl, Jl, Li, Hb, Im, Fu, Yn, Gs, JA, R, EU, SA, NA
Inocybe praetervisa Quel.	mixed forests	P	*Pinus* spp.	Zh, Hl, Jl, Fu, Gd, AS, EU, NA
Inocybe radiata PK.	mixed forests	P	*Pinus, Betula* spp.	An, Zh, Hb, Js, Hn, Sh, Yn, Xi, EU
Inocybe rimosa (Bull.: Fr.) Quel.	mixed forests	P	*Pinus, Betula* spp.	Gu, Bj, Qg, Hl, Jl, Li, Hb, Sa, Js, Hn, Yn, Tb, Qh, Xj, JA, EU, NA
Inocybe umbrinella Bres.	forests	P	*Pinus* spp.	Gu, An, Qg, Hl, Jl, Hb, Gd, Sh, Yn, Tb, JA, R, EU, NA
Cortinarius multiformis Fr.	pine and mixed forests	E	*Pinus, Betula* spp.	Gu, Lp, Hl, Jl, Li, Sa, Tw, Hn, Sh, Tb, JA, R, EU, NA
Cortinarius purpurascens (Fr.) Fr.	mixed or broad-leaf forests	E	Pinus massoniana	Xg, Hp, Lp, Hl, Jl, Hn, Sh, Tb, JA, R, EU, NA
Rozites caperata (Pers.: Fr.) Karst.	mixed forests		?	Tu, Hl, Jl, Li, Js, Yn, Qh, JA, R, EU, NA
Boletales				
Paxillaceae				
Phylloporus rhodoxantus (Schw.) Bres.	mixed forests	E	*Pinus massoniana, Quercus* spp.	Gu, An, Xi, Bj, Jl, Js, Zj, Ah, Fu, Hn, Gd, Ha, Gg, Sh, Yn, Tb, AS, A, NA
Gomphidiaceae				

Table 1. (Continued) Ectomycorrhizal basidiomycete fungi associated with forest trees in Guizhou Province showing their habitat, utilisation, associated trees and geographic range.

Species	Habitat	Use	Host tree	Range
Gomphidius roseus (Fr.) Gill.	pine or mixed forests		*Pinus* spp.	Gu, An, Zh, Rn, Jl, Li, Hn, Sh, Yn, Tb, JA, EU
Strobilomycetaceae				
Strobilomyces floccopus (Vahl: Fr.) Karst.	mixed forests	M	*Pinus* spp.	Gu, Rn, An, Zh, Sh, Wo, Tz, Tn, Hl, Jl, Js, Zj, Ah, Fu, He, Hn, Gd, Ha, Gg, Sh, Yn, Tb, JA, R, EU, NA
Strobilomyces glabriceps Chiu	pine forests		Pinus yunnensis	Xi, Xg, Yn
Boletellus ananas (Curt.) Murr.	pine forests	P	Pinus massoniana	Tz, Zh, Fu, Gd, Ha, Gg, Sh, Yn, Tb, AS, NA
Boletaceae				
Boletinus kunmingensis Chiu	pine forests		*Pinus* spp.	Gu, An, Zh, Pn, Xi, Yn
Boletinus pictus Pk.	pine forests		*Pinus armandii*	Gu, Rn, An, Hl, Jl, Js, Zj, Ah, Hb, Hn, Gd, Ha, Sh, Yn, JA, EU, AS, NA
Boletinus pinetorum (Chiu) Teng	pine and mixed forests		*Pinus, Quercus* spp.	Gu, Rn, Gi, Hu, An, Zh, Xi, Pu, Px, Xg, Hj, Lp, Hj, Jl, Fu, Hn, Yn, EU, NA
Boletus aereus Bull.: Fr.	mixed forests		Pinus massoniana, P. yunnensis, Quercus fabri	Gu, An, Zh, Ky, Xi, Pn, Zu, Tu, Zh, Sh, Yn, EU
Boletus brunneissimus Chiu	forests		?	Gu, An, Xi, Zu, Sh, Yn
Boletus edulis Bull.: Fr.	forests	M	*Pinus, Quercus* spp.	Gu, An, Zh, Xi, Pn, Px, Ky, Zu, Tu, Zn, Hl, Jl, Li, Js, Ah, Fu, Tw, Gd, Tw, Ha, Yn, Tb, Xj, EU, NA
Boletus griseus Frost.	mixed forests		Pinus, Quercus	Xi, Gd, Ha, Gg, Sh, Yn, NA
Boletus instabilis Chiu	conifer forests		*Pinus* spp.	Gu, An, Sh, Yn
Boletus luridus Fr.	forests		*Quercus* spp.	Gu, Xi, Px, Gl, Sq, Fu, Gd, Sh, Yn, EU, NA
Boletus margificus Chiu	pine forests		Pinus yunnensis	Xi, Yn
Boletus regius Krombh.	*Quercus* forests	M	*Quercus* spp.	Gu, Xi, Pn, Sh, Yn, EU, NA
Boletus speciosus Frost	broad-leaf & mixed forests	M	Pinus yunnensis	Gu, Xi, Jp, Js, Zj, Gd, Ha, Sh, Yn, Tb, EU, NA
Boletus sylvestris Petch	mixed forests	E	*Quercus* spp.	Xg, Yn, AS
Boletus taianus Chiu	mixed forests	E	*Pinus, Quercus* spp.	Xi, Pn, Yn
Boletus tomentipes Earle	pine forests		Pinus massoniana	Ky, Yn, NA
Boletus umbrinus Pers.	mixed forests		Pinus yunnensis, Quercus acutissima	Xi, Pn, Sh, Yn, EU
Boletus violaceo-fuscus Chiu	forests		?	Gu, An, Xi, Tz, Gd, Yn
Leccinum aurantiacum (Bull. ex St. Amans) Gray	mixed forests		*Pinus, Quercus* spp	Xi, Hl, Jl, Li, Hb, Zj, Hn, Gd, Yn, Tb, JA, R, EU, NA
Leccinum crocipodium (Letellier) Watling	broad-leaf forests		*Pinus, Quercus* spp.	Xi, Zu, Wu, Js, Zj, Ah, Fu, Tw, Hn, Gd, Sh, Yn, AS, EU, NA
Leccinum nigrescens (Richon & Roze) Sing.	mixed forests		*Pinus, Quercus* spp	Gu, An, Zh, Xi Xg, Zu, Tu, Lp, Js, Sh, Yn, AS, AF, EU
Leccinum rubropunctum (Pk.) Sing.	*Quercus* forests		*Quercus* spp.	Px, Yn, NA
Leccinum rugisiceps (Pk.) Sing.	*Quercus* forests		Quercus fabri	Pn, Yn, Tb, EU, NA

Table 1. (Continued) Ectomycorrhizal basidiomycete fungi associated with forest trees in Guizhou Province showing their habitat, utilisation, associated trees and geographic range.

Species	Habitat	Use	Host tree	Range
Leccinum scabrum (Fr.) Gray	forests		*Pinus* spp., *Fagus lucida*	Tu, An, Zh, Xi, Hl, Jl, Hb, Js, Zj, Ah, Sh, Yn, Tb, Sa, Qh, R, EU, NA
Pulveroboletus ravenelii (Berk. et Curt.) Murr.	mixed forests	P	Pinus massoniana, P. armandii, Betula luminifera	Gu, Xu, An, Bj, Xi, Tz, Wo, Ky, Sq, Js, Ah, Fu, Gd, Ha, Gg, Sh, Yn, Tb, AS, EU, AF, NA, SA
Suillus bovinus (L.: Fr.) O. Kuntz.	conifer forests	M	*Pinus, Quercus* spp.	Gu, Rn, Pn, Xi, Tu, Li, Jl, Zj, Ah, Fu, Jx, Tw, Ha, Gg, Yn, Tb, JA, R, AF, EU, NA
Suillus flavus (With.) Sing.	pine forests		Pinus armandii	Gu, Xg, Xi, Pn, He, Sh, Yn, Sa, EU
Suillus granulatus (Fr.) Kuntz	pine or mixed forests	M	Pinus massoniana, P. armandii	Gu, Xi, Ar, Pn, Ky, Zu, Bj, Pt, Hl, Jl, Li, Hb, Js Zj, Ah, Sd, Hj, Hn, Tw, Gd, Gg, Yn, Tb, JA, R, EU, NA
Suillus luteus (Fr.) Gray	pine or mixed forests	M	Pinus massoniana, P. armandii	Gu, An, Ky, Xi, Bj, Zu, Ls, Hl, Jl, Li, Hb, Sa, Js, Hn, Sh, Yn, AS, EU, AS, NA
Suillus placidus (Bon.) Sing.	pine forests	P	*Pinus* spp.	An, Zh, Zh, Qg, Ky, Xf, Pb, Sh, Yn, EU, NA
Suillus subaureus (Pk.) Snell	mixed forests		Pinus armandii	Ky, Sh, Tb, EU, NA
Tylopilus alboater (Schw.) Murr.	*Quercus* forests		*Quercus* spp.	Gu, Zu, Jl, Ah, Fu, Gd, Gg, NA
Tylopilus eximius (Pk.) Sing.	forests		Pinus armandii	Cz, Sh, Yn, Tb, NA
Tylopilus felleus (Bull.: Fr.) Karst.	mixed forests	P	Pinus massoniana, P. armandii	Gu, Xi, Jl, Hb, Js, Ah, Fu, Gd, Ha, Sh, JA, EU, NA
Tylopilus plumbeovilaceus (Snell et Dick) Sing.	mixed forests		*Quercus acutissima, Q. allena, Populus davidiana*	Gu, Fu, Ha, Yn, AS, NA
Tylopilus sinicus (Chiu) Tai	pine forests		*Pinus* spp.	Gu, Xi, Rn, Yn
Xerocomus badius (Fr.) Gilb.	pine forests	P	*Quercus acutissima, Pinus yunnensis*	An, Pb, Qg, Xi, Xg, Pn, Jl, Hb, Js, Zj, Ha, Sh, Yn, Tb, JA, R, EU, NA.
Xerocomus chrysenteron (Fr.) Quel.	mixed forests		*Pinus, Quercus* spp.	An, Su, Hl, Jl, Li, Hb, Js, He, Gd, Ha, Sh, Yn, Tb, JA, R, EU, NA
Xerocomus spadiceus (Fr.) Quel.	pine forests		*Pinus yunnensis*	Xi, Sh, Yn, Tb, EU, NA
Xerocomus subtomentosus (L.: Fr.) Quel.	mixed forests		*Pinus, Quercus* spp.	Xi, An, Hl, Jl, Li, Js, Zj, Ah, Fu, Tw, Hn, Gd, Ha, Sh, Yn, Tb, Xj, JA, R, EU, NA
Xerocomus yunnenensis (Chiu) Tai	mixed forests		*Quercus* spp.	Xi, Pn, Yn
Russulales				
Russulaceae				
Lactarius camphoratus (Bull.) Fr.	*Quercus* forests	E	*Quercus* spp.	Gu, An, Xi, Mo, Px, Ch, Ds, Rn, Pt, Jl, Js, Gd, Gg, Sh, Yn, R, JA, EU, NA
Lactarius deliciosus (L. Fr.) Gray	forests	M	Pinus massoniana, P. armandii	Gu, An, Zh, Pn, Ky, Qg, Pb, Xf, Xu, Xi, Xg, Zu, Tu, Ch, Xs, To, Qh, Bj, We, Tn, Yp, St, Ka, Rj, Wo, Lc, Jh, Tj, Gi, Rn, Jl, Li, Hb, Js, Ah, Zj, Tw, He, Gd, Sh, Tb, Gs, Qh, JA, R, EU, NA
Lactarius fuliginosus Fr.	forests		*Pinus, Quercus* spp.	Gu, An, Xi, Bj, Ja, Zu, Ch, Hl, Jl, Li, Ah, Sh, Yn, R, EU
Lactarius hatsudake Tanaka	pine & mixed forests	M	*Pinus massoniana, P. yunnensis, Quercus* spp.	Gu, An, Xi, Zh, Qg, Ky, Pd, Gl, Dj, Ds, Hl, Jl, Li, Hb, Fu, Tw, Hj, Gd, JA

Table 1. (Continued) Ectomycorrhizal basidiomycete fungi associated with forest trees in Guizhou Province showing their habitat, utilisation, associated trees and geographic range.

Species	Habitat	Use	Host tree	Range
Lactarius hygrophoroides Berk. et Curt.	forests		*Pinus yunnensis, Fagus lucida*	Gu, Xi, Px, Zu, Tu, Tn, Yp, Jl, Li, Hb, Js, Ah, He, Jx, Sh, Yn, Gg, Tb, Sa, EU
Lactarius insulsus Fr.	forests		*Quercus* spp.	Xi, Xg, An, Gu, Zu, Tu, Qg, Ky, Xf, Bj, Tn, Jl, Li, Hb, Js, Ah, He, Yn, Sh, Sa, Sa, R, EU, NA
Lactarius mitissimus Fr.	forests		Pinus massoniana	Gu, An, Qg, Ky, Xu, Xf, Rn, Pt, Jl, EU
Lactarius obliquus Fr.	pine forests		*Pinus massoniana, P yunnensis*	Gu, An, Xi, Pn, EU
Lactarius pallidus (Pers. : Fr.) Fr.	pine forests	E	*Pinus yunnensis*	Bj, Hb, He, Yn, Tb, Sa, EU
Lactarius poperatus (L.: Fr.) Gary	forests	E	*Pinus yunnensis, Quercus* spp.	Gu, An, Qg, Ky, Pb, Zh, Zh, Xi, Xg, Tz, Hl, Jl, Li, Im, Hb, Js, Ah, Zj, Fu, Tw, Hn, Sh, Yn, Tb, Sa, R, JA, EU, NA
Lactarius scrobiculatus (Scop.: Fr.) Fr.	forests	P	*Pinus* spp.	Xi, An, Hl, Jl, Sh, Qh, R, JA, EU, NA
Lactarius subdulcis (Pers.: Fr.) Gray	forests	E	*Pinus, Quercus* spp.	An, Jl, Hb, Js, Ah, Zj, Fu, Hn, Gg, Gs, R, EU, NA
Lactarius torminosus (Fr.) Gray	mixed forests	P	Betula luminifera	Xi, Hl, Jl, Li, Hb, Sa, Fu, Gd, Gg, Sh, Yn, Tb, JA, R, EU, NA
Lactarius vellereus (Fr.) Fr.	mixed & broad-leaf forests	P	*Pinus, Quercus* spp.	Gu, An, Qg, Ky, Xu, Bj, Js, Xi, Hl, Jl, Li, Hj, Ah, Fu, Hn, Yn, Tb, Sa, JA, R, EU, NA
Lactarius volemus (Fr.) Fr.	mixed forests	E	*Pinus, Quercus* spp.	Gu, An, Qg, Pb, Xy, Xg, Zf, Bj, Qn, Ja, Sh, Zu, Tu, Hl, Mt, Tn, Ka, Rj, Ls, Dj, Pt, Gi, Rn, Hj, Jl, Js, Zj, Fu, Ah, Gd, Ha, Gg, Sh, Yn, Tb, R, EU, NA
Russula adusta (Pers.) Fr.	pine forests	M	*Pinus yunnensis, P. massoniana*	Gu, An, Xi, Ar, Ky, Zu, Ti, Hb, Jl, Js, Jx, Hn, Gd, Gg, Ha, Sh, Yn, Tb, AS, EU, NA
Russula alutacea (Pers.) Fr.	mixed forests	M	*Pinus yunnensis, P. massoniana, Quercus* spp.	Gu, An, Gg, Sh, Bj, Zu, Tn, Ka, Lp, Dj, Hl, Jl, Li, Hb, Sa, Gs, Js Ah, Fu, He, Gd, Ha, Yn, Tb, R, EU, NA
Russula amoena Quel.	mixed forests		*Pinus massoniana, Quercus* spp.	Fm, Jl, Hn, JA, EU
Russula atropurpurea (Krombh.) Britz.	pine forests		Pinus massoniana	Gu, Li, Hb, Sa, Sh, Yn, Tb, JA, EU, NA
Russula aurata (With) Fr.	forests		Quercus acutisissima	Gu, Zi, An, Xi, Hp, Tz, Hl, Jl, Ah, He, Sh, JA, R, EU
Russula crustosa Pk.	mixed forests		*Pinus yunnensis, P. massoniana, P. armandii, P. Taeda, Quercus myrsinaefolia*	Gu, An, Xi, Ar, Zh, Zh, Pb, Rn, Gi, Pt, Ky, Zu, Mt, Ja, Jl, Hb, Sa, Js, Ah, Fu, Gd, Gg, Yn, Sh, EU, NA
Russula cyanoxantha (Schaeff.) Fr.	mixed forests		*Quercus fabri, Fagus lucida, Pinus massoniana*	Gu, An, Pu, Xi, Bj, Tu, Rn, Lb, Yp, Jl, Li, Js, Ah, Fu, Gd, Gg, Ha, Sh, Yn, Tb, Sa, Qh, Xj, AS, EU, NA
Russula cyanoxantha (Schaeff. ex Schw.) Fr.	broad-leaf & mixed forests		Quercus glauca	Tu, Gd, Ha, Fu, EU
Russula decolorans Fr.	mixed forests		*Fagus lucida, *Camellia oleifera*	Tu, Jl, Hb, Js, Tb, NA

Table 1. (Continued) Ectomycorrhizal basidiomycete fungi associated with forest trees in Guizhou Province showing their habitat, utilisation, associated trees and geographic range.

Species	Habitat	Use	Host tree	Range
Russula delica Fr.	conifer, broad-leaf & mixed forests		*Pinus massoniana, P. yunnensis, P. elliotii, *Camellia oleifera, Betula luminifera*	Gu, An, Xi, Sh, Bj, Zu, Tu, Yw, Tn, Sb, Ka, Pt, Rn, Qg, Hl, Jl, Li, Hb, Js, Zj, Ah, Hn, Gd, Gg, Sh, Yn, Tb, Gs, Xj, AS, EU, NA
Russula densifolia (Secr.) Gill.	pine & mixed forests		*Pinus* and *Quercus* spp.	Gu, An, Bj, Zh, Xi, Tn, Dj, Jl, Hb, Js, Fu, Jx, Ah, Hn, Gd, Gg, Ha, Sh, Tb, R, JA, EU, NA
Russula emetica (Schaeff.: Fr.) Pers. ex Gray	conifer, broad-leaf & mixed forests		*Pinus, Quercus* spp.	Gu, An, Xi, Zu, Tu, Tn, Ka, Dj, Pt, Hl, Jl, Li, Js, Ah, Fu, Hn, Sh, Yn, R, JA, EU, A, NA
Russula foetens (Pers.: Fr.) Fr.	forests	P	*Pinus massoniana, P. armandii, Betula luminifora*	Gz, Hl, Jl, Li, Hb, Sa, Js, Ah, Zj, Fu, Gd, Ha, Hn, Sh, Yn, Tb, AS, EU, NA
Russula fragilis (Pers.) Fr.	forests	P	*Pinus, Quercus* spp.	Gu, An, Xi, Sh, Fm, Rn, Hl, Jl, Li, Js, Zj, Fu, Ah, Hj, Hn, Gd, Tw, Yn, Tb, R, JA, EU, AS, NA.
Russula furcata (Pers.) Fr.	forests	E	*Quercus* spp.	Gu, Zh, Jl, Js, Fu, He, Gd, Sh, Yn, JA, EU, NA
Russula integra (L.) Fr.	forests	E	*Pinus* spp.	Gu, An, Xi, Bj, Tn, Rn, Pt, Jl, Li, Hb, Sa, Js, Fu, He, Gd, Sh, Yn, Sa, Xj, JA, EU, NA
Russula lepida Fr.	forests	E	*Quercus fabri, Q. glauca, Pinus armandii.*	Gu, An, Xi, Bj, Ja, Zu, Tu, Sq, Tn, Kr, Pt, Ar, He, Jl, Li, Js, Fu, Hn, Gd, Gg, Sh, Yn, Tb, R, JA, EU, NA
Russula lilacea Quel.	mixed forests	E	*Pinus massoniana, Euonymus** sp.	Gu, Tu, Fu, Gg, Ha, Yn, EU
Russula lutea Fr.	*Quercus* & pine forests	E	*Fagus lucida, Pinus massoniana*	Gu, Tu, Ka, Jl, Js, Ah, Sh, Yn, EU, NA
Russula nigricans (Bull.) Fr.	forests	P	*Quercus* spp.	Gu, An, Zh, Xi, Zu, HEU, Jl, Js, Ah, Fu, Hb, Hn, Gd, Ha, Gg, Yn, Sa, R, JA, EU, NA
Russula ochroleuca (Pers.) Fr.	forests	E	Betula luminifera	An, Tn, Tz, Hl, Jl, Js, Ah, Fu, Hn, Gd, Ha, Sh, Yn, Tb, Gs, JA, EU, NA
Russula pseudoaura Chiu	pine forests		*Pinus massoniana, P. yunnensis*	Xi, Zh, Yn
Russula pulchella Borsz.	broad-leaf forests	E	Betula luminifera	Gu, An, Jl, Js, Yn, Tb, EU, NA
Russula senecis Imai	broad-leaf & mixed forests	P	*Pinus massoniana, Alnus nepalensis*	Gu, Xi, Ar, An, Wo, Ky, Jx, Gd, Gg, Sh, JA
Russula subdepallens Pk.	mixed forests	E	*Pinus* spp., *Fagus lucida*	Gu, Zh, Pn, Xi, Tu, Hl, Jl, Fu, Sh, Yn, Tb, NA
Russula vesca Fr.	broad-leaf & mixed forests	E	Pinus massoniana	Gu, An, Zh, Xi, Pn, Fm, Js, Fu, Gd, Ha, Gg, Yn, Tb, AS, EU, NA
Russula vinosa Lindbl.,	broad-leaf forests	E	Fagus lucida	Tu, Fu, Gd, Ha, EU
Russula virescens (Schaeff.) Fr.	pine & mixed forests	E	*Pinus, Quercus* spp.	Gu, An, Zh, Xi, Xg, Pn, Bj, Sh, Zu, Tn, Ka, Sb, Lp, Dj, Rn, Hl, Jl, Li, Js, Fu, Hj, Gd, Ha, Gg, Sh, Yn, Tb, R, JA, EU, NA
Russula viridi-rubrolimbata Ying,	pine forests		*Pinus* spp.	Gu, Ky, An, Yn, Gg
Russula xerampoelina (Schaeff.) Fr.	mixed forests	E	*Pinus, Quercus, Betula* spp.	Gu, Zh, Xi, Xg, Bj, Hl, Jl, Li, Js, Hj, Gd, Sh, Yn, Xj, JA, EU, NA

Table 1. (Continued) Ectomycorrhizal basidiomycete fungi associated with forest trees in Guizhou Province showing their habitat, utilisation, associated trees and geographic range.

Species	Habitat	Use	Host tree	Range
GASTEROMYCETES				
Sclerodermatales				
Sclerodermataceae				
Scleroderma bovista Fr.	forests		*Pinus, Quercus* spp.	Gu, An, Xi, Rj, Dj, Hl, Li, Jl, Hb, Sd, Js, Hb, Hn, Gd, Ha, Gg, Sh, Yn, Tb, Sa, Gs, I, NZ, AS, AF, EU, NA, SA
Scleroderma cepa Pers.	mixed forests	E	*Pinus, Quercus* spp.	Wg, Js, Zj, Fu, He, Hb, Hn, Gd, Gg, Sh, Yn, JA, T, I, EU, AF, NZ, AS, NA
Scleroderma citrinum Pers.	mixed forests		*Pinus massoniana, P. yunnensis, Quercus* spp.	Rn, Hu, Zh, Xi, Fu, Hn, Gd, Ha, Gg, He
Scleroderma polyrhizum Pers.	forests	E	*Pinus massoniana, P. yunnensis*	Gz, Js, Zj, Jx, Fu, Hn, Gd, Ha, Gg, Sh, Yn, Tw, JA, AF, EU, AS, NA
Pisolithus tinctorius (Mich.: Pers.) Cke ex Couch	forests & disturbed habitats	E	coniferous & deciduous trees	Hu, Su, Ld, Xi, Li, Js, Zj, Ah, Fu, Tw, He, Hb, Hn, Gd, Ha, Sh, Ha, Gg, Sh, Yn, Tb, JA, T, AS, NZ, AF, EU, NA

Abbreviations for uses: E = edible, P = poisonous, M = Medicinal use. * = May not be ectomycorrhizal

Abbreviations used for provinces: An = Anshun, Ah = Anhui, Ar = Anrong, Bj = Bijie, Ch = Chishui, Cz = Chizhang, Dj = Dujun, Ds = Dushang, Fm = Fanjing Mountain, Fu = Fujian, Gd = Guangdong, Gi = Guiding, Gg = Guangxi, Gl = Guanglin, Gs = Guangshun, Gu = Guiyang, Gz = Guizhou, Ha = Hainan, Hb = Hubei, He = Henan, Hj = Huanjingshan, Hl = Helongjang, Hn = Hunan, Hp = Huangpin, Hu = Huishui, Im = Inner Mongolia, Ja = Jinsha, Jh = Jianghe, Ji = Jinlin, Jl = Jilin, Jp = Jingping, Js = Jiangsu, Jx = Jiangxi, Ka = Kaili, Ky = Kaiyang, Kr = kairen, Lb = Libo, Lc = Leichu, Ld = Loudian, Li = Liaoning, Lp = Liping, Ls = Leishang, Mo = Mowang, Mt = Meitang, Pb = Pinba, Pd = Puding, Pn = Puan, Pt = Pingtang, Pu = Puning, Px = Panxiang, Qg = Qingzheng, Qh = Qinghai, Qn = Qiangxi, Qs = Qabgsu, Qu = Quangxi, Rj = Rongjiang, Rn = Rongli, Sa = Shaanxi, Sb = Shibing, Sc = Sichuan, Sd = Shangdong, Sh = Shuichueng, Sj = Shongjie, Sq = Shiqian, St = Shongtao, Su = Shangdu, Tb = Tibet, Tj = Tujunpingtang, Tn = Tongreng, To = Tongzhi, Tu = Tuoyang, Tw = Taiwan, Tz = Tiangzhu, Wg = Wangmiu, Ww = Weining, Wu = Wuchuan, Xf = Xifeng, Xg = Xingreng, Xi = Xingyi, Xj = Xingjiang, Xn = Xingning, Xs = Xishui, Xu = Xiouwen, Xy = Xiaoyi, Yn = Yunnan, Yp = Yuping (Yuqing), Yw = Yuwqing, Zf = Zhenfang, Zh = Zhenning, Zi = Zhijin, Zj = Zheijiang, Zn = Zhengan, Zu = Zhunyi

Abbreviations used for countries: AU = Australia, AS = Asia, AF = Africa, EU = Europe, I = India, JA = Japan, K = Korea, NA = North America, NZ = New Zealand, R = Russia, SA = South America, T = Thailand

Conclusions

Some issues concerning the research required to harness the potential of ectomycorrhizal fungi in China are discussed below.

The selection of superior fungus isolates for forestry

China has a high diversity of fungal resources. So far, there have been more than 500 species of ectomycorrhizal fungi recorded. Effects of ectomycorrhizas on the growth of trees vary greatly with tree species, environmental conditions, the distribution and growth characteristics of the fungi concerned. For example, *Russula delica, Laccaria laccata, Scleroderma polyrhizum, Suillus luteus* and other species of fungi widely distributed in China, and thus apparently are highly adaptable, can form ectomycorrhiza with more than one tree species. However, there are other species, such as *Pisolithus tinctorius, Cantharellus purpureus,* which exhibit habitat preferences and are associated with particular tree species. For example, *Pisolithus tinctorius* occurs south to the Yellow River and *Cantharellus purpureus* is only found in Guizhou Province. Consequently, the selection of fungal species should take into account *in situ* conditions, tree species, ectomycorrhizal distribution, and symbiotic characteristics, in order to select the most suitable fungi for a particular situation.

Publicising information about ectomycorrhizal associations

People in the forest industry know about mycorrhizal associations, but have a limited understanding of their importance. In order to promote and spread mycorrhizal technology, the first step is to ensure that mycorrhizal infomation is widely disseminated. As there is inadequate information on mycorrhizal roots, it is recommended that the Ministry of Forestry organise relevant scientists to compile a publication on ectomycorrhizal fungi associated with forest trees in China. It is also recommended that mycorrhizal technology is adopted as a measure for reforestation and the improvement of low-production forests. In the meantime, mycorrhizal research should be continued to increase scientific understanding and investigate practical applications.

The preservation of fungal isolates

The preservation of fungal isolates in culture is one method to conserve genetic fungal resources. The biodiversity of natural ecosystems is declining due to the destruction of forests from logging and industrial pollution. For example, in the 1950s, 25.7% of the land on Hainan Island was covered with forests, but the forest coverage has now decreased to 7.2%; 55% of Xishuangbanna was covered with primitive forests in the 1950s, but only 28% of them remain. A similar trend has occurred in other provinces. The forest destruction rate in China greatly exceeds the world average rate of 1% per year.

Some fungal species can be preserved in natural conservation areas. However, in other areas where the natural ecosystems have been destroyed, tree-associated mycorrhizal fungi will be lost. For this very reason, it is recommended that a centre of mycorrhizal resource preservation be established, to provide mycorrhizal resources for future reforestation, scientific research, education and international exchange.

The culture of edible mycorrhizal fungi

It is important to improve the economic efficiency of ectomycorrhizal applications. Among the 159 species of ectomycorrhiza reported in this paper, 119 species are edible and 50 species have medical uses (Table 1). Culture of edible ectomycorrhizal fungi, especially some rare delicacy species, has greatly increased. In Italy and France, *Tuber* spp. have been successfully cultured and commercially grown. They have also been introduced into the USA, Japan, New Zealand and other countries. *Tricholoma matsutake, Lyophyllum decastes* and *Rhizopogon rubescens* have been experimentally grown in China and Japan. The method of edible ectomycorrhizal culture is to inoculate tree seedlings with the selected fungal isolates at suitable sites, with ectomycorrhizal spores or hyphae. The culture of edible ectomycorrhizal fungi can be developed as a economic tool alongside reforestation programs. Some rare species of edible ectomycorrhizal fungi, such as *Tuber magnatum* Pico and *T. melanosporum* Vitt., are very expensive and cost as much as US$1000/kg in 1990. Forests growing on calcareous soil in the subtropical area of southwestern China may be suitable for the growth of *Tuber* spp. A domestic culture base may be established in the above area to cultivate *Tuber* spp. and other species, in addition to the investigation of local fungal resources. The culture of edible ectomycorrhizal fungi is important to improve the overall economic benefit from mycorrhizal technology.

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Taxonomy, Morphology and Ecology of *Russula lepida* in the Pubei Region of Guangxi Province, China

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Abstract

Russula lepida is an ectomycorrhizal fungus that is abundant in the *Castanea hystrix* woodland in the Pubei region of Guangxi Province in China. This edible fungus is very important to the local forest fungus industry. In this study, the morphology of this fungus is described and results of an investigation on its ecology are reported, to provide information for the eventual artificial cultivation of this important mushroom.

Key words: *Russula lepida*, ecology, morphology, ectomycorrhizal fungi, edible fungus

THERE is a great area, consisting of more than 1.3 million ha of *Castanea hystrix* dominated woodland distributed over Pubei County of Guangxi Province, which is rich in several species of wild edible red mushrooms, called Hongzhui Jun by the locals. One particular red mushroom, which is the dominant fruiting species of commercial value, produces 6–10 tonnes a year during a relatively short mushroom season. This fungus, which has now been identified as *Russula lepida*, is considered to be an ectomycorrhizal fungus, as are all members of its genus (Singer 1986). This species, which belongs to the subdivision Basidiomycotina, order Agaricales, family Russulaceae, genus *Russula*, is classified as *R. lepida* (Fr.) Fries. It is reported that this edible mycorrhizal fungus forms ectomycorrhizal associations mainly with the roots of *C. hystrix* in the family Fagaceae (Ying Jian Xin 1984; Shang Guan Zhou Jian 1994).

Pubei County is located in Guangxi Province in the south of China (109° 31'–109° 51'E, 21° 51'– 22° 41' N, 60 km north of Beibu Bay). This region is on the west side of the Liuwan Mountain, with northeast to southwest running ridges with generally increasing topography from south to north. The Wuhuang Range is in the middle of this hilly region and is composed mainly of small hills 200–500 m high with 20–35° slopes.

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This report summarises knowledge on *R. lepida* which was gathered to help ensure continued benefits from this biological resource. These investigations, which began in 1989, include examinations of the symbiotic association of this fungus and field observations of its morphology and ecology in Pubei County. A series of research experiments concerning the isolation, pure culture cultivation and mycelial habitat requirements of this fungus, were also initiated, but will be reported elsewhere.

Morphologic Characteristics

The pileus of *R. lepida* from Pubei is 4–12 cm wide, broadly convex with a sunken centre, red to dark-red, and is not sticky. Its surface tends to be inflexible and often becomes cracked. The flesh is thick and white and often has signs of feeding by small creatures. The attached, closely spaced gills are equally spaced but vary in length, and are white in youth, but become greyish when ageing, and develop a reddish tint toward the margin of the cap when older. The stalk is 3–6 cm long by 0.5–1.5 cm in diameter, and is even from top to bottom with a pinkish surface. The stalk is solid, becoming stuffed with age. The spore print is whitish. The spores are colourless when viewed in water under a microscope, 7–8 × 6–7 μm, ornamented with puctations and net-like reticulations. The basidium is typically club-shaped, 45–60 × 11–14 μm, bearing 4 spores each. The cystidia are club-shaped, 90–110 × 8–12 μm in size, and are abundant.

Russula lepida produces two types of mycelium in the soil, narrow filaments and fan-like growths of hyphae. The hyphae are white and velvety. Isolated mycelium grown in pure culture, produced reddish secretions and had two kinds of growing patterns, aerial hyphae and hyphae on the surface of the media.

During the mushroom season, *R. lepida* mycelia twist together to form small dull brown primordia about 1 cm below the soil surface. Primordia usually occur in pairs or in groups. After emerging from the earth, the mushroom cap expands, gradually turning red. After being picked from the soil, the cap stops expanding, even if kept moist.

The Ecological Environment

Habitat characteristics

We observed *Russula lepida* to be widespread in Pubei County. However, commercial harvesting is greatest in a hilly, low elevation zone around Wuhuang in central Pubei. This fungus was especially abundant at Maojia of Longmen village and Qianling of Zhanghuang village which are adjacent to the southeast of the Wuhuang Range.

Russula lepida often occurred in hilly areas at a low elevation of about 120–300 m with a slope of 20–38°. Generally speaking, it was found in habitats with a shady—sunny exposure, half way up the hill where it is unlikely to be waterlogged by rain, but will keep moist when it is dry. There is little or no *R. lepida* production on hill ridges, in gullies, or on hillsides entirely shaded from the sun.

Associated vegetation

Fruiting of *R. lepida* often occurs near wood or rotten stumps of *Castanea hystrix*, that were 1.0–1.5 m in height. Some fruiting bodies also occur near other species of trees, such as *Pimela*. Most *R. lepida* fruiting occurs in undegraded woodland habitats where there are few weeds.

Pubei County is covered with southern subtropical monsoonal vegetation. These conditions favour *C. hystrix*. However, over-lumbering in this area has produced mixed forests with a simplified composition and a canopy cover of 60–90%. Vegetation in these woodlands can be divided into tree, shrub and herbaceous plant strata. The tree canopy is uniform and dominated by *C. hystrix* (55% of trees). Other trees, listed in order of importance, are *C. carlesic*, *Styrak suberifolia*, *Schima crenata*, *Schfflera octophylla*, *Canarium pimela* and *Meliosma fordic*. The shrub level, although greatly shaded by the trees, is vigorous and uniformly distributed, with 75% cover. This layer is composed of large plants (1.0–2.5 m), most of which are shrubs that can tolerate the humid shady environment, and saplings of upperstorey trees. *Ardisia quinquegnais* is the dominant species, *C. hystrix* saplings are also common and *Psychotria ruba* is present. The herbaceous plant level is 20–30 cm in height and has an average cover of 20% that is sparsely distributed with some species of grasses and pteridophytes, such as *Lophatherum gracile*, *Adiantum flabellulatum*, *Scirpus grossus*, *Microstogium biforme* and *Dicranopteris linearis*.

Climatic factors

Lying south of the Tropic of Cancer, Pubei County has a southern subtropical monsoonal climate with an average annual temperature of 21.5°C, with extreme temperatures of 38.6°C and –1.9°C and the highest average monthly temperature in July (28°C). This district witnesses lots of typhoons and abundant rainfall during summer and autumn. The average annual precipitation is 1763 mm, much of which falls in the period between April to September, with a peak of 270–380 mm per month between June and August. Evaporation surpasses precipitation except between April and May. The climate in Pubei has marked seasonal differences in humidity, and dry periods in spring and autumn are likely. Typically fructification of *R. lepida* in this region peaks during stiflingly hot showery weather (April–August and especially May–July).

Soil composition

The soil of Pubei belongs to the south subtropical monsoon laterite zone and is a reddish soil rich in iron and aluminium. Soil sampled from spots where there is abundant fructification of *R. lepida* has a relatively loose texture and has the characteristics shown in Table 1. These soils often have up to 40% gravel, an acidic pH and an average of 2.8% organic matter content in the topsoil.

Table 1. Typical properties of soil from locations where *Russula lepida* fruits abundantly.

Property	Value
Organic matter (%)	3.7
Organic carbon (%)	2.2
C/N ratio	13:1
Rapidly soluble P (μg/g)	1.9
Rapidly soluble K (μg/g)	136.4
Exchangeable Ca (μg/g)	8.6
Exchangeable Mg (μg/g)	2.1
Available Cu (μg/g)	0.7
Available Zn (μg/g)	5.2

Conclusions

Although *Russula lepida* is known to fruit throughout Pubei County, only the hilly area of the Wuhuang supports a commercially viable industry based on this fungus. Evidently, many factors, such as topography, vegetation, climate and soil composition, could influence the productivity of this mycorrhizal fungus. By describing the morphological characteristics and investigating the ecological environment of *R. lepida*, we have laid a foundation for the promotion of production by this fungus by management of its habitat. Needless to say, the further study of the unique ecological properties of this mushroom, as well as the physiological and biochemical mechanism resulting in its fructification will be required as groundwork for its artificial cultivation.

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Mycorrhizal Associations in Disturbed and Natural Habitats in Tropical Australia

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Abstract

The diversity and distribution of Glomalean fungi forming vesicular-arbuscular mycorrhizal (VAM) associations were examined in undisturbed natural habitats (savanna woodland, hill, rainforest and wet sites) and disturbed mine site habitats in the vicinity of Kakadu National Park in the Northern Territory of Australia. Pot-culture isolation procedures were used to supplement spore occurrence data in this survey and provided much additional information about VAM fungi. Results showed that spores of VAM fungi were absent from locations in mine sites without vegetation, but were more abundant in other mine site habitats with dense vegetation cover than in undisturbed plant communities in the region. The diversity of Glomalean fungi was lower in disturbed sites than in natural habitats.

The capacity of propagules of VAM and ectomycorrhizal (ECM) fungi to form associations in soils from the same disturbed and undisturbed habitats were examined. Propagules of VAM fungi were detected in all sites, but were rare in disturbed areas with sparse plant cover, and occurred in exceptionally high numbers in soil from a rainforest site and some fully vegetated mine site habitats. Fungi capable of forming ECM associations with *Eucalyptus* seedlings were only absent from disturbed areas and wetland sites where suitable hosts did not occur. In disturbed habitats, the probability of seedlings encountering VAM or ECM inoculum increased with the cover of suitable hosts. Bioassay results suggest that the activity of mycorrhizal fungi can be substantially higher in patches of early-successional vegetation than in undisturbed habitats.

Key words: mycorrhizal fungus diversity, inoculum potential, disturbance, succession, tropical habitats

Two major types of mycorrhizal associations, vesicular-arbuscular mycorrhizas (VAM) and ectomycorrhizas (ECM) occur in the roots of the majority of plants in natural ecosystems in Australia and throughout the world (Brundrett 1991; Brundrett and Abbott 1991). The occurrence of these associations has been well documented in many plant communities, but there is little information about their role in plant nutrient uptake in nature, or how habitat changes resulting from disturbance affect this process. It has been shown that in some cases disturbance can eliminate mycorrhizal fungi from soils and their absence can affect the growth and survival of recolonising vegetation in a variety of habitats (Danielson 1985; Jasper et al. 1992; Pfleger et al. 1994).

Glomalean fungi are responsible for VAM associations and are obligate mutualists that can only be propagated in association with the roots of a growing host plant. However, knowledge of the diversity and biology of these ubiquitous and important soil fungi in Australia or globally is very limited. Indeed, there is a paucity of knowledge about all Australian fungi, with only about 10% of these organisms having been named, while an estimated 80% remain undiscovered (Pascoe 1991). There is little information about the importance of biodiversity in communities of soil organisms. However, we would expect the taxonomic diversity of mycorrhizal fungi present in soil to be correlated with their functional diversity, since different species are likely to have evolved different strategies to survive adverse soil conditions or provide nutrients to associated plants.

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Inoculum potential is defined as the energy for growth of an organism at the surface of its host, and is a consequence of the numbers of active propagules of that organism and their nutritional status (Garrett 1956). Bioassays using bait plants grown in intact soil cores provide a better estimate of mycorrhizal inoculum potential than assays using mixed soil or methods for counting propagules such as spores (Abbott and Robson 1991; Jasper et al. 1991; Brundrett and Abbott 1994). Propagules of VAM fungi are thought to include spores, dead root fragments and other colonised organic material, as well as networks of hyphae in soil (Brundrett 1991). Propagules of ECM fungi include networks of mycelial strands, old mycorrhizal roots, sclerotia and basidiospores (Skinner and Bowen 1974; Ba et al. 1991).

Major objectives of this research were to compare (1) the diversity of Glomalean (VAM) mycorrhizal fungi and (2) the capacity of VAM and ECM fungi to form associations (inoculum potential), in different habitats. These habitats included natural sites with different vegetation types and soil properties and disturbed mine sites located in or near Kakadu National Park in the Northern Territory of Australia.

This paper provides a summary of a series of mycorrhizal experiments. The results of these experiments will be presented in greater detail elsewhere.

Materials and Methods

During two surveys in the wet season in 1991 and 1992, soil samples and intact soil cores were collected from the same locations in 31 natural and disturbed habitats, with different types of soil and vegetation, in or near Kakadu National Park. Natural habitats included savanna woodlands, rocky hill woodlands, wet sites, disturbed areas and a rain forest habitat. Disturbed habitats included partially vegetated waste rock dump (WRD) material at Ranger, Nabarlek and Coronation Hill mine sites and woodland plots with different fire histories at the CSIRO Kapalga research station. Topsoil samples were collected to quantify VAM fungus spores and intact soil cores were taken for subsequent bioassays, along a transect at each site (see Fig. 1).

To identify Glomalean fungi, spores were separated from soil samples by wet-sieving and centrifugation and counted under a dissecting microscope by sorting them into visual categories. Additional fungi were identified when they sporulated in pot cultures started from soil, roots or seedlings grown in intact soil cores (Fig. 1). The mycorrhizal inoculum potential of soils was estimated by bioassays where seedlings were grown in 1 L intact soil cores (Fig. 1). Seedlings of clover (*Trifolium subterraneum*) were grown in soil cores for 4 weeks as bait plants for VAM fungi, along with *Eucalyptus* seedlings (*E. tetrodonta* or *E. miniata*) which were used as bait plants for ECM fungi. Cleared and stained roots of these plants were examined microscopically to measure the rate of mycorrhizal colonisation. This was used to provide an estimate of inoculum potential. Soil samples were also used for chemical and physical analysis. Methods used for spore separation from soil, clearing and staining of roots, the assessment of mycorrhizal colonisation and bioassays to measure mycorrhizal inoculum potential, are explained in greater detail elsewhere (Brundrett et al. 1994).

Results

Sixteen species of VAM fungi were identified by comparing microscopic features of spores separated from soil samples (Table 1). Seven additional fungi were found by using various methods to start pot cultures from Kakadu soils. Undisturbed sites contained between 5 and 13 species of VAM fungi, but disturbed sites had a much lower diversity (Table 1). It was noted that spores of VAM fungi were absent from mine site WRD soil samples taken from areas without vegetation, but occurred in exceptionally high numbers in samples from under early successional vegetation at these sites. Most VAM fungi were widespread, but several were restricted to wetland sites or disturbed habitats, suggesting that their distribution can be influenced by soil conditions (Table 1).

The chemical composition and texture of soils varied between sites, but only data on P levels is presented here. Available soil phosphorus was substantially higher in WRD sites than in undisturbed habitats (Fig. 2A).

Propagules of VAM fungi were detected in all sites. Bioassay results indicated that these fungi occurred in exceptionally high numbers in soil from a rainforest site and some vegetated mine site WRD areas, but were rare in other mine site WRD areas with sparse plant cover (Fig. 2B). Fungi capable of forming ECM associations with *Eucalyptus* seedlings were only absent from disturbed areas and wetland sites where suitable hosts did not occur (Fig. 2C). In WRD sites, ECM fungus inoculum was often associated with patches of *Acacia* spp. (which were found to have dual VAM/ECM associations when their roots were examined).

In disturbed habitats, the probability of seedlings encountering VAM or ECM inoculum was observed to increase with the cover of suitable hosts and had similar distribution patterns. Bioassay measurements of inoculum levels of VAM fungi were generally well correlated with numbers of spores in Kakadu soils, but there were some sites where high bioassay levels were associated with low spore numbers. The activity of ECM fungi (but not VAM fungi) appeared to be substantially curtailed by the hot annual fire regime in the CSIRO Kapalga trials (Fig. 2).

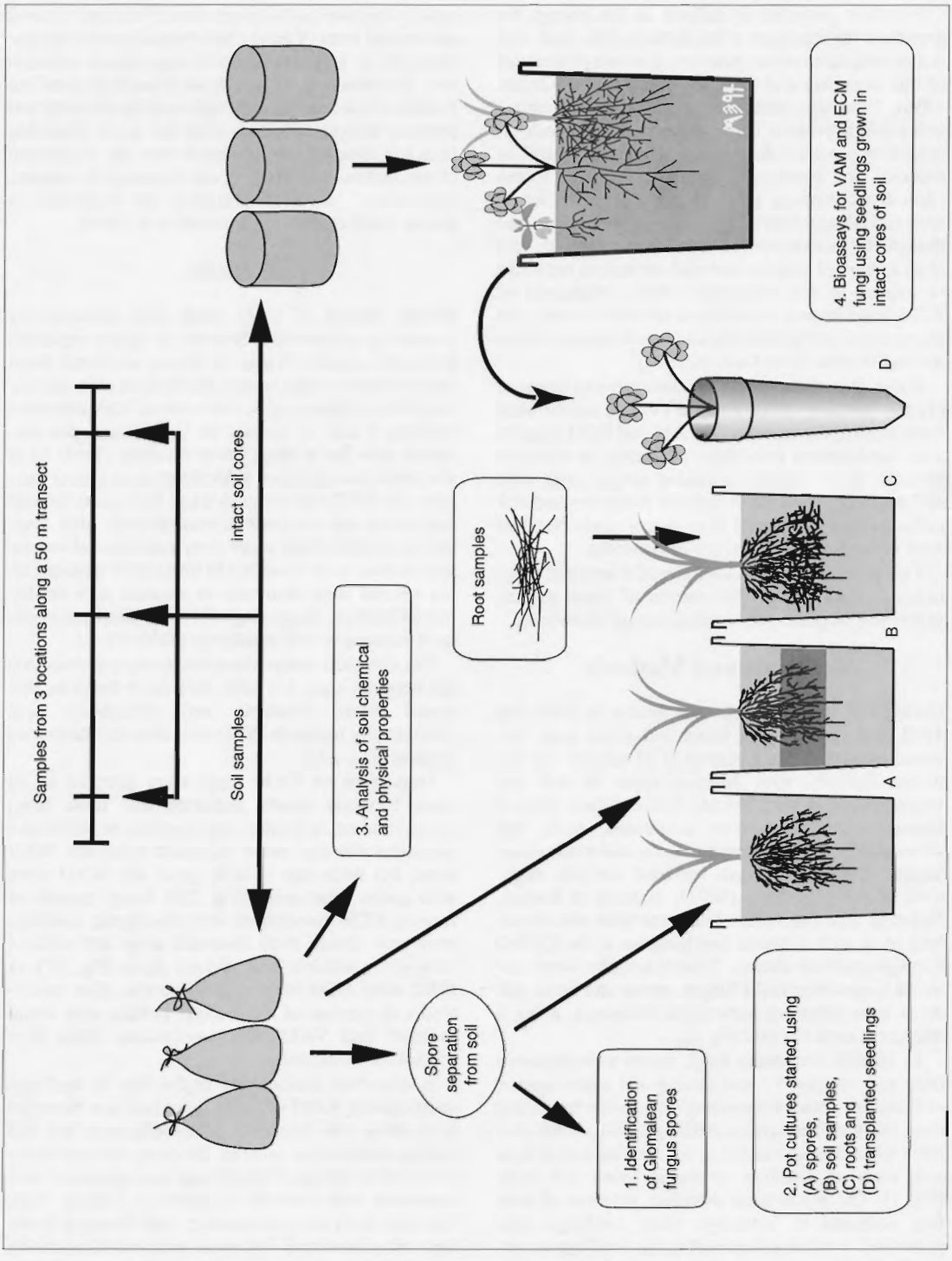

Figure 1. Summary of procedures used in the field collection of intact soil cores and soil samples, and methods used to identify Glomalean fungi and quantify mycorrhizal fungus propagules in soil from tropical Australian habitats.

Table 1. The diversity of VAM fungi present in ARR habitats, identified from spores separated from soil samples, or isolated in pot cultures using soil or plant roots.

Genus			Spore		Occurrence (number of sites)						
#	Size (μm)	Colour	Wall structure, etc.	Abundance[a]	Wetlands	Rainforest	Woodlands	Hills	Disturbed	Mine site	Total
Scutellospora			(Total sites)		(3)	(1)	(12)	(6)	(2)	(7)	(31)
1	300–600	black	reticulate	1–54	1	1	5	3		1	11
2	120–600	orange-brown	dull	1–132	2		12	6	2	4	26
3	150–500	white	dull	1–20	2	1	11	3	1	1	19
4	80–130	clear	irregular, thin	1–49	1		9	6	1	1	18
5	120–200	orange	smooth	3–20		1	8	2			11
6	120–200	grey-brown	smooth	1–260	2	1					3
7	150–500	clear	smooth, thick	rare	PC						1
8	150–400	yellow	smooth, thin	rare			PC	PC		1	3
Gigaspora											
1	200–600	yellow	smooth	1–19	2	1	10	5		3	21
Acaulospora											
1	80–200	orange	smooth	1–518	3	1	12	6	2	2	26
2	60–100	white	pitted 'golfball'	331–650	2	1	10	6	1	2	22
3	100–150	white	deep pitted	1–257			10	4	1	2	17
4	100–150	white	tinny pits	rare		1		1			2
5	50–90	white	smooth	4–1624	1		2	3	1	2	9
6	100–250	white	shaggy	rare	PC		1, PC	PC		2	11
Glomus											
1	50–120	brown	smooth, aggregated	1–518	2	1	12	6	2	3	26
2	60–150	white	irregular shape	331–650	2						2
3	70–150	orange-brown	smooth, aggregated	1–257						4	4
4	40–120	Type A	thick-walled				PC	PC		PC	7
5	30–70	Type C	thin-walled				PC	PC	PC	PC	6
6	20–40	Type D	thin-walled			PC	PC	PC		PC	6
7	5–20		fine endophyte					PC			1
Total site diversity (*n* species)					5–9	11	8–13	7–13	4–8	2–8	

[a] Average spore numbers per 100 g soil
PC spores not seen, but fungus obtained in pot culture

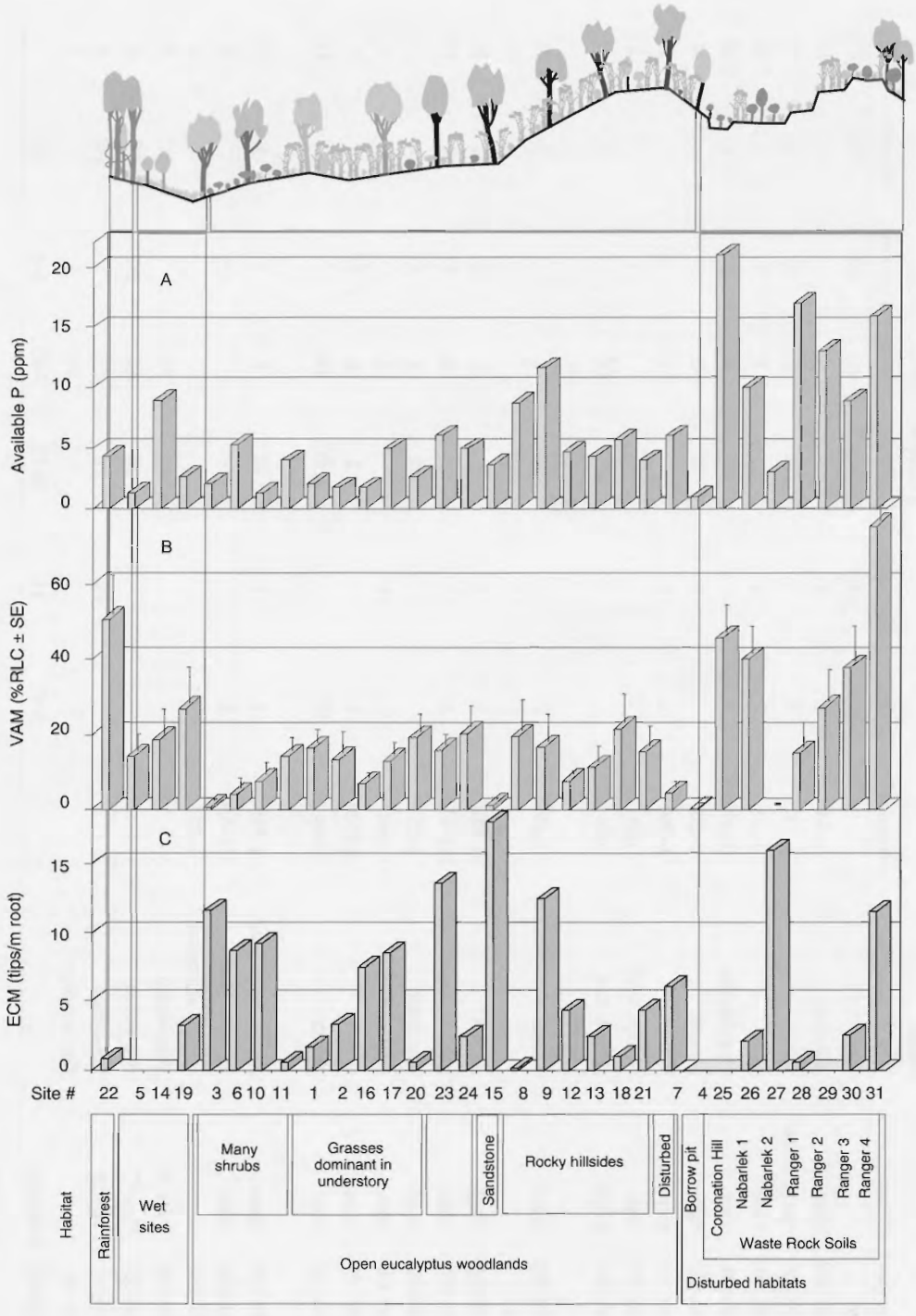

Figure 2. Typical values for (A) available soil phosphorus; and bioassay measurements of (B) root length colonised (RLC) by Glomalean fungi (VAM) and (C) root tips colonised by ectomycorrhizal fungi (ECM), in soils from a wide range of natural and disturbed habitats in tropical Australia.

Discussion

Mycorrhizal fungus diversity

This is the first large-scale population survey of Glomalean fungi within a region and it employed several different methods to enumerate fungi. Most of our knowledge on the distribution of these fungi is based on surveys using spores recovered from field soil samples. However, sporulation by VAM fungi is often poorly correlated with mycorrhiza formation in soils (Abbott and Robson 1991). In the present study, spore occurrence data provided good information about fungi in the genera *Scutellospora, Acaulospora* and *Gigaspora* but greatly underestimated the importance of *Glomus* species in soils. Glomalean fungi present in soils can also be identified by characteristics of their growth patterns within the roots of bioassay plants and by their eventual sporulation within pot cultures started from soil samples (Brundrett et al. 1994). These later procedures established that *Glomus* isolates were the dominant fungi in most soils even when living spores of these fungi were not present.

In this survey, different VAM fungus isolation procedures produced complimentary results and several methods were required to isolate or identify the full diversity of fungi present in a soil sample. The methods used for isolating and identifying fungi relied on different propagules produced by the fungi. Bioassay experiments measured all fungal propagules, especially active networks of mycelium; spore enumeration measured only soil-borne spores; and isolation procedures utilised spores, fungal structures in roots, associations formed by living bait plants, or mixed propagules in bulk soil. Comparisons of the results of bioassay, spore survey and culturing experiments using the same soils, demonstrated major differences in the propagule strategies of Glomalean fungi for survival and spread within tropical Australian soils. In particular, species in the genera *Scutellospora, Acaulospora* and *Gigaspora* apparently relied heavily on spores in the soil to survive the dry season, while most *Glomus* species had few spores, but survived as other propagules (perhaps as hyphal networks, or root fragments containing vesicles).

Without much additional taxonomic work, most fungi could only be assigned to genera. However, individual species of fungi could be distinguished by features of spores, soil hyphae and mycorrhizal associations, and were assigned numbers within genera (Table 1). Spores of VAM fungi were more abundant under host plants in early successional habitats than in climax plant communities. However, these fungi were absent from other disturbed habitats, where host plants did not occur, and their diversity was always substantially lower in disturbed sites than in natural habitats. This reduced diversity in young sites may have resulted from limitations in their dispersal mechanisms resulting in delays in fungal introductions, the absence of appropriate host plants, or in the inability of fungi to adapt to site conditions resulting in establishment failure (Malajczuk et al. 1994). There is some evidence that wind action, larger animals which consume fungi and small soil animals can function as vectors for Glomalean fungi (Allen 1991; Brundrett 1991; Claridge and May 1994). However, there is little information on the time required for these processes to introduce fungi into virgin habitats.

There was some evidence of soil specificity for some fungi (which preferred wet or disturbed habitats), but most species of Glomalean fungi occurred in a wide range of habitats. However, it is likely that local isolates of the widespread species of Glomalean fungi have adapted to the prevailing soil conditions in different sites (moisture, pH, texture, etc.). More research is urgently required to compare the capacity of fungus isolates from a range of habitats to promote the growth of associated plants in different soils, and to investigate their capacity to tolerate changes in climatic and soil conditions.

Mycorrhizal fungus propagules

Bioassays detected propagules of VAM fungi wherever patches of vegetation were established on WRD sites. Apparently, populations of these essential soil microbes have the capacity to recover rapidly in disturbed habitats after their introduction by dispersal mechanisms. The activity of mycorrhizal fungi (measured as spore numbers, or by bioassays) was substantially higher in patches of early-successional vegetation in mine sites than in undisturbed habitats. It is not known if mycorrhizal fungi produced higher numbers of propagules in early successional habitats (perhaps to compensate for harsh environmental conditions), or if these propagule numbers reflect enhanced mycorrhizal fungus productivity (perhaps due to a more rapid root turnover or higher soil fertility).

The relatively high fertility of mine site (WRD) substrates relative to woodland sites may result in a reduced requirement of plants to form mycorrhizal associations in these young soils. There was also a predominance of weedy plants (including many non-mycorrhizal species) in the disturbed habitats sampled (Brundrett et al., in press). These factors suggest that plants in these mine-site habitats may receive less benefit from mycorrhizal associations than plants in intact natural habitats, as has been reported, in other disturbed ecosystems (Janos 1980; Allen 1991). However, there are many other questions that must be answered before the role of mycorrhizal associations in disturbed habitats can be ascertained (Jasper 1994). In particular, the time-course of mycorrhizal fungus dispersal to new sites and recovery of their activity in

virgin soils relative to the establishment of vegetation are unknown. It is also uncertain whether the reduced diversity of mycorrhizal fungi, or limits in their ability to adapt to soil conditions can reduce their symbiotic effectiveness in disturbed sites. Answers to these questions are required before the potential value of technology to speed up the introduction of mycorrhizal fungi to disturbed habitats can be evaluated.

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Vesicular-Arbuscular Mycorrhizal Associations in Three Forest Types in the Dinghushan Biosphere Reserve, Subtropical China

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Abstract

Vesicular-arbuscular mycorrhizal (VAM) associations of 107 plant species in 3 major forest types of the Dinghushan Biosphere Reserve in subtropical China were investigated. The primary result showed that 44 plant species had VAM associations, accounting for 41% of the plants surveyed. This includes 26 genera of plants whose mycorrhizal associations had not previously been reported. Spores of VAM fungi occurring in soils the Dinghushan area, included *Gigaspora* and *Glomus* species. These results are significant for the conservation, administration and utilisation of VAM fungus resources, and also for the study on forest ecosystems.

Key words: vesicular-arbuscular mycorrhiza, forest communities, natural ecosystem, mycorrhizal roots, ecology

VESICULAR-arbuscular mycorrhizal (VAM) associations are caused by fungi in the Zygomycete order Glomales which colonise the roots of many plants species and have an important role in plant mineral nutrition. Studies of VAM associations in natural ecosystems in China began in the 1980s. Chen et al. (1981) reported that 96 species of plants had VAM in central subtropical China; Cao et al. (1985) demonstrated that 17 species of plants had VAM in a desert habitat; Niu (1990) found 55 species of VAM plants in Dinghushan, Guangdong; and Yi et al. (1990) found 105 species of plants with VAM in tropical and subtropical artificial forests.

Dinghushan Biosphere Reserve (DBR) is located at 23° 09' N, 112° 30' E, in the southern subtropical region of China. The mean annual air temperature, precipitation and relative humidity in DBR are 21.5°C, 1927 mm and 80%, respectively (Huang and Fan 1982). The three main soil types are lateritic red earth, yellow earth and mountain shrubby meadow soil, distributed at different elevations (He et al. 1982). Natural vegetation in the reserve can be divided into 8 types. Among them, coniferous forest, mixed forest and monsoonal evergreen, broad-leaved forest are the most important, forming a successional series in this zone. The monsoon evergreen, broad-leaved forest has a diverse flora with a complex community structure and is the typical vegetation type in this area (Wang et al. 1982). A survey of VAM associations of forest plants was undertaken to provide information which would be of value for the conservation and administration of natural resource and for the study of biodiversity in DBR.

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Materials and Methods

This survey was carried out in April 1994 in the three major forest communities in DBR and investigated 107 species (from 86 genera and 52 families) of the 148 total plant species which occur in the DBR. These include 18 species which have previously been reported to have VAM (Niu 1990).

Fine, relatively young, roots of each species were sampled in the field and processed in the laboratory. Root samples were cleaned in water, cut into 1–1.5 cm segments and fixed with a FAA fixative (formalin 5 mL, acetic acid 5 mL, 70% alcohol 90 mL, glycerine 5 mL). Samples from 30–50 root segments belonging to 1–3 individuals were used for each species. These samples were stained with Trypan blue and proportion of roots colonised by mycorrhizas was assessed under a dissecting microscope (Phillips and Hayman 1970). Fungus spores were collected using a wet sieving and centrifugation technique (Kuo and Bi 1989). Seven replicates of each soil type were used.

Results and Discussion

VAM associations

The results of this survey (Table 1) showed that 44 species of plants, belonging to 39 genera and 31 families, had roots containing VAM associations. Thus VAM was detected in 41% of the surveyed plants. Among the 39 genera of plants with VAM, 26 genera were not previously reported in China (Kuo and Bi 1989; Niu 1990; Yi et al. 1990). Although no mycorrhizal symbiosis was observed in *Schima superba* during this study, it has been reported to have VAM (Niu 1990).

The plants with VAM included dominant species in the forest community, such as *Castanopsis chinensis, Machilus kwangtungensis* and *Syzygium levinei* in monsoonal, evergreen broad-leafed forests, *Castanopsis chinensis* and *Ardisia crenata* in mixed forests and *Rhodomyrtus tomentosa* in coniferous forests. Other species were typical plants of southern subtropical regions, including *Garcinia multiflora, Bowringia callicarpa, Ficus fistulosa, Tetrastigma planicaule* and *Calamus rhabdocladus.* Species of nitrogen-fixing plants, like *Ormosia semicastrata* which has root nodules and *Ardisia crenata* which has leaf nodules, were also included (Chen et al. 1985; Ding et al. 1993). Plants of economic value included *Aquilaria sinensis,* which is used as a medicine, and *Glochidion wrightii* and *Evadia lepta,* which are used to produce oil. All five species of *Cyperaceae* investigated in this study were not mycorrhizal, in agreement with many earlier reports that mycorrhizas rarely occur in this plant family (Harley and Smith 1983).

Table 1. Vesicular-arbuscular mycorrhiza of plants in the Dinghushan Biosphere Reserve in three forest types: monsoonal evergreen, broad-leafed forests (B), mixed forests (M) and coniferous forests (C).

Family	Genus species	VAM	Life form	Distribution B	M	C	References for VAM records
Dicksoniaceae							
	Cibotium barometz	-	Fern	+	+	+	
Lindsaeaceae							
	Lindsaea orbiculata	-	"	+	+	+	
	Schizoloma intertextum	-	"	+			
Adiantaceae							
	Adiantum capillus-veneris	-	"	+	+	+	
	A. flabellulatum	-	"	+	+	+	
Athyriaceae							
	Diplazium donianum	+	"	+			
Asleniaceae							
	Asplenium sp.	-	"	+			
Blechnaceae							
	Blechnum orientale	-	"	+	+	+	
Dryopteridaceae							
	Arachniodes exilis	-	"	+			
Aspidaceae							
	Hemigramma decurrens	-	"	+			
Annonaceae							
	Fissistigma glaucecens	-	Liana	+	+		
Lauraceae							
	Cryptocarya chinensis	-	Tree	+	+		
	C. concinna	-	"	+	+		
	Lindera chunii	-	"	+	+		
	L. metcalfiana	-	"	+	+		
	Litsea verticillata	-	"	+	+	+	

Table 1. (Continued) Vesicular-arbuscular mycorrhiza of plants in the Dinghushan Biosphere Reserve in three forest types: monsoonal evergreen, broad-leafed forests (B), mixed forests (M) and coniferous forests (C).

Family	Genus species	VAM	Life form	Distribution			References for VAM records
				B	M	C	
	Machilus breviflora	+	"	+	+	+	Niu 1990
	M. chinensis	-	"	+	+		
	M. kwangtungensis	+	"	+	+		Niu 1990
	M. phoenicis	+	"	+	+	+	Niu 1990
	Neolitsea cambodiana	+	"	+	+		Niu 1990
	N. umbrosa	-	Shrub	+			
Illigeraceae							
	Illigera platyandra	+	Liana	+			
Menispermaceae							
	Hypserpa nitida	+	"	+	+		
Piperaceae							
	Piper arboricola	-	"	+			
	P. hancei	-	"	+	+		
Chloranthaceae							
	Scarcandra glabra	-	Shrub	+	+		
Polygalaceae							
	Xanthophyllum *hainanensis*	-	Tree	+			
Thymelaeaceae							
	Aquilaria sinensis	+	Tree	+	+		Yi et al. 1990
	Wikstroemia nutans	+	Shrub	+	+		
Dilleniaceae							
	Tetracera asiatica	-	Liana	+	+		
Samydaceae			+				
	Casearia glomerata	+	Tree				
	C. villilimba	+	Tree	+			
Theaceae							
	Eurya macartheyi	-	Shrub	+			
	Schima superba	+	Tree	+	+	+	Niu 1990
Myrtaceae							
	Rhodomyrtus tomentosa	+	Shrub		+	+	Yi et al. 1990
	Syzygium levinei	+	Tree	+		+	Yi et al. 1990
Melastomaceae							
	Melastoma dodecandrum	+	Herb		+	+	Niu 1990; Yi et al. 1990
Gutiferae							
	Calophyllum membranaceum	-	Shrub	+	+		
	Garcinia multiflora	+	Tree	+	+	+	
	G. oblongifolia	-	"	+	+	+	

Table 1. (Continued) Vesicular-arbuscular mycorrhiza of plants in the Dinghushan Biosphere Reserve in three forest types: monsoonal evergreen, broad-leafed forests (B), mixed forests (M) and coniferous forests (C).

Family	Genus species	VAM	Life form	Distribution B	M	C	References for VAM records
Elaeocarpaceae							
	Elaeocarpus nitentifolius	-	"	+	+		
Sterculiaceae							
	Sterculia lanceolata	-	"	+	+		
	Pterospermum lanceaefolium	+	"	+			
Euphorbiaceae							
	Antidesma japonicum	+	Shrub	+	+		
	Aporosa yunnanensis	-	"	+			
	Croton lachnocarpus	+	"	+	+		
	Glochidion wrightii	-	Tree	+	+		
	Macaranga sampsonii	+	"	+	+		
Rosaceae							
	Photinia prunifolia	-	"	+	+	+	
	Pygeum topengii	+	"	+			
	Rhamnus crenata	-	Shrub	+	+		
Papilionaceae							
	Bowringia callicarpa	+	"	+	+		
	Ormosia semicastrata	+	Tree	+			
Hamamelidaceae							
	Eustigma balansae	+	"	+			
Fagaceae							
	Castanopsis chinensis	+	"	+	+	+	Yi et al. 1990
Ulmaceae							
	Gironniera subaequalis	-	"	+			
Moraceae							
	Artocarpus styracifolius	-	"	+			
	Ficus fistulosa	+	Shrub	+			Niu 1990; Yi et al. 1990
	F. variolosa	+	"	+	+	+	Niu 1990; Yi et al. 1990
	F. virens	-	"	+			
Aguifoliaceae							
	Ilex memecylifolia	-	"	+			
	I. triflora	+	Tree	+	+		Kuo and Bi 1989
Santalaceae							
	Henslowia frutescens	-	Liana		+	+	
Vitaceae							
	Tetrastigma planicaule	+	"	+			
Rutaceae							
	Evadia lepta	+	Shrub		+	+	

Table 1. (Continued) Vesicular-arbuscular mycorrhiza of plants in the Dinghushan Biosphere Reserve in three forest types: monsoonal evergreen, broad-leafed forests (B), mixed forests (M) and coniferous forests (C).

Family	Genus species	VAM	Life form	Distribution			References for VAM records
				B	M	C	
Burseraceae							
	Canarium album	+	Tree	+			Kuo and Bi 1989
Sapindaceae							
	Nephelium chryseum	+	"	+			
Sabiaceae							
	Meliosma rigida	+	"	+			
Connaraceae							
	Rourea microphylla	+	Liana	+			
Ebenaceae							
	Diospyros eriantha	-	Tree	+			
	D. morrisiana	+	"	+	+	+	Kuo and Bi 1989
Myrsiaceae							
	Ardisia crenata	+	Shrub	+	+	+	
	A. punctata	+	"	+	+	+	
	A. quinquegona	-	"	+	+		
	Maesa salicifolia	-	"	+	+		
	Rapanea neriifolia	-	"	+	+		
Symplocaceae							
	Symplocos adenopus	-	"	+			
Oleaceae							
	Jasminum pentaneurum	-	"	+	+	+	
Apocynaceae							
	Melodinus fusiformis	-	Liana	+	+	+	
Rubiaceae							
	Canbium dicoccum	-	Tree	+			
	Canthium horridum	-	Shrub	+			
	Hedyotis lancea	+	Herb		+	+	
	Ixora chinensis	-	Shrub	+	+	+	
	Lasianthus chinensis	+	"	+			
	Pavetta hongkongensis	-	"	+			
	Psychotria serpens	-	Liana	+	+	+	
	Randia canthiodes	-	"	+			
	R. spinosa	-	Shrub	+	+		
	Tarenna mollissima	-	"	+	+		
Convolvulaceae							
	Erycibe obtusifolia	-	Liana	+			
Zingiberaceae							
	Alpinia chinensis	-	Herb	+	+		
Liliaceae							
	Dianella ensifolia	+	"		+	+	

Table 1. (Continued) Vesicular-arbuscular mycorrhiza of plants in the Dinghushan Biosphere Reserve in three forest types: monsoonal evergreen, broad-leafed forests (B), mixed forests (M) and coniferous forests (C).

Family	*Genus species*	VAM	Life form	Distribution B	M	C	References for VAM records
Smilacaceae							
	Smilax corbularia	-	"	+	+		
	S. glabra	+	Shrub	+	+	+	Niu 1990
	S. lanceaefolia	-	"	+	+	+	
Palmae							
	Calamus rhabdocladus	+	Liana	+	+		
	Caryota ochlandra	-	Tree	+			
Cyperaceae							
	Carex sp.	-	Herb	+			
	C. cryptostachys	-	"	+			
	Gahnia tristis	-	"	+	+	+	
	Hypolytrum nemorum	-	"	+	+		
	Scleria terrestris	-	"	+	+		
Gramineae							
	Indocalamus longiauritus	-	Shrub	+			
	Lophatherum gracile	-	Herb	+	+	+	
	Arundinella hirta	+	"			+	
	Miscanthus floridulus	+	"		+	+	

Spores of VAM fungi

The average density of spores in forest soil was 3.7 per g of soil ± 2.2 (SD). Spores of two genera of VAM fungi, *Gigaspora* and *Glomus*, were predominant.

Conclusions

Of the plant species from DBR examined, 44 had VAM associations in their roots, but 63 other species did not. Since these results were obtained from a single survey, mycorrhizal associations for some of the species listed in Table 1 may have been discovered if additional samples had been taken. Therefore, further research on the occurrence of mycorrhizal associations in the DBR is needed, to allow the impact of seasonal changes in the monsoonal climate of this region to be considered.

Acknowledgments

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Ectomycorrhizal Roots and Fungi of Philippine Dipterocarps

N. M. Pampolina*, R. E. de la Cruz*† and M.U. Garcia*

Abstract

A study was carried out of common ectomycorrhizal fungi associated with a typical dipterocarp forest on Luzon Island in the Philippines. The forest was dominated by the following dipterocarp species, *Shorea contorta, S. guiso, Parashorea malaanonan, Anisoptera thurifera ssp. thurifera, Hopea pligata* and *Dipterocarpus grandiflorus* and the fungi collected included species belonging to seven genera of basidiomycetes, *Russula, Lactarius, Scleroderma, Amanita, Boletus, Paxillus, Cantharellus* and *Russula* species dominated many of the collections, while *Lactarius* was less common. Fruiting of these fungi occurred during the onset of the wet monsoonal season when the relative humidity reached over 75% and a mean temperature of 28°C. Roots samples collected at the site were examined macroscopically and microscopically and showed typical ectomycorrhizal root development. They had monopodial to ramiform branching, were velvety in texture and whitish to brownish in colour. Colonisation of the root seldom extended into the cortex, forming a typical Hartig net. The fungal mantle was observed to range from 32–56.5 μm in thickness. Hyphae were generally septate and clamp connections were commonly observed. Cleared and stained roots did not contain evidence of vesicular-arbuscular mycorrhizal infection, suggesting therefore that these dipterocarps only had ectomycorrhizal associations.

Key words: dipterocarps, ectomycorrhizal infection, diversity, basidiomycetes

DIPTEROCARPS are high-quality, wood producing trees that are widely distributed in the tropical rainforests of Southeast Asia. Unfortunately, their present status is in danger because of uncontrolled deforestation (Mayers 1988). In the Philippines major deforestation in the past 50 years has reduced this resource to only a million hectares of old growth dipterocarp forest (Factoran 1990). Recently, forest policies have been put into place to rehabilitate denuded forest land through a strategy known as Assisted Natural Regeneration, where enrichment planting of denuded sites is carried out as well as enhancing growth of current regeneration (Factoran 1989). This method also protects remnant dipterocarp mother trees that allow natural dispersal of seed to these denuded areas.

It has recently been shown that ectomycorrhizas can play a pivotal role in the growth and survival of dipterocarp seedlings in degraded sites (de la Cruz et al. 1988), by mobilising plant water and nutrient uptake via hyphae and increasing resistance to environmental stresses (Harley and Smith 1983; de la Cruz et al. 1987; Mejstrick 1989; Kowalski et al. 1989). Such benefits would assure the growth, development and survival of planted dipterocarp seedlings in the field. Presently, there are only few investigations on the mycorrhizal status of dipterocarps (de la Cruz 1988; Sangwanit and Sangthian 1992; Egam 1993). This paper presents the results of a morphological and anatomical investigation of the mycorrhizal infection of dipterocarp roots collected in the field.

Materials and Methods

The second growth dipterocarp forest in Mt Makiling Botanical Garden at the College of Forestry, University of the Philippines, Los Baños, was sampled for basidiocarp in 1991–92. Site information is provided in Table 1. Fruiting bodies were collected during the rainy season and identified macro- and microscopically to genus (Pampolina et al. 1993). Samples of roots were washed, cut into segments and fixed in formalin acetic acid (Sass 1958) in preparation for clearing in KOH and staining with trypan blue to view internal root structures (Koske and Gemma 1989). About five samples of dipterocarp roots per species were randomly replicated to measure the inner and outer layer of fungal mantle. Photomicrographs of specimen were taken to record associations.

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Table 1. Properties of the soil and habitat in the dipterocarp forest at Mt Makiling Botanical Garden.

Criteria	Description
A. Soil properties	
pH	5.9
Organic matter (%)	3.2
Available phosphorous (ppm)	1.6
Exchangeable potassium (me/ 100 g soil)	2.3
Sand (%)	30.3
Silt (%)	44.6
Clay (%)	25.1
Texture	loamy
B. Site properties	
Topography (%)	0–15
Monthly mean rainfall (mm)	262.9
Relative humidity (%)	79.0
Vegetation	Dominated by dipterocarp trees

Results and Discussion

Fourteen species of ectomycorrhizal fungi were found associated with the dipterocarps (Table 2). They appeared to form typical ectomycorrhizas with short, swollen roots that had rounded lateral ends and were covered with an extensive mantle of hyphae (Figure 1). *Parashorea malaanonan* appeared to be an exception where roots had pointed ends (Table 3). The manner of root branching ranged from monopodial to pyramidal in form. There was an absence of root hairs on the root surface and mycelial strands present ranged from white to brown in colour. First and second order roots were seldom covered by a fungal sheath.

Tranverse sections of dipterocarp roots observed under SEM revealed intercellular hyphal penetration. This is exemplified by roots of *Shorea pligata, Anisoptera thurifera* ssp. *thurifera* and *Parashorea malaanonan* where hyphal extension in the cell wall formed a Hartig net characterisitic of ectomycorrhizas (Figure 1). Fungal infection in sampled roots seldom extended past cell walls in the cortex region and never reached the stele.

The fungal mantle surrounding roots appeared to be pseudoparenchymatous inwardly and had different layers of compact and densely arranged hyphae outwardly. The two layered mantle ranged in thickness from 32–56.5 mm (Table 4). Roots of *Hopea pligata* appeared to be thicker than those of *Parashorea malaanonan* and *A. thurifera* ssp. *thurifera*. The outer layer of the fungal mantle was found to be thicker than the inner layer.

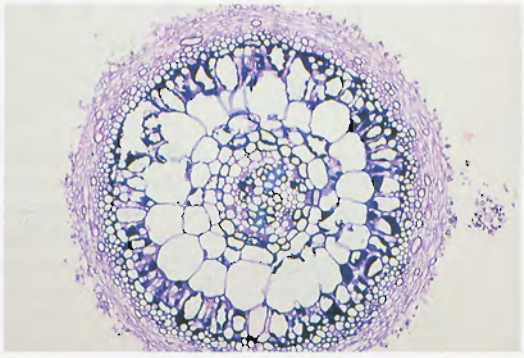

Figure 1. Cross-section of an ectomycorrhizal root of *Shorea* sp. and *Lactarius* sp. showing the thick mantle and epidermal Hartig net.

In longitudinal section, the most striking feature was the oblique expansion of the elongated epidermal cells which had a well formed Hartig net. In addition the root cap was found to be absent below the apical root meristem, being replaced by sheathing mycorrhiza.

Hyphal structures observed were all septate with clamp connections. These observations conclusively verify that the mycorrhizal associations were initiated by basidiomycetes. Further investigation, using root samples which were cleared and stained, confirmed the absence of vesicular-arbuscular mycorrhizal associations. The dipterocarps species in this study only initiated ectomycorrhizal associations.

Conclusions

Investigation of dipterocarp roots in the Philippines revealed that all fine roots were typically ectomycorrhizal. The results of the taxonomic study showed that there were at least fourteen species of putative ectomycorrhizal found to be associated with the dipterocarps. The predominance of *Russula* and *Lactarius* species with this forest suggest that they are potential strains for the climax stage in the development of the forest community when managing human-made or natural forest. This may also imply that dipterocarp seedlings could benefit from these fungi, since regeneration of seedlings is common under older stands.

Table 2. Ectomycorrhizal fungi collected from a dipterocarp forest showing their distinct features, growth habit and host dipterocarps (Pampolina et al. 1993).

Ectomycorrhizal fungus	Distinct features	Growth habit
Lactarius piperatus	yellowish, peppery taste, creamy sap, sphaerocyst present, brittle	solitary to gregarious
L. volemus	reddish brown, creamy sap, sphaerocyst distinct	solitary to gregarious
L. velereus	whitish, peppery taste, creamy sap, brittle	gregarious
Russula pectinatoides	pale brown, margin, striated, brittle, sphaerocyst distinct	solitary
R. subnigricans	grayish violet, brittle, sphaerocyst distinct	solitary
Russula sp. 1	grayish, brittle, sphaerocyst distinct	solitary
Russula sp. 2	fuschia pink, brittle, sphaerocyst distinct	solitary
Russula sp. 3	whitish, brittle, sphaerocyst distinct	solitary
Russula sp.4	orange, brittle, sphaerocyst distinct	solitary
Scleroderma bovista	brownish, gasteroid, leathery peridium, stipe nearby buried	solitary to gregarious
Boletus sp.	reddish brown, cap umbonate and viscid with pores underneath, slender stalk	solitary
Cantharellus cibarius	pale brown, funnel shaped, tough, gills well spaced and decurrent	solitary
Paxillus sp.	orange, tough, pileal margin inrolled	solitary to scattered
Amanita sp.	brownish, viscid cap, annulus and volva	solitary

Table 3. Morphological characteristics of ectomycorrhizal roots from the dipterocarp forest at Mt. Makiling Botanical Garden.

Host species	Fungus	Manner of root branching	Characteristics of lateral tips	Color
Hopea pligata	*Russula* sp.	monopodial to ramiform	short, swollen, rounded	whitish to light brown
Anisoptera thurifera ssp. *thurifera*	*Russula pectinatoides*	monopodial to ramiform	short, swollen, rounded	light brown
Parashorea malaanonan	*Lactarius piperatus*	ramiform	short, swollen, rounded or pointed	brown to black
Anisoptera thurifera ssp. *thurifera*	*Scleroderma bovista*	ramiform	short, swollen, rounded	brownish to black
Shorea guiso	*Russula* sp.	ramiform	short, swollen, rounded	whitish to light brown
Pentacme contorta	*Amanita* sp.	monopodial	short, swollen, rounded	brownish

Table 4. Mean thickness of the inner and outer layers of fungal mantle coating sample of dipterocarp roots from the forest at Mt. Makiling Botanical Garden.

Host species	Ectomycorrhizal fungi	Thickness of mantle (μm)		
		Inner layer	Outer layer	Total
Hopea pligata	*Russula* sp.	13.0	37.5	56.5
Anisoptera thurifera ssp. thurifera	*Russula pectinatoides*	14.0	19.0	32.0
Parashorea malaanonan	*Lactarius piperatus*	23.0	19.0	42.0

Acknowledgments

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The Physiology and Ecology of Mycorrhizal Associations

Optimising Growth Conditions for *Pisolithus tinctorius* Inoculum Production

Chen Lianqing and Pei Zhida*

Abstract

Cultural conditions for the growth of a strain of *Pisolithus tinctorius* associated with Masson pine (*Pinus massoniana*) were studied. Experiments using different temperatures and media compositions, showed that in pure-culture, the optimum growth temperature was 25°C; the optimum pH in the culture solution was 5.5, and the most effective sources of carbon and nitrogen were glucose and inorganic ammonium nitrogen respectively. These data provide information for the formulation of a suitable medium to produce inoculum of *P. tinctorius* for use with Masson pine in nurseries.

Key words: *Pisolithus tinctorius*, inoculum production, cultural conditions, *Pinus massoniana*

MASSON pine (*Pinus massoniana*) is a fast growing timber species in subtropical regions of China. It is widely distributed in southern China and has a high economic value. This tree is also resistant to adverse environmental conditions characteristic to this region, such as poor soils. This species is typically ectomycorrhizal, and earlier research has shown that inoculation of Masson pine seedlings with mycorrhizal fungi can improve their outplanting survival performance and growth (Chen Lianqing1989; Chen Lianqing and Pei Zhida 1990).

Pisolithus tinctorius (Pers) Coker & Couch is a common ectomycorrhizal fungus associate of Masson pine forming typical yellow mycorrhizal roots. It has been shown to be effective in colonising roots on young seedlings in a number of nursery experiments (a 75.7% mycorrhizal inoculated rate has been achieved; Chen Lianqing and Pei Zhida 1990). The purpose of this study was to determine optimum growth parameters for the efficient sterile-culture cultivation of this fungus to produce mycorrhizal inoculum.

Materials and Methods

The strain RISF-86902 of *Pisolithus tinctorius* was collected and isolated into sterile culture from under a Masson pine stand in Fuyang, Zhejiang Province and this strain was used in all the experiments described below.

An improved synthetic cultural medium was used (Yu Dafu 1975). This medium contained 10 g glucose, 1 g ammonium tartrate, 0.1 g $CaCl_2$, 0.1 g NaCl, 1 g KH_2PO_4, 0.5 g $MgSO_4$, 0.01 g $FeCl_3$ in 1 L of water.

The *Pisolithus* isolate was inoculated into a 250 mL flask with 100 mL of culture medium and then incubated in a temperature-controlled orbital shaker. Culture media experiments were initiated with 3.2 mg (dry weight) of *P. tinctorius* mycelium and were incubated for 30 days. These experiments were replicated 3 times for each of the treatments described below.

- To test the effects of temperature on *P. tinctorius* growth, cultures were incubated at 10, 15, 20, 25, 30, 35 and 40°C in a temperature-controlled environment without agitation.
- In a second experiment, the pH of culture media was adjusted to obtain final values ranging from 2.5 to 8.0 at 0.5 increments, resulting in 12 pH treatments in total. Media pH was adjusted (by adding HCl) before media sterilisation and was measured after fungal growth.
- The effect of using different sugars in media as a carbon source was tested at standardised conditions of pH (5.6) and temperature (20–25°C). The monosaccharides glucose and fructose, the disac-

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charides amylomaltose and saccharose, as well as soluble starch and dextrin were used as carbon sources for *Pisolithus*. These were added to the standard media at 10.0, 9.53, 9.53, 9.03, 8.53 and 8.53 g/L respectively. The control treatment consisted of fungi grown without a carbon source.

- The effect of different nitrogen sources on *P. tinctorius* growth were compared using media containing inorganic ammonium nitrogen in the form of $(NH_4)_2SO_4$, KNO_3, $Ca(N0_3)_2$, NH_4NO_3 and organic nitrogen in the form of tartaric acid, urea, peptone, and yeast maceration extract. These were added to the standard media at 0.70, 0.55, 1.25, 0.43, 1.0, 0.32, 1.03 and 2.16 g/L respectively. Ammonium tartrate was the control treatment for this experiment. Cultures were grown at 20–25°C and media pH was adjusted to 5.6 by adding HCl.

For the culture experiments described above, fungal biomass was measured by taking the weight of mycelia dried at 50, 80 then 105°C. This mycelia was separated from 100 mL of medium by filtration on qualitative filter paper and rinsed with distilled water. The diameter of fungal colonies on plates was measured using a ruler under a dissecting microscope. The pH of culture liquid media was measured by taking a 25 mL sample of media.

Results and Discussion

A. The effect of temperature

At 25 and 30°C, *P. tinctorius* mycelium started to grow within 2–3 days, with rapidly proliferating, dense hyphae and a normal colony appearance. The average daily growth of mycelium was 1.2 mm/day. The sprouting time for hyphae could be delayed by either raising or lowering the temperature. At temperatures 10°C above or below 25°C (15°C or 35°C), the average growth of mycelium was only one tenth of that at 25°C. Temperature also influenced the time required for hyphal growth to start, which was 2–3 days at 30°C, but increased to 18 days at 10°C. At 40°C the mycelium did not grow (Table 1).

B. The effect of pH on the growth of mycelium

All pH values cited in this paper are initial measurements taken before inoculating the culture media. Sterilisation of culture media resulted in slight pH increases at pH values below 4.6 and pH decreases at higher pH values (Table 2). High-pressure sterilisation also produced a brown sediment due to chemical reactions in less acidic culture media, which resulted in poor growth of *Pisolithus* mycelium. However the experimental treatments resulted in a wide range of pH values which were relatively stable during culture growth. There was a tendency for pH to decline in cultures with the most fungal growth, probably as a result of organic acid secretion by fungi (Table 2). There was strong correlation between the initial pH of culture media and that after fungal growth (r = 0. 970).

The initial pH of the growth media was an important factor affecting mycelial metabolism. *Pisolithus tinctorius* was able to tolerate a wide range of pH values between 2.5 and 8.0. However, the proliferation of mycelium was favoured by pH values between 4.0 and 5.5 and the optimum initial media pH was 5.5 (Table 2). These results showed that the growth of *P. tinctorius* mycelium increased with increasing pH in the range from 2.5 to 5.5 and there was a significant linear correlation between pH and biomass ($y = -7.317 + 16.508x$, $r = 0.952$). With pH values of 6–8, the growth of mycelium decreased as pH increased and there was a significantly negative correlation ($y = 202.8 - 23.91x$, $r = -0.994$).

C. The effect of carbohydrate source on the growth of mycelium

Sugars are the main nutrient source for the growth of fungal mycelia in sterile culture. They are required to synthesise carbohydrates and amino acids and to provide an energy source. The shape and size of mycelial colonies varied with the use of different kinds of sugars in the medium (Table 3). Mycelial biomass was greater than that of the control treatment (without carbohydrate) for all carbohydrates except fructose. The monosaccharide glucose produced the most mycelial balls, the highest biomass (16 times greater than the control) and resulted in hyphae with a normal appearance. Of the disaccharide sugars used, amylomaltose was the best. The carbohydrate sources were ranked according to their efficiency in promoting *P. tinctorius* growth as follows: glucose > amylomaltose > dextrin > saccharose > polyose soluble starch > control > fructose. *Pisolithus* mycelium was able to absorb soluble starch only after it was decomposed by starch amylase. The poor results using fructose as a sugar source require further study.

D. The effect of nitrogen sources

Nitrogen is an essential element for fungal growth and is required for synthesising proteins, nucleic acids, etc. Among the nitrogen sources tested, ammonium sulfate was the most readily utilised by *Pisolithus* mycelia, followed by ammonium nitrate, potassium nitrate and calcium nitrate (Table 4). Organic nitrogen sources were less effective in promoting *Pisolithus* growth, resulting in weak mycelial development. Yeast extract was ineffective (Table 4).

Table 1. The growth of *Pisolithus* mycelia at different temperatures.

Temperature (°C)	Start of growth (days)	Colour of growth media	Colour of mycelium	Colony diameter (mm)	Mycelial growth (mm day^{-1})	Morphology of aerial hyphae[a]
10	18	light brown	brown-yellow	7.0	0.04	sparse, weak hyphae
15	9–11	brown–yellow	brown	11.9	0.1	dense, fluffy hyphae, some adhering
20	6–7	darkish brown	brownish	17.2	0.3	dense, tidy, adhering
25	3–4	brown–yellow	golden yellow	38.0	1.2	dense, flocculate, adhering
30	2–3	yellow–brown	yellow	26.0	0.4	tidy, dense, adhering
35	6	yellow–brown	yellow	12.5	0.1	dense, tidy and short fluffy
40	no growth	—	—	—	—	—

[a] Adhering refers to growth on sides of the vessel

Table 2. The effect of culture media pH on the proliferation of *Pisolithus* mycelia.

Media pH			Media colour		Mycelial colonies			Fungal dry weight (mg)	Time for growth (days)
Unsterile	Sterile	Used media	As prepared	After use	Colour	Diameter	Shape		
2.5	2.6	2.5	translucent	translucent	yellow	0.2–1.5	short	25.2	7.9
3.0	3.0	2.8	translucent	yellow	yellow	0.5–2.0	fine	44.3	13.8
3.5	3.5	3.2	translucent	yellow	yellow	0.5–2.0	long	55.9	17.5
4.0	4.0	3.5	translucent	yellow	brown	1–3	long	65.2	20.4
4.5	4.5	4.0	translucent	yellow	brown	1–3	long	70.1	21.9
5.0	5.0	4.0	translucent	brown	brown	1–3	long	74.2	23.2
5.5	5.4	4.1	translucent	brown	brown	1–3	long	77.1	24.1
6.0	6.0	4.5	translucent	brown	brown	1–3	flat	59.1	18.5
6.5	6.4	5.1	yellow	brown	brown	1–2	flat	48.9	15.3
7.0	6.5	6.3	yellow S	yellow	yellow	0.5–1	short	32.2	10.1
7.5	6.9	6.7	yellow S	yellow	yellow	0.5–1	short	26.0	8.1
7.9	–	6.9	brown S	yellow	white	0.5–1	short	14.5	4.5

S = sediment produced in media

Table 3. The effect of different carbohydrate sources on the growth of *Pisolithus* mycelia.

Carbohydrate source	Media colour		Mycelial colonies			Fungal growth	
	Before inoculation	After growth	Colour	Diameter (cm)	Shape	Dry weight (mg)	Relative growth (x)
Glucose	translucent	brown	yellow–brown	1–2	long	62.7	19.6
Fructose	translucent brown	yellow	yellow	0.5–1	flat	2.3	0.7
Amylomaltose	translucent	yellow–brown	yellow	0.5–1.5	untidy	24.7	7.7
Saccharose	translucent	brown	yellow	0.5–1.5	flat	13.0	4.1
Soluble starch	milky	pale yellow	yellow	0.5–1.0	flat	4.7	1.5
Dextrin	translucent	brown	yellow	0.5–1.5	flat	16.4	5.1
Control (nil)	translucent	translucent	yellow	0.5–1.5	flat	4.4	1.4

Note: Numerical values are averages from 3 samples

Table 4. The effect of different nitrogen sources on *Pisolithus* growth in sterile culture.

Nitrogen source	Media colour		Mycelial colonies			Fungal growth	
	Before inoculation	After growth	Colour	Diameter (cm)	Shape	Dry weight (mg)	Relative growth (x)
$(NH_4)_2SO_4$	translucent	brown translucent	brown	1.0–2.0	flat	51.6	23.5
KNO_3	translucent	brown	brown	1.0–2.0	fluffy ball	35.5	16.1
$Ca(N0_3)_2$	translucent	light brown	brown	1.0–2.0	fluffy ball	34.5	15.7
NH_4NO_3	translucent	brown	brown	1.0	fluffy ball	39.9	18.1
Tartaric acid	translucent	brown	yellow–brown	0.5–1.5	fluffy ball	37.7	17.1
Urea	translucent	white sediment	white	0.5	irregular flat	3.0	1.3
Peptone	brown–yellow	brown–yellow	yellow	0.5	regular flat	3.1	1.4
Yeast extract	yellow milky	—	—	—	—	—	—

Note: Numerical values are averages from 3 samples

Conclusions

The experiments presented here showed that, in artificial culture media, the optimum growth temperature for *Pisolithus* was 25°C, while temperatures of 20–30°C were suitable for growth and some growth could occur at 10–35°C. The optimum pH of the culture solution was 5.5, but this isolate of *Pisolithus* could grow at a wide range of pH values. When carbohydrate sources were compared, the monosaccharide sugar glucose was most effective, other carbohydrates were less effective, and fructose was not readily utilised. Comparisons of nitrogen sources demonstrated that inorganic nitrogen sources were more easily utilised than organic nitrogen sources, and that ammonium was better that nitrate. These results help to define media which can be used to produce inoculum of *Pisolithus tinctorius* for use with Masson pine, but additional research may be required to help optimise cultural media conditions for this fungus, and to develop methods of inoculum production.

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The Influence of Nutritional Supplements on the Growth of Ectomycorrhizal Fungi in Culture and Associated Tree Seedlings in the Nursery and Field

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Abstract

Studies on the effect of Chinese nutritional supplements on the growth of ectomycorrhizal fungi in pure culture, the formation of ectomycorrhizal roots and the growth of associated host plants in the nursery are reported here. These supplements included the products Liquid F, Liquid Z and Nongle. They generally had a beneficial effect on ectomycorrhizal fungi such as *Boletus*, by stimulating hyphal production in liquid culture. They also stimulated the formation of ectomycorrhizal roots and promoted the growth of associated *Pinus elliottii* and *P. tabulaeformis* seedlings. The application of supplements increased the survival of *P. tabulaeformis* seedlings and boosted the growth of *Larix olgensis* seedlings in afforestation sites.

Key words: ectomycorrhizal fungi, inoculum production, nutritional supplements, afforestation

SOME compounds are currently being used to increase the yield of edible fungi in culture. These compounds include rare earths, mineral elements, 'N-FIX' nutrient supplement. (Wang Jinhui and Yuan Jiaye 1991; Yang Ruiai et al. 1991; Ouyang Zeng and Chang Guolong 1991; Wang Yilei 1991). In China, nutrient supplements such as rare earth minerals and 'N-FIX', are widely used in agriculture and have produced good results. These supplements have also been used to help the culture of edible fungi such as *Pleurotus ostreatus* and *Flammulina velutipes* (Wang Jinghui and Yuan Jiaye 1991; Ouyang Zeng and Chang Guolong 1991; Wang Yilei 1991). These results lead the authors to study the effects of nutritional supplements on mycorrhizal associations. Experiments were designed to study the effects of various nutritional supplements on the growth of mycorrhizal fungi in culture, mycorrhizal formation and the growth of associated host plants.

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Materials and Methods

Biological materials

The ectomycorrhizal fungi used in experiments were *Boletus* sp. *B. edulis, Suillus grevillei, S. luteus, Gomphidius viscidus, Xerocomus chrysenteron* and *Cortinarius* sp., which were isolated from fungal fruitbodies. An unidentified fungus isolated from *Larix* sp. roots (89112) was also used. The tree species that were used in experiments were *Pinus tabulaeformis, P. elliottii* and *Larix olgensis.*

Nutritional Supplements

The following nutritional supplements were used in experiments: 'Nongle' (a compound containing trace elements), chelated rare earth elements, 'Liquid F' (humic acid salts), 'Liquid Z' (a composite micronutrient solution) and 'Compound liquid'. These supplements are commercially available in China except for Liquid Z, which was developed by the authors and is currently being evaluated.

Plate culture and liquid culture methods were used to study the effect of nutritional supplements on mycorrhizal fungus growth in sterile culture. Potato dextrose agar media (PDA), made from 200 g of boiled potato extract, 20 g of sucrose and 18 g agar per litre of water, and potato and dextrose liquid (PD), with-

out the agar, were used for the sterile culture of mycorrhizal fungi. Media were sterilised by autoclaving for 30 min at 121°C before use.

Plate cultures

The following supplements were added to plate culture media: (1) PDA + 100 ppm Nongle; (2) PDA + 200 ppm Nongle; (3) PDA + 100 ppm rare earth chelate; (4) PDA + 200 ppm rare earth chelate; (5) PDA + 500 ppm Liquid F; (6) PDA + 1000 ppm Liquid F; (7) PDA + 1000 ppm Liquid Z; and (8) PDA only. The mycorrhizal fungi *Boletus* sp., *B. edulis, Suillus grevillei, S. luteus* and *Gomphidius viscidus* were grown on 5 replicates of each media. Cultures were incubated for 7 days at 25°C, before fungal growth was estimated by measuring the diameter of colonies.

Liquid culture experiments

Culture media was made by combining 100 mL PD liquid media with 1000 ppm of Liquid F, or 100 mL Liquid PD with 1000 ppm Liquid Z in 250 mL flasks before sterilisation. Fungi were cultured for 6 days on a rotating bed incubator at 120 rotations per minute. After growth fungal mycelia was removed from flasks, washed with distilled water, dried at 80°C and then weighed. Each treatment was replicatted 3 times.

Nursery seedlings

Seeds were sterilised with 0.5% $KMnO_4$ before germination on agar in petri dishes. Germinated seedlings of *Larix olgensis*, *Pinus elliottii* and *P. tabulaeformis* were used. One germinated seedling was transferred to each pot. Pots were kept in a nursery and used for the following experiments.

Nursery experiments

Pinus tabulaeformis and *P. elliottii* seedlings were removed from the soil 10 days before watering with 100 mL/pot of Liquid F in water, then inoculated with 5 g of cultured inoculum of *G. viscidus* per pot. At 7 and 14 days after inoculation, some seedlings were watered with Liquid F, applied in water. Experimental treatments were as follows: (1) Liquid F only; (2) inoculum of *G. viscidus* only; (3) *G. viscidus* + Liquid F (double treatment); and (4) the control (water and sterilised culture media only). Every treatment was replicated 3 times with 45 seedlings.

The effect of Liquid F on the storage of *P. tabulaeformis* seedlings in liquid culture was examined using one-year-old seedlings. Seedling roots were washed clear of soil and surface sterilised for 3 minutes using 0.2% chloramine T, before rinsing with water. Seedling roots were kept in 1000 ppm Liquid F in water, or just in water (controls). Each treatment consisted of 20 seedlings.

The effect of compound liquid on *Larix olgensis* afforestation

This experiment treatments were as follows: a 500 g inoculum of (1) *Boletus* sp.; (2) *Cortinarius* sp.; or (3) *X. chrysenteron* was applied in 800 mL of compound liquid (1:40 dilution). The control treatment (4) had sterile culture medium without fungi. These mixtures were applied to 2-year old *Larix olgensis* bare-root seedlings from the nursery, by dipping their roots before planting. There were 3 replicates and 700 seedlings per treatment, that were randomly arranged in the field. The afforestation site was in Helong Province, China.

Measurement of plants

For laboratory experiments, the dry weight of fungal mycelium produced in liquid culture was measured after it was filtered from the medium and dried at 60°C. The diameter of colonies grown on solid media in petri dishes were measured. For nursery experiments, seedling growth (height, diameter, number and length of needles) was measured 1 month after inoculation. Five months after inoculation, plants were harvested and seedling height, basal diameter and dry weight were measured; the number of lateral roots and ectomycorrhizal roots was quantified; and leaf chlorophyll levels and the N, P, and K concentrations in seedlings were measured using standard procedures. After *Larix olgensis* seedlings were planted at the afforestation site, the height of trees, their diameter and the length of new branches were measured. Analysis of variance tests were used to compare experimental results.

Results and Discussion

The effect of nutritional supplements on ectomycorrhizal fungi biomass

Laboratory trials showed that various nutritional supplements could promote the growth of mycorrhizal fungi, but they had different effects on different groups of fungi (Table 1). In liquid culture, Liquid F, Liquid Z had clear stimulatory effects on *B.* sp, *B. edulis, S. grevillei, S. luteus* and *G. viscidus* (Table 1). Hyphal dry weight of *S. grevillei* was increased by 1.21 times, while that of *G. viscidus* increased by 2.3 times due to the application of Liquid F. Growth stimulation due to Liquid Z were from 1.07 to 2.45 times. Statistical comparisons demonstrated differences between nutritional supplement treatments and controls (Table 1). Liquid F had the largest effect on *G. viscidus, B.* sp. and *S. luteus* and less effect on *B. edulis* and *S. grevillei*. Liquid Z promoted the growth of *B.* sp., *B. edulis* and *G. viscidus*, but not the *Suillus* species.

Table 1. Effect of various nutritional supplements on the liquid culture growth of ectomycorrhizal fungi in liquid culture on PD media.

Treatment	Average dry weight of fungus mycelium				
	B. sp	*B. edulis*	*S. grevillei*	*S. luteus*	*G. viscidus*
PD+ Liquid F	365**	290.1*	303.1*	451.3**	1048.8**
PD+ Liquid Z	566**	440.8**	269.9	382.5	674.4**
PD only	231	205.9	250.5	209.3	455.2

** = P < 0.1%, * = P< 0.05%

On solid culture media, all supplements (except 1000 ppm Liquid Z) increased the growth of some fungi (Table 2). However, the two *Boletus* isolates did not respond much to these treatments. There have been reports on the stimulation of edible fungus growth by Nongle (Wang Jinhui 1991), but this is the first report of the effect of this supplement on ectomycorrhizal fungi.

The effect of supplements on the growth of *Pinus elliottii* and *P. tabulaeformis* seedlings

As shown in Table 3, seedling size 1 month after inoculation was greater than that of the control for every treatment. The effect of treatments on *Pinus elliotti* was more substantial than on *P. tabulaeformis*. Analysis of variance established that differences in seedling height, diameter and needle length between *Gomphidius viscidus* or Liquid F treatments and the control were significant. Treatment with both *Gomphidius viscidus* and Liquid F was better than single treatments. In the double treatment, the dry weight of *Pinus elliottii* was greater than the control by 2.4 times, while that of *P. tabulaeformis* increased by 1.7 times. The response of seedlings to treatments after 5 months (Table 4) was better than results after 1 month (Table 3). Increases in seedling yield were related to the number of ectomycorrhizal roots (Table 4). It seems that Liquid F was capable of stimulating mycorrhizal formation by *Gomphidius viscidus* as well as indigenous mycorrhizal fungi.

Effect of nutritional supplements on the N, P, K and chlorophyll concentrations in *Pinus elliottii* and *P. tabulaeformis* needles

From Table 5, the concentrations of P, K and chlorophyll in needles were greater than for controls for all treatments, but N levels were not affected. The effect of the ectomycorrhizal fungus inoculation with Liquid F was greater than either treatment alone. Increases in the P, K and chlorophyll in needles should be beneficial to seedlings by increasing their growth potential and resistance to disease.

The effect of supplements on the survival of *P. tabulaeformis* seedlings in water

After removal from soil, seedlings of *P. tabulaeformis* can survive storage for 10 days, or so in water, but their longevity can be prolonged greatly by adding 1000 ppm of Liquid F to the water. After 4 days in water, nearly one-third of the untreated seedlings died and most were dead after 13 days. However, treatment with Liquid F, reduced seedling mortality to one-third after 10 days in water and two-fifths after

Table 2. Effect of various nutritional supplements on the growth of ectomycorrhizal fungi on solid media.

Treatment	Diameter of fungal colonies (mm)				
	B. sp.	*B. edulis*	*S. grevillei*	*S. luteus*	*G. viscidus*
100 ppm Nongle +PDA	59	90	90	86	90
200 ppm Nongle +PDA	57	90	90	87	90
100 ppm rare earth chelate +PDA	55	90	90	85	90
200 ppm rare earth chelate +PDA	51	43	90	57	65
500 ppm Liquid F +PDA	54	90	90	89	90
1000 ppm Liquid F +PDA	60	90	90	89	90
1000 ppm Liquid Z +PDA	50	90	25	53	53
PDA only	53	90	63	48	59

13 days. Observations of seedling root systems showed that most roots in water died after 13 days, but many of the roots in water with Liquid F survived this long and some seedlings grew new roots.

The effect of treatments on *Larix olgensis* growth in afforestation sites

Mycorrhizal fungus inoculation and compound liquid treatments resulted in clear increases in the growth of *Larix olgensis* over the controls (Table 6). The effect of compound liquid in combination with *B.* sp. or *X. chrysenteron* was the most substantial.

Discussion

Results from laboratory and field tests, indicate that nutritional supplements can stimulate the growth of mycorrhizal fungi in culture, increase the formation of mycorrhizal roots, and promote the growth of ectomycorrhizal plants. Liquid F was found to have a synergistic effect on mycorrhizal formation by *Pinus elliottii* and *P. tabulaeformis* seedlings, and Compound Liquid promoted the growth of mycorrhizal *Larix olgensis* after planting in an afforestation site. There have been few studies on interactions between ectomycorrhizal associations and nutritional supplements in China or elsewhere. The nutritional supplements used by the author were inexpensive and effective, costing as little as 1.5 yuan/100 g. It is suggested that there should be further studies in this area to produce inocula to meet the needs of the forestry industry to help with seedling production and plantation management.

The use of supplements can also prolong the life of coniferous tree seedlings in water, to facilitate seedling transport and storage. The mechanism for increased *P. tabulaeformis* seedling longevity due to Liquid F application apparently results from protection of the root system, particularly mycorrhizal roots. There is still much work required to study the mechanisms of these interactions before definite conclusions can be made about the role of Liquid F or other supplements in plant growth or mycorrhizal formation.

Table 3. Effect of synergist on seedlings of *Pinus elliottii* (E) and *P. tabulaeformis* (T) 1 month after inoculation in the nursery.

Treatment	Height (cm)		Needle length (cm)		Number of needles	
	E	T	E	T	E	T
Control	3.2	2.6	0.97	2.28	72	30
F	3.6*	3.1	1.19*	2.65	90**	34
GV	3.3	3.2	1.14	2.57	76	34
GV+F	4.2**	3.7*	1.24**	2.59	94**	34

Notes: Statistical procedures as in Table 1. GV *Gomphidius viscidus*; F Liquid F

Table 4. Effect of supplements and mycorrhizal inoculation on seedlings of *Pinus elliotti* (E) and *P. tabulaeformis* (T) after 5 months of growth.

	Shoot height (cm)		Stem diameter (mm)		Shoot weight (g/plant)		Number of lateral roots		Ectomycorrhizal formation[b]	
Treatment[a]	E	T	E	T	E	T	E	T	E	T
Control	4.3	3.7	1.68	1.84	0.16	0.25	6	9	22	27
F	5.3*	4.3	1.85*	2.01*	0.28	0.28	11	12	34	38
GV	5.5*	4.5*	1.87*	2.01*	0.30	0.32	13	14	70	71
GV+F	6.0**	5.0**	2.05**	2.16**	0.39	0.44	14	16	93	84

[a] Statistical procedures as in Table 1 and experimental treatments are as in Table 3
[b] Ectomycorrhizal formation = number of seedlings with > 30% mycorrhizal root length

Table 5. Effect of supplement and mycorrhizal fungus applications on the N, P, K and chlorophyll concentrations in *P. elliotti* and *P. tabulaeformis* needles.

	Pinus elliottii				*P. tabulaeformis*			
Treatment[a]	N (%)	P (%)	K (%)	Chlorophyll (mg/g)	N (%)	P (%)	K (%)	Chlorophyll (mg/g)
Control	1.31	0.30	0.14	0.69	1.02	0.29	0.25	0.71
F	1.01	0.42	0.25	0.78	1.10	0.35	0.51	0.82
GV	1.16	0.58	0.29	0.82	1.16	0.40	0.41	0.89
F+GV	1.25	0.67	0.30	1.07	1.07	0.44	0.71	1.10

[a] Experimental treatments are the same as Table 3

Table 6. Effect of mycorrhizal fungus inoculation, with or without compound liquid application, on the size and annual growth of *Larix olgensis* in an afforestation site.

Inoculum	Compound liquid			Water		
	Height (cm)	Diameter (mm)	Growth (cm/year)	Height (cm)	Diameter (mm)	Growth (cm/year)
Botelus sp.	36.8	4.66	23.9	20.0	3.75	11.1
Cortinarious sp.	26.8	4.42	19.0	24.5	4.10	15.9
X. chrysenteron	25.8	4.22	17.8	20.7	3.66	9.8
Control	17.0	2.71	9.2	16.0	2.63	9.1

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Research on Mycorrhizal Associations of Poplar

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Abstract

Considerable research worldwide has shown that more than 20 poplar species have mycorrhizal associations. In some cases ectomycorrhizas were the predominant form, whereas in others, both ectomycorrhizas and vesicular-arbuscular mycorrhizas (VAM) were found in the same root system. Research has concentrated on the following topics: (1) investigations into the understanding of the biodiversity and distribution of poplar ectomycorrhizal fungi; (2) determining the frequency of occurrence of VAM and ectomycorrhizal associations of poplar and factors influencing these associations; and (3) physiological and biochemical aspects of the relationship between ectomycorrhizal fungi and their host plant.

Key words: *Populus*, ectomycorrhiza, vesicular-arbuscular mycorrhiza, dual associations, growth responses

POPLAR species are widely distributed in the Northern Hemisphere occurring as major tree groups in Asia and North America. With an ever-increasing demand for timber and timber products, poplars are becoming more important in the local forestry of these countries, including China. A wide range of research activities on these species has been conducted in countries where poplars occur naturally, especially America, China, South Korea, Italy, France, Ireland and India. The main species studied include *Populus tremuloides, P. deltoides, P. tomentosa* and poplar hybrids. To date, some 20 poplar species have been shown to form mycorrhizas. Of these, a few formed ectomycorrhizas, such as *P. tremuloides* (Godbout and Fortin 1985; Cripps and Miller 1993), *P. alba* (Meotto and Carraturo 1988) and *P. fremontii* (Vozzo and Hacskaylo 1974). Others have been reported to form both ectomycorrhizas and vesicular-arbuscular mycorrhizas (VAM) on the same root system, including *P. canadensis* (Vozzo and Hacskaylo 1974; Guo et al. 1983), *P. deltoides* (Lodge 1989; Lodge and Wentworth 1990), *P.* x *euramericana* cv. 'I-214' (Aguillon and Garbaye 1990), *P. tomentosa* (Zhao et al. 1994a,b), *P. balsamifera* (Helm and Carling 1993) and *P. grandidentata* (Vozzo and Hacskaylo 1974). Only one report has indicated a lack of mycorrhizas in the poplar species *P. heterophylla* (Vozzo and Hacskaylo 1974).

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The following summary of poplar mycorrhizal research considers three topics: the biodiversity and distribution of the ectomycorrhizal fungi; the effect of ectomycorrhizas on host plants; and interactions between VAM and ectomycorrhizal associations.

The Occurrence of Poplar Ectomycorrhizal Associations

Poplar species naturally occur, and are often planted as pioneer tree species, in sites with extreme climatic or edaphic conditions. For example *P. diversifolia, P. pruinosa, P. ariatia* and *P. litwinowiana* are the only native tree species in desert areas of the Sinkiang region of China (Xue 1988). *Populus cathavana* and *P. simonii* are often transplanted in the arid sites of Shaanxi, Gansu and Qinghai provinces in the northwest of China. Poplar can even grow in some sites with seriously polluted soils, such as abandoned mines, eroded soil and areas polluted by heavy metals. In these situations mycorrhizas are always associated with the poplar roots (Cripps and Miller 1993; Schramm 1966). One would assume that these associations play a significant role in the survival and growth of poplars in harsh sites.

Ectomycorrhizal types of poplar

Ectomycorrhizas formed by different fungi have unique characteristics in morphology and anatomy (Agerer 1986). Godbout and Fortin (1985) have

investigated the ability of *P. tremuloides* to form ectomycorrhizas with 54 spp. of fungi and showed that structural and morphological characteristics of ectomycorrhizas are unique to the genera of mycorrhizal fungal species and independent of the host plant. Heslin (1985) has examined the roots of *Populus* spp. from 5 locations in Ireland and classified the five most frequently encountered root types. Studies by Zhao et al. (1993) found 14 distinct types of ectomycorrhizas on *P. tomentosa* roots from central China. Eight common Chinese poplar ectomycorrhizal types are described in Table 1.

Table 1. Description of some of the major ectomycorrhizal association types observed on *P. tomentosa* roots from central China.

Fungus (association type)	Features
***Tricholoma columbetta* (1)**	
colour of fungal sheath:	brown–yellow
mycorrhizal form:	club-shaped
fungal sheath:	thick plectenchymatous layer
external hyphae:	not seen
Hartig net:	weak, develops in the first cortex layer
evaluation:	beneficial ectomycorrhiza
***Laccaria laccata* (2)**	
colour of fungal sheath:	fresh material is golden yellow
mycorrhizal form:	pyramidal and irregularly pyramidal
structure of the fungal sheath:	well developed, plectenchymatous
external hyphae	sometimes visible
Hartig net:	weak, develops in the first cortex layer
evaluation:	beneficial ectomycorrhiza
***Cenococcum geophilum* (4)**	
colour of fungal sheath:	coal black
mycorrhizal form:	club-shaped
fungal sheath:	thick pseudoparenchymatous
external hyphae	hair-like
Hartig net:	only in the first cortex layer
evaluation:	beneficial ectomycorrhiza
Unknown fungal species (5)	
colour of fungal sheath:	light yellow, translucent, with brown spots
mycorrhizal form:	club-shaped, unbranched, or branched
fungal sheath:	thin plectenchymatous layer
external hyphae:	visible
Hartig net:	develops throughout the cortex
evaluation:	non-effective ectomycorrhiza
Unknown fungal species (6)	
colour of fungal sheath:	light to dark brown, with brown spots
mycorrhizal form:	club or club with branches
structure of the fungal sheath:	thick pseudoparenchymatous layer
external hyphae:	brown

Table 1. (Continued) Description of some of the major ectomycorrhizal association types observed on *P. tomentosa* roots from central China.

Fungus (association type)	Features
Hartig net:	develops throughout the cortex
evaluation:	non-effective ectomycorrhiza
Unknown fungal species (7)	
colour of fungal sheath:	light yellow
mycorrhizal form:	pyramidal or irregular pyramidal
structure of the fungal sheath:	well developed, plectenchymatous
external hyphae:	brown
Hartig net:	extensive, separating cells in cortex
evaluation:	non-effective ectomycorrhiza
Unknown fungal species (8)	
colour of the fungal sheath:	brown–black
mycorrhizal form:	pyramidal or irregular pyramidal
structure of the fungal sheath:	pseudoparenchymatous
external hyphae:	hairy brown
Hartig net:	cells in cortex are separated by it
evaluation:	non-effective ectomycorrhiza
***Scleroderma* sp. (11)**	
colour of fungal sheath:	silver, root tip often protruding
mycorrhizal form:	club-shaped or pyramidal
structure of the fungal sheath:	thick plectenchymatous layer
external hyphae:	silver
Hartig net:	develops in 1–2 layers in the cortex
evaluation:	beneficial ectomycorrhiza

The ecology of poplar ectomycorrhizas

Ectomycorrhizas in North America were investigated by Cripps and Miller (1993) in *P. tremuloides* (aspen) stands in the Rocky Mountains at 1800–2000 m above sea level. The two sites in southwestern Montana and one site in southeastern Idaho varied in size, age of trees, type of soil, drainage patterns and to a certain extent climate. The results show that 43 species of ectomycorrhizal fungi were found associated with aspen. The *Cortinariaceae* were a dominant component of the mycoflora, including at least seven species of *Inocybe*. Fourteen species of ectomycorrhizal fungi occurred on all three sites in association with aspen, and the dominant or characteristic mycorrhizal species varied among sites. The early colonisers *Inocybe lacera* and *Laccaria laccata* were characteristic of the smelter-acidified, nutrient-poor soil of the site near Butte, Montana. Late stage fungi such a *Amanita muscaria* and *Lactarius controversus* were more prevalent in the older, undisturbed aspen stands. Schramm (1966) showed that amongst the early plants colonising anthracite waste in the United States were ectomycorrhizal trees including seedling of *P. tremuloides*. Fruit bodies of several well-known ectomycorrhizal fungi were associated with it. These included *Scleroderma aurantium, Amanita rubescens, Pisolithus tinctorius, Thelephora terrestris* and *Inocybe lacera*.

Investigation of ectomycorrhizal fungi in a poplar stand in Korea showed that 21 species (11 genera) of ectomycorrhizal fungi were associated with *Populus* (Lee and Kim 1986). The major genera were *Amanita, Russula, Laccaria* and *Leccinum* (Lee and Kim 1983). Zhao et al. (1993) showed that *Tricholoma columbetta, Laccaria laccata, Cenococcum geophilum and Scleroderma sp.* form beneficial ectomycorrhizas with *P. tomentosa* in soils with pH values less than 7.5. These species were absent in alkaline soils, where only a few ectomycorrhizal types were present and a pseudomycorrhiza (unknown fungal associations) was common.

The Role of Ectomycorrhizal Associations of Poplar

Evaluation of symbiotic relationships between ectomycorrhizal fungi and poplar

Meyer (1984) showed that the anatomical structure of ectomycorrhiza could reflect its symbiotic relationship with the host plant. According to Meyer (1984) the non-effective ectomycorrhizas have the following anatomical characteristics: cell walls of outer cortex are filled with tannins; Hartig net formation results in separation of epidermal cells; and hyphae enter intracellular spaces. Meyer (1984) thought that suberisation and tannin secretion by host cells could increase their resistance to fungal penetration, and may help to limit the spread of ectomycorrhizal fungi to intercellular spaces. Studies of *P. tomentosa* by Zhao et al. (1993) showed that most of the 14 observed ectomycorrhizal types associated with *P. tomentosa* had features which are characteristic of plant responses to invasion. Whether or not some of these associations were detrimental to the health of the plant remains to be determined.

Specificity of ectomycorrhizal fungi for poplar species

Godbout and Fortin (1985) demonstrated that out of 54 species of ectomycorrhizal fungi associated with aspen, 29 formed ectomycorrhizas on aspen seedlings in the field. Aspen seems to display little specificity for ectomycorrhizal fungi or alternatively is host to a wide range of fungal symbionts. During comparative studies on the composition of ectomycorrhizal fungi in pine and poplar stands, Lee and Kim (1983) discovered that 13 of 16 ectomycorrhizal fungal species collected under poplar were similar as those associated with a nearby pine stand. However, research on the development of ectomycorrhizas in a poplar hybrid (*P. trichocarpa* × *P. tacamahaca cv.* TT32) under aseptic conditions Helsin and Douglas (1986) showed that only 5 of 18 mycorrhizal fungi common with poplars formed an association with the host plant under experimental conditions.

Effects of ectomycorrhizas on growth and mineral nutrition of poplar

Lee and Koo (1985) observed that *Populus alba* × *P. glandulosa* cuttings planted in a nursery and inoculated with *Pisolithus tinctorius* mycelium were 19% taller and 48% heavier in above ground dry weight, than uninoculated cuttings after one growing season. Zhao et al. (1994) showed that in soils with a pH 7.0, inoculation of *Populus tomentosa* with *Tricholoma columbetta, Laccaria laccata* or *Scleroderma sp.* increased the height and diameter of plants; whereas in alkaline soils only *Scleroderma* was associated with the host plant cuttings. Inoculated plants were 14.6% taller and 11.2% greater in diameter. Above ground biomass increased by 54.2% over uninoculated controls. The P content in leaves and roots of the inoculated seedlings was increased by 11.1% and 14.8% respectively when compared with the uninoculated plants.

Dual VAM and Ectomycorrhizal Associations of Poplar

Lodge and Wentworth (1990) have examined mycorrhizal associations of natural populations of *Populus deltoides* growing in the eastern United States, and discovered that there is a negative correlation between VAM and ectomycorrhizal colonisation of the root system. However, this relationship varied with ectomycorrhizal morpho-types present on the roots and was influenced by soil moisture (Lodge 1989). Similar results were obtained by Zhao et al. (1994b) in studies of mycorrhizas of outplanted *P. tomentosa* cuttings in the field. Their results indicated that the initial infection of cuttings were by VAM fungi, but these were rapidly replaced by ectomycorrhizas. This negative interaction between VAM and ectomycorrhizal fungi became more apparent with age of the transplanted cuttings. Soil pH appeared to affect this relationship. On alkaline sands, the percentage of VAM root length was greater than in the natural forest soil.

Conclusions

Research has shown that more than 20 species of *Populus* can form mycorrhizas. Of these some form only ectomycorrhizas, but others form both ecto- and VAM in the same root system. There is a range of ectomycorrhizal fungi that occur with poplars on sites with different soil pH, nutrient status and climatic conditions. It is probable that this diversity ensures the survival and growth of poplar in these sites and hence the adaptability of poplars for planting on difficult sites. It has been shown that growth and P absorption of poplar is greatly increased by inoculating them with mycorrhizas in nursery beds, and that the use of different species can significantly affect this relationship. This further demonstrates a need to select appropriate mycorrhizal fungi for inoculation of nursery plants. Interactions between VAM and ectomycorrhizal associations appears to be important and further work should be carried out to determine their significance.

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The Effect of Vesicular-Arbuscular Mycorrhizas on the Resistance of Poplar to a Canker Fungus *(Dothiorella gregaria)*

Ming Tang and Hui Chen*

Abstract

Biochemical index changes of *Populus* sp. (x *'beijingensis')* bark induced by the formation of associations with vesicular-arbuscular mycorrhizal (VAM) fungi and their relation to canker resistance were studied. The results showed that mycorrhizal formation is positively correlated with relative turgidity, enzyme activities (peroxidase and polyphenoloxidase), available phosphorus and total phenolic content of bark, but negatively correlated with total soluble sugars in bark. The growth of *Dothiorella gregaria* was negatively correlated with VAM formation. VAM fungus increased canker tolerance of poplars by promoting the uptake of water and nutrients (mainly phosphorus), which in turn increased the growth and vigour of trees. Induced peroxidase and polyphenoloxidase activities of poplars should result in increases in substances such as phenolics which are likely to be important parts of the canker-resistance mechanisms of poplars.

Key words: vesicular-arbuscular mycorrhizal fungi, poplar, *Dothiorella gregaria,* canker resistance

THE potential biocontrol of root diseases with mycorrhizal fungi is currently being investigated (Dehne et al. 1978; Hu Zhengjia and Wang Ping 1993; Liu Runjing and Qiu Weifan 1994), but there is less information on mycorrhizal interactions with trunk diseases of trees. Poplar canker is one of the important and widespread tree diseases in China. The bark structure, chemical composition and enzyme activity of poplar in relation to disease resistance has been studied (Xang Yuing and Hua Xiaomei 1981; Yang Chuanhe et al. 1989; Hu Jingjiang et al. 1990; Zhu Wei and Jing Yao 1990), but there have been no reports of the effect of vesicular-arbuscular mycorrhizal (VAM) fungi on canker resistance of poplars.

Field investigations of the relation between mycorrhizal colonisation and the disease index of poplar canker under natural conditions, suggested the inoculation of trees with VAM fungi could decrease the occurrence of canker (Tang Ming and Chen Hui 1994b). This study explores mechanisms for VAM fungus-induced changes in canker resistance, by investigating physiological and biochemical changes in poplar that could be related to canker resistance.

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Materials and Methods

Sandy soil collected in the Yanling area was mixed with washed river sand (1.1 v/v) and sterilised with formalin (Zhang Ling and Ran Chengxi 1989). The soil had the following properties: pH 7.7 (in water); available phosphorus 10.4 ppm; available potassium 14.5 ppm; total nitrogen 0.08%; and organic matter 1.08%.

One-year-old seedlings of *Populus* sp. *'beijingensis'* (*P. nigra* L. var. *italica* (Moench.) Koenne x *P. Catherana* Rehd.), a species which is often infected by the canker fungus, were grown in pots in a greenhouse. The temperature range was kept between 10°C and 24°C, with a mean night minimum of 10°C and a mean daily maximum of 24°C. Spores of the VAM fungus *Glomus mosseae* (Nichol. and Gerd.) Gerd. and Trappe were isolated from rhizosphere soil of *P.* sp. *'beijingensis'* by wet sieving and decanting (Gerdemann and Nicolson 1963) and propagated in pot cultures using clover (*Trifolium repens* L.) as the host. The infected root fragments, spores and soil from these pot cultures were used as inoculum. The pathogenic fungus, *Dothiorella gregaria* Sacc., used in experiments was isolated from canker tissue of poplar.

Determination of relative turgidity of bark

The potted transplants were divided into 4 different water content levels (A > B > C > D) by controlling their water supply, by watering to 20%, 15%, 10% or

5% soil water by weight respectively. Each water treatment group contained 30 seedlings, half of which were inoculated with *G. mosseae* and the remainder of which were used as controls. After 6 months, bark samples were collected at 1 m height to determine their relative turgidity (RT) using the following formula:

$$RT(\%) = \frac{\text{fresh weight} - \text{dry weight}}{\text{saturated weight} - \text{dry weight}} \times 100$$

Assessment of mycorrhiza and disease formation

Quantification of mycorrhizal infection was done with root samples collected from 3 points on the lateral root system of each seedling by taking 4 cm diameter × 10–15 cm deep soil cores. Roots were removed from the soil then cleared with 10% KOH and stained with trypan blue (Phillips and Hayman 1970). The gridline intersect method was used to measure root colonisation by mycorrhizal fungi (Giovanetti and Mosse 1980; Schenck 1982).

Determination of the disease index (DI) was performed using a graded standard of canker disease and a disease index was calculated according to Tang Ming and Chen Hui (1994b).

Biochemical determinations

Peroxidase (PO) and polyphenoloxidase (PPO) activities were determined using seedlings of C level water content inoculated with *G. mosseae* and/or *D. gregaria* (VAM + pathogen, VAM, or pathogen treatments). These plants were used to determine PO and PPO activities during the early growing stage of the second year. Annual branches were collected and their bark was peeled off immediately after rinsing and cold storage, then fixed by freezing, before enzyme activities were determined (Hu Jingjiang et al. 1990).

Bark samples, collected at 1 m height from seedlings, were used for the determination of total phenolics, soluble sugars and available phosphorus contents. This material was rinsed, dried at 48–50°C, ground, sieved and kept in stoppered bottles before use. These samples were then used to measure total phenolics — with phosphomolybdic acid–sodium tungstate colorimetry, soluble sugars — with flavone colorimetry, and available phosphorus — with molybdenum and antimony colorimetry (Zhu Wei and Jing Yao 1990).

Results and Discussion

Effect of VAM fungi on turgidity

Relative turgidity and mycorrhizal infection rate of seedlings inoculated with *Glomus mosseae* (Gm) or uninoculated control treatments of different moisture contents are presented in Table 1.

Table 1. Relative turgidity of bark, and VAM fungus colonisation of roots, from plants inoculated with *Glomus mosseae* and grown at different moisture levels (A–D).

Moisture level (% water)	Relative turgidity (%)		Mycorrhizal colonisation (%)[a]
	+VAM	–VAM	
A (20%)	92.3	91.1	18.1
B (15%)	89.7	86.3	26.2
C (10%)	84.4	71.2	41.5
D (5%)	79.8	63.8	54.6

[a] Reported for +VAM treatment only, –VAM seedlings were non-mycorrhizal.

The relative turgidity of VAM-inoculated seedlings was higher than that of the uninoculated controls at all 4 water levels and followed the order of D < C < B < A. The influence of mycorrhizal associations on the absorption of moisture by seedlings was substantial at low water contents, but was less when water supply was higher (Table 1). Mycorrhizal colonisation was inversely correlated with the water content of seedlings. The relative turgidity of bark also increased gradually as mycorrhizal formation increased at the same water content level (Fig. 1).

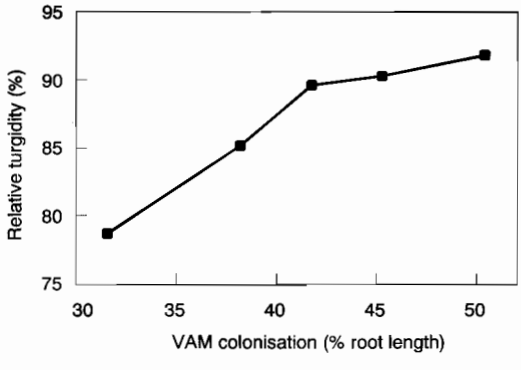

Figure 1. Relation between relative turgidity of bark and VAM formation.

Water content of plants is an important influencing factor on disease resistance (Jing Yoa and Tang Ming 1986; McPartland 1984; Xiang Yuing and Hua Xiaomei 1981). The research by Yang Chuanhe et al. (1989) pointed out that water content influences phenylalanine ammonia-lyase (PAL) activity and further the synthesis of lignin, phenolics and flavonoids in the phenylpropanoid metabolic pathway. Some phenolic constituents (e.g. catechol, p-hydroxybenzoic,

protocatechuic acid) in bark decreased as the water content decreased. Increases in the relative turgidity of bark resulted in increases in catechol content and consequently in increased disease resistance. Resistance factors of the host are most effective in turgid tissues. The water content of living tissues in annual bark can be used as a canker-resistance index for poplar. Cankers are more likely to form when relative turgidity is less than 80% (Beir 1964).

Effect of VAM and a pathogenic fungus on peroxidase and polyphenoloxidase activities in poplar bark

The results of enzyme activity determinations of tree seedlings inoculated with a VAM fungus and/or pathogen (Table 2) showed that the PO and PPO activities were highest in the dual inoculated plants. In single inoculated samples the enzyme activities (PO and PPO) were increased compared to controls, indicating that both the pathogenic and VAM fungi induced a higher enzyme activity of the host. Enzyme activities were *highest* and disease index was *lowest* in dual inoculated seedlings (VAM + pathogen) but in seedlings inoculated with pathogen only the opposite was observed.

Table 2. The effect of inoculation with a VAM fungus (*G. mosseae*) and/or a pathogenic fungus (*D. gregaria*) on peroxidase (PO) and polyphenoloxidase (PPO) activities in the bark of poplar seedlings.

Treatment	PO activity (enzyme units)	PPO activity (enzyme units)	Disease index
VAM + pathogen	1707	142.4	1.7
VAM	1111	84.7	0
Pathogen	981	73.4	14.7
Control	727	46.2	0

The relation between enzyme activities and disease index showed that as the enzyme activities increased the disease index decreased and the mycorrhizal infection rate increased (Fig. 2). Both mycorrhiza formation and enzyme activities were positively correlated with canker resistance in poplar. Peroxidase and polyphenoloxidase activities are known to be high in resistant cultivars of poplar (Hu Jingjiang et al. 1990). Canker resistance of poplars is positively correlated with mycorrhizal infection rate (Tang Ming and Chen Hui 1994b) and VAM fungi are able to induce the production of more then 10 pathogenesis-related proteins, promote terpenoid aldehyde contents and chitinase activity, thereby enhancing the resistivity of plants to pathogenic fungi (Liu Runjing and Qiu Weifan 1994; Dehne et al. 1987).

The effect of VAM on the chemical composition of poplar bark

The available phosphorus, total soluble sugars and total phenolic contents in mycorrhizal poplar bark were different from those of non-mycorrhizal controls. Available phosphorus and total phenolic contents increased and total soluble sugars decreased in inoculated seedlings. Analysis of variance tests indicated that the differences in available phosphorus, total phenolics and soluble sugar contents were all significant between inoculated plants and the controls ($P > 0.05$). Available phosphorus and total phenolic contents increased and total soluble sugars decreased as mycorrhizal formation increased in inoculated poplar seedlings (Fig. 3).

Mycorrhizal fungi are known to promote nutrient uptake and plant growth (Gou Xiuzhen and Bi Gouchang 1989; Tang Ming et al. 1993). Increased phosphorus uptake would result in increasing phospholipid content and thus decreased permeability of plant cell membranes. These factors should reduce the susceptibility of plants to pathogenic fungi. We would expect that the growth of *D. gregaria* would be inhibited by higher phosphorus concentrations (Tang Ming and Chen Hui 1994). Therefore, it seems logical that VAM fungi would induce canker resistance of host plants by increasing their phosphorus and phenolic contents. Harris and Paul (1987) and Bevege et al. (1975) showed that 4–14% of the total carbon resulting from photosynthesis is distributed to mycorrhizal fungi associated with the host plant, while total soluble sugars decreased. Conidia germination and colony growth of *D. gregaria* requires a high soluble sugar supply (Zhu Wei and Jing Yao 1990). Therefore, reduced soluble sugar contents resulting from high mycorrhizal colonisation rates, could reduce the impact of canker disease on poplar.

Conclusions

The formation of VAM associations influences the physiological and biochemical aspects of poplar, including water uptake, phosphorus content, enzyme activities, and the content of substances that inhibit canker formation. Inoculation with VAM fungi apparently enhances water uptake by seedlings, especially under conditions of low water supply. VAM associations also enhanced peroxidase and polyphenoloxidase activities in poplar bark, which were associated with reduced canker disease index. VAM fungus inoculation also caused increases in the

total phenolic content and decrease in total soluble sugars that are unfavourable to the growth of *D. gregaria*. VAM associations also increased canker resistance by promoting nutrients uptake and strengthening the vigour of trees, and by increasing the content of fungistatic substance (such as phenolics, lignin and phenylpropanoids) in bark. These mechanisms may work together to cause the VAM fungus-induced canker resistance of poplars that was observed.

Poplars are simultaneously infected by both VAM and ectomycorrhizal fungi in nature (Zhao Zhong, this volume) and these associations are negatively correlated with disease index of canker fungus. Infection by ectomycorrhizal fungi has shown a stronger direct influence on this disease than that of VAM fungi (Tang Ming and Chen Hui 1994a,c), and further study of the effect of ectomycorrhizal associations on the induced resistance of poplars to the canker fungus is required.

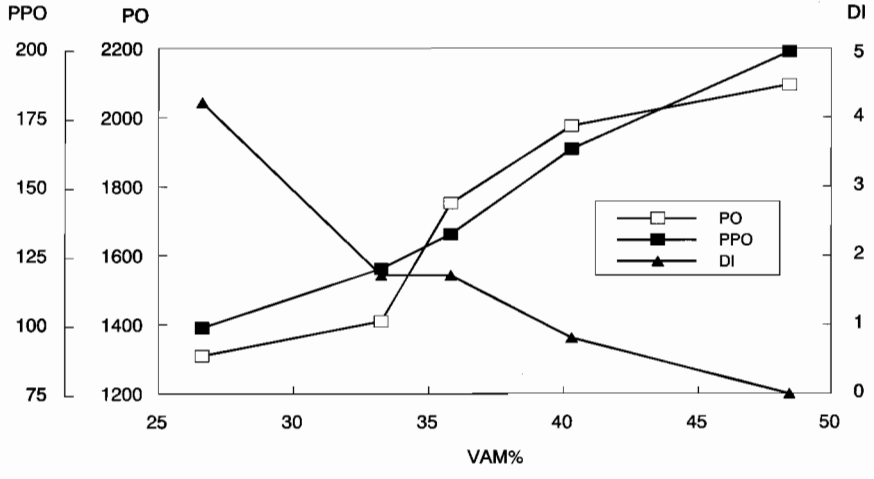

Figure 2. Effect of the degree of mycorrhizal formation (VAM%) on peroxidase (PO) and polyphenoloxidase (PPO) optical density enzyme activity and the disease index (DI) of poplar.

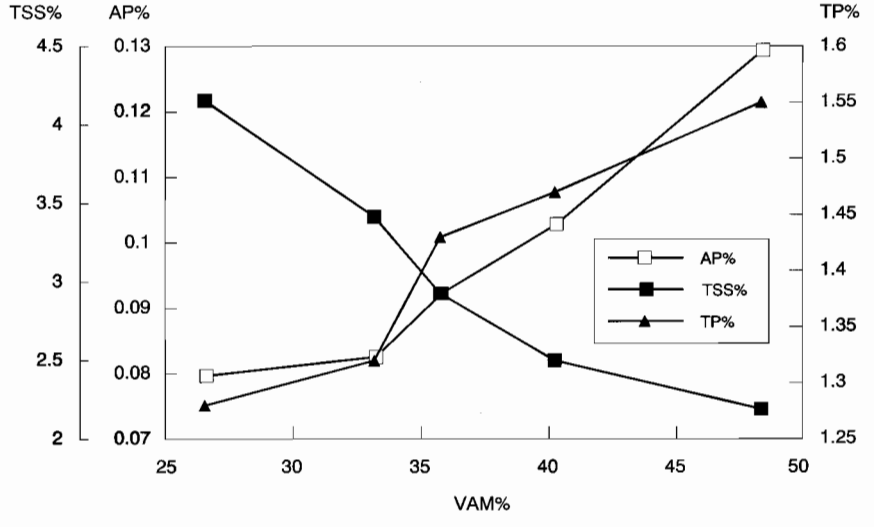

Figure 3. Effect of mycorrhizal formation (VAM%) on available phosphorus (AP%), total soluble sugars (TSS%) and total phenolics (TP%) in poplar bark.

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Inoculation of *Pinus massoniana* with Ectomycorrhizal Fungi: Growth Responses and Suppression of Pathogenic Fungi

Xiaohong Li, Bingfu Fu and Jingyong Yu*

Abstract

The present study investigated the inoculation of *Pinus massoniana* with pure cultures of ectomycorrhizal fungi. The aim of this research was to demonstrate the value of mycorrhizal technology for forest production in Guizhou Province. Inoculation of *Pinus massoniana* plants with pure culture inoculum of *Suillus grevillei* and *Boletus* sp. was successful, resulting in 95–100% mycorrhizal root formation in the pine seedlings. Plant height, basal diameter and dry matter of the inoculated seedlings increased substantially compared to the uninoculated plants. In a subsequent experiment, pine seedlings inoculated with the same ectomycorrhizal fungi were infected with the pathogenic fungi *Fusarium sodani* and *Rhizoctonia solani* to test antagonistic interactions. It was found that the infection rates by these pathogens in ectomycorrhizal pine seedlings decreased by 10–46 % relative to the uninoculated seedlings. Inoculation of pine plants with ectomycorrhizal fungi, such as *Suillus grevillei* and *Boletus* sp., is recommended to enhance plant growth and resistance to pathogenic fungi.

Key words: Ectomycorrhizal fungi, growth promotion, *Pinus massoniana*, pathogen resistance, mycorrhizal formation, *Fusarium sodani*, *Rhizoctonia solani*

THE Masson pine *Pinus massoniana* Laub. is an important tree species for the production of timber and pine resin in southern China, with widespread uses and high economic values. This species plays an important role in the reforestation of the southern provinces of China, and as a pioneer tree species for reforestation in Guizhou Province.

Masson pine is associated with ectomycorrhizal fungi in the field and can not grow without mycorrhizal roots (Hua Xiaomei and Jiao Luzheng, pers. comm.). As a result, it is important to understand how to utilise ectomycorrhizal fungi in the preparation of Masson pine seedlings for reforestation. Mycorrhizal inoculation is a routine practice during seedling culture for reforestation in some countries and has the potential to increase tree growth and timber production (Guo Xiouzheng and Bi Guochang 1989). In order to promote the application of ectomycorrhizal fungus technology for forestry in Guizhou Province, a series of experiments on the ectomycorrhizal associations of pine trees in the central and southeastern areas of Guizhou Province were started in 1989.

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Materials and Methods

Fungal resources

Cultures of the ectomycorrhizal fungi *Boletus* sp. (B) and *Suillus grevillei* (S) were provided by Professor Guo Xiouzhen of the Chinese Forestry Institute. Another *Boletus* species (Bn) was isolated by the Forestry Institute of Guizhou Province. Cultures of the pathogenic fungi *Fusarium sodani* (F) and *Rhizoctonia solani* (R) were supplied by the Microbiological Institute of the Science Academy of China.

Mycorrhizal inoculation experiment

In mid-March 1989, seeds were sterilised with a 0.5% $KMnO_4$ solution for 2 hours, and then rinsed with water. They were soaked and germinated in water for 2 days before sowing.

Growth bags were made from 20 cm by 14 cm polyethylene sheets, with several holes punched in the bottom of each bag for drainage. These bags were filled with a potting mix composed of peat, vermiculite and surface loam soil (volume ratio 1:1:2). The potting mix was fumigated with formalin solution (50:1 v/v) for 4 days and watered with acidified water (pH 5) several times before and after seeding.

Fungal isolates, that were maintained as slope cultures on agar media, were subcultured to produce inoculum as follows using a culture media containing 80% vermiculite, 18% corn meal, 1% glucose, 1% $CaSO_4.2H_2O$, 0.1% KH_2PO_4, 0.1% K_2HPO_4, 0.1% NH_4Cl and 0.1% $CaCl_2$ in water. The culture media was mixed and 500 g of this solid culture substrate was put in each culture bottle, which was sealed with cotton and paper. The bottles with culture media were sterilised. After autoclaving (121°C for 45 minutes) and cooling to 30°C, bottles were inoculated with fungal isolates and incubated at 25°C for 25 days.

Pure culture inoculum, prepared as described above, was mixed with the bagged potting mix at a ratio of 1:10. Pure culture media without fungal inoculum was also mixed with the potting mix in a 1:10 ratio for the control treatment. Two germinated seeds were then transplanted into each of the growth bags. There were 3 treatments in this experiment, a control (without inoculation) and fungal treatments with *Boletus* sp., and *S. grevillei* inoculum. Each treatment was replicated 3 times and there were 20 growth bags within each replication. All replicates were randomly arranged in a glasshouse.

The plants were sampled on July 30, 1989. Sixty plants were harvested in each treatment (20 plants per replicate). The parameters determined were plant height, basal diameter, the number of lateral roots, dry matter, shoot growth and the level of mycorrhizal colonisation of roots. Mycorrhizal formation was recorded as the percentages of mycorrhizal roots relative to the total number of lateral roots, using 5 categories — level 0: 0%, level 1: 1–25%, level 2: 26–50%, level 3: 51–57% and level 4: 76–100%.

Laboratory study of antagonism between mycorrhizal and pathogenic fungi

These experiments were conducted from March 1990 to January 1991. Agar plates containing potato dextrose agar (PDA) culture medium (Yu Daba 1959) were inoculated with the mycorrhizal fungi B, S, and Bn and the pathogen fungi F and R on the same petri dishes in a randomised design, to examine antagonistic interactions. Each treatment was replicated 3 times. The growth of hyphae and colonies were monitored.

Nursery study of antagonism between mycorrhizal and pathogenic fungi

Seed germination treatments and the fungus inoculum production procedures were the same as described above. Each of the seedling beds was 1 m^2 in area and 15 cm in height, with a 30 cm gap between any two neighbouring seedling beds. Four rows were prepared for sowing seeds in each seedling bed. There were 7 treatments including inoculation with the same 3 species of mycorrhizal fungi and 2 species of pathogen fungi used in other experiments, individually and in combination (3 by 2), and the control (without inoculation). Each treatment was replicated three times (small blocks) and the replicates of the treatments were randomly arranged. A replicate block was a 1 m^2 seedling bed which was inoculated with 50 g of pure culture inoculum of mycorrhizal fungi B, S and Bn. Pathogenic fungi were isolated and 5 mL of inoculum from an agar slant of each was transferred into test tubes with water which was evenly sprayed onto each of the replicate blocks (one tube per block). The same medium without fungi was applied to control blocks. Management practices were the same for all the treatments.

After tree seedlings emerged, their growth was measured and pathogen infection was monitored (seedlings infected by pathogenic fungi were pulled out when observed). These observations continued until the end of June and 20 seedlings were sampled in each of the replicates on 17 November 1990. Measurements were made of seedling height, basal diameter, dry matter and lateral root number, and the level of root mycorrhization (as described above). Statistical analyses were performed to compare experimental treatment effects, on tree seedlings.

Experimental methods for mycorrhizal seedling growth for reforestation

Seedlings were grown in a 0.13 ha area of nursery beds. Seeds were sown in mid-March 1992 in sowing ditches of seedling beds which were 13 cm apart. The seed sowing rate was 75 kg/ha. Inoculation was achieved by thoroughly mixing fungal culture inoculum with fine soil and evenly spreading this mixture on seedling beds at a rate of 450 kg inoculum per ha when seeds were sown. The same culture media–soil mixture without fungus was spread onto seedling beds for the control treatment. Seedlings were planted on a 1.5 m × 1.5 m grid.

There were 3 inoculation treatments in this experiment, using the mycorrhizal fungi B, S and Bn. The inoculation treatments were arranged in a paired pattern for both seedling growth and reforestation experiments. Certified seedlings were judged according to the standard for Class I seedlings used in Guizhou Province. Only tree seedlings from beds, where more than 90% of the seedlings met Class I standards and root mycorrhization rates were more than 95%, were used in the reforestation trial.

On 20 November 1992, 240 plants in each treatment were sampled. The parameters measured were plant height, basal diameter, lateral root numbers, and the level of root mycorrhization. A reforestation area

of 10 ha was planted using these seedlings in January 1993. The survival rate of the transplanted seedlings was determined 3 months after transplanting. At the end of 1993, the rates of survival of young trees and new shoot branching were measured in 256 plants grown in a field of 0.07 ha.

Results

Inoculation of seedlings in growth bags

The inoculated tree seedlings had a very high level of mycorrhizal formation (Table 1). From the measurements and investigation, *P. massoniana* formed an association with all of the mycorrhizal fungi tested. It indicated that this pine species is compatible with the mycorrhizal fungus species used (*Boletus* sp., and *S. grevillei*). The infection rate in the inoculated seedlings reached 100% and the proportion of seedlings with level 3 and 4 colonisation was 83.3 and 86.7% respectively, but only 19.2% of the control plants had mycorrhizas (3.4% with level 3 or 4 infection rates) (Table 1). Mycorrhizal inoculation had a substantial effect on the growth of tree seedlings (Table 1).

Table 1. The growth parameters measured in pine plants grown in growth bags inoculated with *Boletus* sp. (B) or *Suillus grevillei* (S) or not inoculated (control).

	Inoculation treatment[a]		
Growth parameter	B	S	Control
Plant height (cm)	20.0**	19.4**	16.6
Basal diameter (cm)	1.3**	1.2**	1.1
Lateral roots (number)	15.1**	14.7**	11.5
Dry weight (g)	0.6**	0.6**	0.5
Mycorrhizal roots[b]	86.7	83.3	3.4

** The *t*-test values indicate a significant difference from the control at P = 0.01

[a] Values are means of three replicates

[b] Percent of level 4 mycorrhizal roots (see methods)

Antagonism between mycorrhizal and pathogenic fungi *in vitro*

Antagonism tests in plate culture demonstrated significant antagonistic interaction between the mycorrhizal fungi B, S and Bn and the pathogenic fungi F and R (Table 2). In these experiments growth of the pathogenic fungi stopped in the presence of mycorrhizal fungi, which grew vigorously in zones of overlap and eventually occupied the whole plate. Both types of fungi grew well when they were cultured on their own. When fungal hyphae from zones of overlap between pathogenic and mycorrhizal fungi were used to start new plate cultures, most of the resulting colonies were of mycorrhizal fungi (Table 3).

Antagonism between mycorrhizal and pathogenic fungi in seedling beds

Antagonistic effects of mycorrhizal fungi on pathogenic fungi were studied by examining the infection of tree seedlings by pathogenic fungi 15 days after their emergence. The results shown in Table 4 indicate that the mycorrhizal fungi tested in the present experiment had an inhibitory effect on the pathogenic fungi R and F. This was consistent with the results from the plate culture experiments in the laboratory. Seedlings inoculated with mycorrhizal fungi had significantly lower infection rates by the pathogenic fungi than the plants in the control treatment.

At the end of the nursery interaction experiment, various growth parameters of the tree seedlings were measured (Table 5). There was a significant difference between the plants inoculated with the mycorrhizal fungi and those without inoculation (a *t*-test showed the significant level as $P < 0.01$). The growth of plants inoculated with both pathogenic and mycorrhizal fungi appeared normal, but was significantly different from the plants in the control ($P < 0.05$ from *t*-test). The experimental results demonstrated that the mycorrhizal fungi tested here not only had certain inhibitory effects on pathogenic fungi, but also had stimulatory effects on plant growth.

Reforestation experiment with tree seedlings inoculated with mycorrhizal fungi

At the end of this experiment seedling plants were sampled and their growth was examined (Table 6). The results show that mycorrhizal inoculation of the cultured seedlings resulted in a high level of mycorrhizal colonisation and significantly increased plant biomass and seedling quality.

In early 1993, mycorrhizal seedlings from the nursery inoculation trial were used in a reforestation trial. The survival rates of the transplanted seedlings were investigated after 3 months of growth. The results showed that root mycorrhization greatly increased the survival rate of the transplanted seedlings. Their survival rate almost reached 100%. In mid-November 1993, the survival rates and the growth increments of newly-initiated shoots were investigated (Table 7). Seedlings produced by standard procedures (controls) were also examined and were planted at the same time as the mycorrhizal seedlings.

Table 2. Effects of mycorrhizal fungi[a] on the time to death (hours) of pathogenic fungi[b] in an antagonistic culture experiment.

Mycorrhizal fungus	Pathogen R		Pathogen F	
	Exposed hyphae	Hyphae in medium	Exposed hyphae	Hyphae in medium
B	72	76	48	48
S	84	84	72	72
Control	48	48	48	48

[a] Mycorrhizal fungi: B = *Boletus* sp; S = *Suillus grevillei*
[b] Pathogenic fungi: R = *Rhizoctonia solani*; F = *Fusarium sodani*

Table 3. The number of viable colonies resulting from fungal hyphae subcultured from zones of overlap in an antagonistic culture experiment.

Mycorrhizal fungus:	S	S	B	B	Bn	Bn
Pathogenic fungus:	F	R	F	R	F	R
Recovered fungus						
S	80	110				
B			100	100		
Bn					110	100
F	20		10		30	
R				10		10

Note: Different combinations of mycorrhizal and pathogenic fungi were used. See Table 2 for key to mycorrhizal and pathogenic fungi; Bn = *Boletus* sp.

Table 4. Pathogenic fungus infection of tree seedlings inoculated with mycorrhizal fungi.[a]

Treatment	Seedlings (no.)	Infection rate (%)	Preventative effect (%)
S + R	1352	21.74	10.13
S + F	1476	15.65	35.30
B + R	1371	16.41	32.16
Bn + F	1016	18.76	22.45
B	1398	13.52	44.11
S	1390	12.94	46.51
Bn	1382	14.33	40.76
Control	951	24.19	

[a] For key to fungal abbreviations see Tables 2 and 3

Table 5. The growth of tree seedlings inoculated with mycorrhizal fungi alone or combined with pathogenic fungi.[a]

	Height (cm)		Basal diameter (mm)		Dry matter (g)		Mycorrhizas[b] (%)
Treatment	Mean	Increase[c]	Mean	Increase[c]	Mean	Increase[c]	
S + R	22.73	20.65	2.44	17.87	1.06	35.90	69.36
S + F	23.50	24.73	3.04	46.86	1.48	89.74	74.98
B + R	20.75	10.14	2.28	10.14	0.82	5.13	50.02
Bn + R	22.01	16.83	2.58	22.64	0.97	24.36	50.70
B	26.03	38.16	3.19	54.11	1.65	111.54	84.31
S	23.55	25.00	2.63	27.05	1.34	71.78	80.25
Bn	23.89	26.80	2.55	23.19	1.21	55.13	70.08
Control	18.84		2.07		0.78		7.1

[a] For key to fungal abbreviations see Tables 2 and 3
[b] Level 3 and 4 mycorrhizal roots
[c] Percentage increases [(treatment – control)/control) × 100]

Table 6. The growth and mycorrhizal formation of tree seedlings inoculated with mycorrhizal fungi[a] in the nursery.

Treatment	Height (cm)	Basal diameter (mm)	Mycorrhizal seedlings (%)	Mycorrhizas[b] (% of roots)
Bn	25.0	4.2	96	72.2
S	25.3	4.3	98	80.0
B	25.2	4.3	97	75.0
Class 1 seedlings[c]	> 25	> 3.5		

[a] See Tables 2 and 3 for key to fungi
[b] Level 3 and 4 mycorrhizal roots
[c] Quality standard explained in methods

Table 7. The survival rates and the average annual growth of transplanted tree seedlings in the reforestation experiment inoculated with 3 mycorrhizal fungi (B, S, Bn) or uninoculated controls.

Treatment	Bn	S	B	Control
Survival (%)	100	100	100	78
New shoot growth (cm)	20.7	22.2	23.0	18.5

Note: The total number of plants investigated was 256

Discussion

The results from the growth bag inoculation experiment showed that the mycorrhizal fungi *Suillus grevillei* and *Boletus* sp. were both able to form a symbiotic association with the pine plants *Pinus massoniana*. Seedlings inoculated with these fungi had high levels of mycorrhizal colonisation and had vigorous root and shoot growth. Pine needles of inoculated seedlings were dark green, in contrast with the uninoculated seedlings, which had slower growth and leaves that were light green in colour.

When mycorrhizal inoculum was applied to seedlings grown in containers, their roots rapidly and consistently developed mycorrhizas. However, when inoculum was applied to seedlings grown in beds, mycorrhizal formation was less efficient and larger amounts of inoculum were required. There were some disadvantages associated with mycorrhizal inoculation to seedlings grown in containers. The horizontal growth of root systems of seedlings in containers was limited and soil conditions in deep soil layers did not favour the development of mycorrhiza. In the future, we should take the above points into consideration when preparing mycorrhizal seedlings in containers.

Plate-culture experiments showed that there were strong antagonistic interactions between the mycorrhizal fungi *Suillus grevillei* and *Boletus* sp. and the pathogenic fungi *Fusarium sodani* and *Rhizoctonia solani*. However, this was only an observation and the mechanism involved in their antagonistic interaction should be explored in future research.

The antagonistic effects of ectomycorrhizal fungi on pathogenic fungi were further confirmed by a nursery experiment with pathogen-infected seedlings. However, when applying mycorrhizal inoculum to soils, a certain amount of culture medium was included and this may have changed the ecological environment of soil microorganisms. Therefore, the effects of different soil treatments on microbiological dynamics requires further study.

The application of mycorrhizal fungi increased the size and the quality of seedlings produced for a reforestation experiment. It also increased the survival rate of transplanted seedlings in the field and stimulated their subsequent growth.

The present study provides further evidence that mycorrhizal inoculation techniques can be used to enhance the timber production of forest trees. In order to promote the use of mycorrhizal technology, further studies should focus on the effects of management practices, agricultural pesticides and fertilisers on mycorrhizal roots.

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Antagonism Between Ectomycorrhizal Fungi and Plant Pathogens

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Abstract

Six ectomycorrhizal fungi, *Boletus edulis, B.* sp., *Suillus grevillei, S. luteus, Chromogomphus rutilus* and *Xerocomus chrysenteron,* inhibited the growth of the root pathogens *Fusarium oxysporum, F. solani, Rhizoctonia solani, Pythium aphanidermatum, P. ultimum* and *Agrobacterium tumefaciens* in laboratory experiments. In culture experiments, mycorrhizal fungi were found to be able to destroy the vegetative mycelia of pathogens and inhibited the formation and germination of their conidia, sporangia or sclerotia. When the mechanisms of antagonism were examined, it seemed that mycorrhizal fungi could secrete non-volatile substances which caused the contraction and lysis of the protoplasm of pathogens such as *A. tumefaciens* even after high temperature treatment (60°C for 30 min.). Volatile active substances were also detected. Several mycorrhizal fungi produced haustoria in the hyphae of *R. solani* and apparently were parasitic on this fungus.

Key words: ectomycorrhizal fungi, plant root pathogens, antagonistic interactions

MANY scholars have reported on the antagonism of ectomycorrhizal fungi to plant root pathogens. Davis et al. (1942) put forward the hypothesis that ectomycorrhizal fungi can protect fine roots from invasion by pathogens. Levisohn (1954) reported that mycorrhizal seedlings of pines are more capable of resisting the invasion of *Rhizoctonia* than non-mycorrhizal seedlings. Zak (1964), Sinclair et al. (1982), Sylvia (1983) and Marx (1972) documented antagonistic interactions between mycorrhizal fungi and pathogens and suggested that ectomycorrhizal fungi should be used as a method of biological protection against root disease. Guo Xiuzhen (1981a, b) and Lei Zengpu et al. (1983) have reported mycorrhiza can help *Pinus tabulaeformis* seedlings resist damping off disease.

Marx (1972) summarised information on antagonistic interactions and concluded that ectomycorrhizal fungi can produce substances which influence the bacterial, fungal and virus resistance of host plants. Most reports of pathogen resistance due to ectomycorrhizal fungi has concerned bacterial disease. Sinclair et al. (1982) and Sylvia (1983) reported ectomycorrhizas suppressed root disease of Douglas-fir seedlings and protected their primary roots. The purpose of this research was to investigate the wide diversity of Chinese mycorrhizal fungi in order to identify species which protect trees by antagonistic interactions with important plant pathogens in China.

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Materials and Methods

The following ectomycorrhizal fungus isolates were used in experiments: *B. edulis, Chromogomphus rutilus, Xerocomus chrysenteron* and *Lactarius deliciosus,* which were isolated by the authors; *B.* sp. and *Suillus grevillei* from the Chinese Forestry Scientific Academy; *S. luteus* from the Liaoning Forestry Scientific Academy; and *L. lignystus* and *Russula aeruginea* from the Guangzhou Botany Research Institute.

Pathogens of plant roots used in experiments were *Rhizoctonia solani, Fusarium oxysporum, F. solani, Pythium aphanidermatum, P. ultimum* and *Agrobacterium tumefaciens.* These organisms were obtained from the Microbiology Institute of the Chinese Scientific Academy.

Culture media used for experiments were PDA medium and PDA-malt medium. This media was made by cooking 200 g of skinned potato in 1000 mL water and adding 10 g of glucose and 18 g of agar to 500 mL of this liquid.

The following culture methods were used in experiments (Lei Zengpu et al. 1983).

1. A confrontation (dual) culture method, where 2 fungi were grown on opposite sides of a petri dish, was used to study pathogen inhibition by mycorrhizal fungi (Tang Wenhua and Lu Shuyun 1981).

2. Spore germination on the surface of a petri dish and plate counting methods were used to measure the inhibition of pathogenic fungus mycelia by mycorrhizal fungus hyphae (Fang Zhongda, pers. comm.).
3. The plate drop counting method (Fang Zhongda, pers. comm.) was used to evaluate the impact of mycorrhizal fungi on the reproductive structures of pathogenic fungi.
4. The Basier method was used to measure inhibitory effects of volatile substances released by mycorrhizal fungus hyphae on pathogenic fungus hyphae (Yang Wang, pers. comm.).
5. The filter culture method (Mu Lixi 1994) was used to measure inhibitory effects of substances secreted by mycorrhizal fungi on pathogenic bacteria.
6. Scanning electron microscopy was used to observe direct parasitism of pathogenic fungi by mycorrhizal fungi.

There were 5–10 replicates for every treatment. Results were analysed by a modified Duncan Multiple Range test (U test).

Results and Discussion

Confrontation culture experiments demonstrated that pathogen fungus growth stopped when they were in close proximity to mycorrhizal fungus hyphae, but mycorrhizal fungus growth continued until the pathogenic fungus colony was totally covered (Table 1). Growth of mycorrhizal fungus hyphae in areas of overlap between pathogenic and mycorrhizal fungi was better than that of controls (mycorrhizal fungus grown alone). Mycelia from these zones of overlap could be used to start new cultures which contained mycorrhizal, but not pathogenic, fungi in most cases. This suggests that hyphae of the pathogens were killed by the mycorrhizal fungi, but pathogenic fungi, such as *R. aeruginea* and *L. lignystus,* did not inhibit mycorrhizal fungi. The time required for mycorrhizal fungi to overwhelm pathogenic fungi was different, *B.* sp. and *S. grevillei* killed *F. oxysporum* in one day, but *X. chrysenteron* required 5 days to kill *R. solani* (Table 1).

Tests of interactions between ectomycorrhizal fungi and plant root pathogens demonstrated that ectomycorrhizal fungi can also inhibit the formation and germination of reproductive structures by pathogens (Tables 2, 3). *Chromogomphus rutilus* can inhibit the formation of *Fusarium* and *Pythium* reproductive structures. This is the first report on the antagonism of *Lactarius* and *Russula* to the pathogen *Fusarium,* but these fungi had little effect on *Pythium.* This observation is in agreement with earlier reports by Marx (1972). Statistical comparisons showed that most differences between mycorrhizal fungal treatments and the control are significant (Table 4).

The mycorrhizal fungi *B. edulis, B.* sp., *S. grevillei, S. luteus* and *Ch. rutilus* inhibited the formation of sclerotia by *R. Solani,* but the mycorrhizal fungi *X. chrysenteron, L. deliciosus, and R. aeruginea* were less effective (Tables 5 and 6).

In another experiment, antagonism between ectomycorrhizal fungi and the root-canker bacterium *A. tumefaciens* was studied. On plate culture, mycorrhizal fungus colonies rapidly covered the *A. tumefaciens* colonies. Samples were taken from these covered colonies a week later and used to start dilution-series cultures (Table 7). These results demonstrated that ectomycorrhizal fungi that were antagonistic to fungal pathogens also had a suppressive effect on *A. tumefaciens.*

Results of preliminary studies suggest that the mechanism of pathogen antagonism by ectomycorrhizal fungi are (1) the production of nonvolatile or volatile antibacterial substances and (2) direct parasitism of pathogenic fungi by mycorrhizal fungi.

Antagonism by non-volatile substances

After microscopic examination of pathogen colonies that were overgrown by mycorrhizal fungi, it was found that hyphae of the pathogens (*F. oxysporum, F. solani, R. solani, P. aphanidermatum,* etc.) showed signs of severe damage. In some experiments the cell walls of pathogens were lysed (such as *B. edulis —R. solani).* In the control cultures, hyphae of pathogens were healthy and grew normally. Whether these nonvolatile substances produced by mycorrhizal fungi are enzymes or antibiotics is not known.

A mycorrhizal fungus (*G. rutilus*) was cultured in liquid potato dextrose medium heated to 60°C for 30 minutes. An 8 mm diameter piece of sterilised paper was dipped in this liquid then placed on a plate used to culture *A. tumefaciens,* to examine antagonism. This liquid extract from a *G. rutilus* culture resulted in a 3.5 mm wide circle where *A. tumefaciens* did not grow.

Antagonism by volatile substances

The Basier method was used to measure the production of volatile substances which inhibit bacteria. Experimental results showed that the mycorrhizal fungi organisms of *B. edulis* and *B.* sp. produced volatile substances which could inhibit hyphal growth by the root pathogenic fungi *P. ultimum* and *P. aphanidermatum.* Krupa and Fries (1971) also reported that *B. variegatus* could produce volatile antagonistic substances.

Antagonism by parasitism

There are many organisms which are parasitic on plant root pathogens (Weindling 1934), but there are no reports on the parasitism of plant root pathogens by mycorrhizal fungi (Guo Xiuzhen 1981). Lei Zengpu et al. (1983) observed by optical microscopy that hyphae of ectomycorrhizal fungi could adhere to the hypha of pathogens, forming finger-like hyphal projections.

Table 1. Time required for mycorrhizal fungus hyphae to kill pathogenic fungus colonies in confrontation culture experiments (days[a]).

Mycorrhizal fungus	Pathogenic fungus					
	R. solani		*F. oxysporum*		*P. ultimum*	
	Aerial[b]	Substrate[b]	Aerial[b]	Substrate[b]	Aerial[b]	Substrate[b]
Ch. rutilus	3	3	5	4	2	2
S. grevillei	2	2	1	1	2	2
B. sp.	2	2	1	1	2	2
X. chrysenteron	5	5	3	3	3	2
L. deliciosus	–	–	–	–	2	2

[a] Times are those required so that only ectomycorrhizal fungus mycelia are present in new cultures isolated from regions of overlap between colonies
[b] Mycelia growing in the substrate and aerial hyphae were examined separately

Table 2. The effect of ectomycorrhizal fungi on the formation of reproductive structures (conidia and sporangia) by 3 pathogenic fungi.

Mycorrhizal fungus	Pathogenic fungus		
	F. oxysporum[a]	*F. solani*[a]	*P. ultimum*[b]
B. edulis	0	0	0
B. sp.	9	0	0
S. grevillei	9	0	0
S. luteus	0	0	0
X. chrysenteron	18	0	0
Ch. rutilus	12	34	0
L. deliciosus	36	10	9
L. lignystus	90	50	5
R. aeruginea	68	11	-
Control (none)	127	84	87

[a] Number of conidia in a 6 mm culture plate
[b] Number of sporangia in one microscope field of view

Table 3. Inhibitory effects of different ectomycorrhizal fungi to the germination of *F. oxysporum* spores.

	B. sp.	*S. grevillei*	*X. chrysenteron*	*Ch. rutilus*	*F. oxysporum* (control)
Germination time (h)	24	22.1	5.8	18.0	2.9
Germination rate (%)	48	33.3	10.7	21.8	91.6

Note: Mycorrhizal fungi were added to plates with a spore suspension of *F. oxysporum*

Table 4. Analysis of variance (ANOVA) statistical comparisons of the effect of ectomycorrhizal fungi on the formation of reproductive structures by pathogenic fungi.

Mycorrhizal fungi	Pathogenic fungus		
	F. solani	*F. oxysporum*	*P. ultimum*
X. chrysenteron	26.52**	-	-
Ch. rutilus	14.03**	21.46**	
L. deliciosus	22.69**	6.05**	1.12
L. lignystus	6.78**	2.42*	3.14**
R. aeruginea	22.54**	10.25**	-

Notes: This table contains ANOVA F values for individual comparisons; ** = $P < 0.01$, *= $P < 0.05$

Table 5. Inhibition of *R. solani* sclerotium formation by different ectomycorrhizal fungi.

Mycorrhizal fungus	Sclerotium formation[a]
B. edulis	0
B. sp.	0
S. grevillei	0
S. luteus	0
X. chrysenteron	1
Ch. rutilus	1
L. deliciosus	3
L. lignystus	3
R. aeruginea	3
R. solani (control)	4

[a] This level refers to the area of sclerotia formation on a culture plate surface, 0 = none, 1 = 1/4 of the plate area, 2 = 1/4 – 1/2, 3 = 1/2 – 3/4, 4 = > 3/4

We have used the scanning electron microscope to make similar observations of parasitic interactions between ectomycorrhizal fungi and plant pathogens (unpublished data).

Conclusions

The results of culture experiments showed that ectomycorrhizal fungi such as *B. edulis, B.* sp., *S. grevillei, S. luteus, Ch. rutilus* and *X. chrysenteron* can be antagonistic to pathogens of plant roots such as *R. solani, F. oxysporum, F. solani, P. aphanidermatum* and *P. ultimum*. Mycorrhizal fungi were able not only to kill mycelia of pathogens, but could also inhibit the formation and germination of spores (conidia, sporangia) and fungal sclerotia. The pathogenic fungi used in this research commonly invade plant roots in China. However, it is not clear if mycorrhizal fungi would also have antagonistic interactions with these pathogens in the field. Field experiments are required to study this potentially valuable role of ectomycorrhizal fungi.

Table 6. Effect of ectomycorrhizal fungi on the germination of *R. solani* sclerotia.

	B. sp.	*C. rutilus*	*X. chrysenteron*	*L. deliciosus*	*L. lignystus*	*R. aeruginea*	Control
Number of sclerotia	56	60	65	80	80	80	80
Germination rate(%)	2.0	0	53.8	61.5	100	100	100

Table 7. The effect of mycorrhizal fungi on dilution-plate recovery of the root-canker bacterium *A. tumefaciens*[a].

Group	*S. grevillei*	*S. luteus*	*B. edulis*	*B.* sp	*G. rutilus*	*X. chrysenteron*	Control
Colonies	5	0	0	0	0	0	235
Inhibition rate (%)	97.9	100	100	100	100	100	

[a] A 3 mm diameter circle of bacterial colonies covered by the fungus was diluted 10^{-6} and replated

The results showed that mycorrhizal fungi such as *B. edulis, B.* sp., *S. luteus, S. grevillei, X. chrysenteron* and *Ch. rutilus,* which can increase the biomass of Chinese pines (Wang Changwen 1985; Lei Zengpu, this volume), have a dual role as antagonists to pathogens. Thus ectomycorrhizal fungi would be superior to other biological products which are only antagonistic to pathogens (Lei Zengpu 1983).

In the present study, it is demonstrated that *L. deliciosus* can enhance the production of sporangia by *Pythium*. It is suggested that this mycorrhizal fungus may not be suitable for nursery inoculation.

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The Relationship Between Cold Tolerance and Ectomycorrhizal Associations of Korean Pine Seedlings

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Abstract

The relationship between winter resistance of evergreen conifers and ectomycorrhizas in natural conditions was studied using two-year old Korean pine (*Pinus koraiensis*) seedlings with different mycorrhizal infection levels chosen from the nursery and axenic Korean pine seedlings inoculated with *Hebeloma mesophaeum*. Using HPLC methods, the cytokinin (Zeatin, IPA) content in needles of the seedlings was assayed. Increased levels of cytokinins, chlorophyll and carotenoids in ectomycorrhizal plants were demonstrated. A potential mechanism for the recovery from winter chlorosis in Korean pine seedlings is discussed.

Key words: Korean pine, ectomycorrhiza, winter injury, cold tolerance, cytokinins, antioxidants

MEMBERS of the family *Pinaceae* depend on ectomycorrhizas for adequate uptake of nutrients and water. Among other benefits to the host attributed to ectomycorrhizas is increased survival potential in adverse environments (Trappe 1977; Maronek 1981; Harley and Smith 1983; Yun Wang and Liu Chen 1988). Korean pine (*Pinus koraiensis* Sieb et Zucc) is one of the main forest species in northeastern China. However, seedlings of this species often die in nurseries and in clear-cut areas during winter. Previous experiments suggest that winter injury is related to photooxidation caused by free radicals which resulted from low temperatures in combination with strong solar radiation (Tao Dali et al. 1988). Slightly injured seedlings are able to recover when the stress is removed in spring. Preliminary experiments have shown that exogenously applied cytokinins can play an important role in the synthesis of chlorophyll during the recovery of injured seedlings (Jin Yuehua, pers. comm.).

Some ectomycorrhizal fungi produce cytokinins in pure culture (Miller 1971; Crafts and Miller 1974). Allen et al. (1980) reported that cytokinin activity increased in both leaves and roots of *B. gracilis* with vesicular-arbuscular mycorrhizal (VAM) infection. Other plants such as *Citrus limon*, *Citrus aurantium* had increased cytokinin levels after inoculation with VAM fungi (Edriss et al. 1984; Dixon et al. 1984).

There have been few experiments concerning winter injury in coniferous trees with ectomycorrhizas. In an experiment by Maronek (1981), overwintered *Pinus strobus* inoculated with *Pisolithus tinctorius* had 80% survival rate when overwintered in a greenhouse at low temperatures (2.6°C), while non-mycorrhizal seedlings only had a 20% survival rate. The aim of this study was to examine the effect of ectomycorrhizal associations on the cold tolerance of Korean pine seedlings and to examine mechanisms for any increases in winter resistance due to mycorrhizas.

Materials and Methods

The experimental site was at the Changbai Mountain Forest Ecosystem Research Station of Academia Sinica. The period when frozen soil and frost-restricted plant growth lasted 5 and 8 months in 1989 and 1990, respectively. In winter, the lowest temperature at this site is below –40°C and the solar radiation can be as high as 2000 μmol $m^{-2}s^{-1}$.

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Fungal inocula

The ectomycorrhizal fungus (*Hebeloma mesophaeum*) was grown on potato dextrose (PD) agar slants at 25°C for two weeks and then was transferred to 500 mL glass bottles containing 150 mL of sterile liquid PD solution with small bits of broken glass in the bottom. The liquid culture was transferred onto sterilised vermiculite + peat moss substrate mixed with liquid modified MMN medium (Molina and Palmer 1982). After one month, the fungus had completely overgrown its substrate.

Seedling culture

Seed of Korean pine was obtained from Chao Hekou forest farm in Liaolin Province and stratified at 4°C for three months. The seeds, surface-sterilised with hydrogen peroxide as described by Molina and Palmer (1982), were then germinated in petri dishes containing a piece of moistened filter paper, rinsed aseptically with distilled water once daily and kept at 25°C. Germinants were transplanted into surface-sterilised culture tubes containing autoclaved perlite (121°C for 1 h). Seedlings were grown in a greenhouse with 14 h photoperiod with a light intensity of 500–1000 μmol $m^{-2}s^{-1}$ at 25–30°C, watered once daily and watered with Knop solution once weekly. After two weeks, seedlings were inoculated with 10 g of fungus culture. Uninoculated seedlings served as controls. All seedlings were transplanted into surface-sterilised pots containing soil the following summer. The potted seedlings were placed in the botanical garden of the Shenyang Institute of Applied Ecology, Academia Sinica, for exposure to natural conditions for hardening and then were transported two months later to the experiment site in Changbai Mountain for overwintering. In the third spring (at the end of March), the potted seedlings were transported back to a greenhouse in Shenyang, watered twice weekly, kept at 12–25°C with 500–1000 μmol $m^{-2}s^{-1}$ light levels and their recovery was observed and survival rates investigated.

Potted bare-root seedling

Two-year-old Korean pine seedlings were provided by Chunlei Nursery in Jilin Province. These bare-root seedlings were graded according to the degree of mycorrhiza formation and classified as mycorrhizal or non-mycorrhizal. Then they were planted with four seedlings per pot in autoclaved (125°C for 1 h) forest soil-sand (3:1 v/v). The potted bare-root seedlings were kept at the experiment site and watered once weekly, until the soil froze from November to March. Seedling survival rate was investigated in May. In the third spring (at the end of March), the potted seedlings were transported to the greenhouse in Shenyang, where they were maintained as described above and their recovery was observed.

Analysis procedures

The procedures used for analysis of needle chlorophyll and carotenoid contents are described by Tao Dali et al. (1988). Extraction and purification of cytokinin was as follows: needles (10 g) were ground (10 000–20 000 rpm) and stirred overnight in 80% (v/v) methanol, filtered, and re-extracted three times with methanol (80, 60, 50 ml for 16, 5, 5 h at 4°C). The combined extracts were reduced to 50 mL in a rotary flash evaporator apparatus at 28°C. Pigments were extracted in petroleum ether, the aqueous phase was adjusted to pH 2.8 with 1N HCl and partitioned three times with equal volumes of ethyl acetate. The aqueous phase was filtered, reduced to 10 mL, adjusted to pH 2.8 and then loaded onto a column of 732 ion exchange resin. Samples were then eluted with 150 ml of 3N NH_4OH and adjusted to pH 8.0. The combined elutriates were partitioned three times with equal volumes of water saturated with n-butanol. The combined n-butanol extracts were reduced to dryness, taken up in 2ml ethanol and were used for HPLC analysis. Partially purified cytokinin sample extracts were separated and quantified by high-performance liquid chromatography (HPLC) on a reverse Novapar C_{18} column, with UV-detector monitoring at 254 nm, as described by Shen Zhende et al. (1984).

Results and Discussion

Observations of winter injury and seedling recovery

The effect of mycorrhizal associations on the winter survival and spring recovery of Korean pine was shown by both potted and axenically grown seedlings. The needles of all potted seedlings gradually lost their green colour from October onwards. When temperatures rose in spring, their needles gradually turned green, but some seedlings could not recover and died. The survival rate of mycorrhizal and non-mycorrhizal bare-root seedlings was 82.6% and 47.2% respectively and the survival rate of axenically-grown seedlings inoculated with *Hebeloma mesophaeum* and uninoculated controls was 75% and 27% respectively. When overwintered seedlings were brought back to the greenhouse, the mycorrhizal seedlings became green within 4–5 days, while non-mycorrhizal seedlings gradually began to turn green after 10 days.

The chlorophyll and carotenoid content of needles

The chlorophyll and carotenoid content of needles of mycorrhizal seedlings were higher than those of non-mycorrhizal seedlings, both in October (before overwintering) and April (during the recovery stage in the greenhouse). Levels of chlorophyll and carotenoid were also higher in inoculated seedlings than in uninoculated controls (Table 1).

Table 1. The chlorophyll and carotenoid content in needles of mycorrhizal and non-mycorrhizal seedlings.

Treatment	Chlorophyll (mg/g dry weight)	Carotenoids (mg/g dry weight)	Chlorophyll/ Carotenoids	Month
A. Bare-root seedlings[a]				
mycorrhizal	3.30	0.55	5.80	10
non-mycorrhizal	1.09	0.33	3.33	10
mycorrhizal	3.01	0.42	7.12	4
non-mycorrhizal	1.48	0.35	5.31	4
B. Axenically-grown seedlings				
inoculated with *H. mesophaeum*	3.70	0.43	4.23	4
uninoculated	2.04	0.37	1.78	4

[a] Mycorrhizal formation in mycorrhizal seedlings was > 50% whereas that of non-mycorrhizal seedlings was < 5% (each number is the mean of 3 replicates)

Cytokinin levels

The main types of cytokinins detected were zeatin and isopentyl adenine (IPA)-like compounds. The cytokinin levels in needles of mycorrhizal seedlings were significantly higher than in non-mycorrhizal seedlings at different stages of growth (October, December and April). These differences are also significant during recovery stages. These results also showed that cytokinin activity decreased in winter and increased in the spring. Cytokinin in the needles of mycorrhiza seedlings decreased slowly in winter and increased rapidly in spring compared with non-mycorrhizal seedlings (Table 2). Needle concentrations of cytokinins were also greater in seedlings inoculated with *Hebeloma mesophaeum* compared with uninoculated controls (Table 3).

Mycorrhizal formation in roots was related to higher chlorophyll content in needles and higher survival rates of Korean pine seedlings, which may have been due to increased cytokinin levels in needles. Cytokinins have been reported to be free radical scavengers, which may protect chlorophyll and other leaf components from degradation, or they may promote chlorophyll synthesis (Lesham et al. 1986). Reductions in chlorophyll content is known to be a good indicator of winter photooxidation injury in Korean pine seedlings (Tao Dali et al. 1988). The higher content of carotenoids in mycorrhizal seedlings would also be beneficial because carotenoids are single-oxygen quenchers. Cytokinins produced by mycorrhizal fungi, or other signals transported from roots to shoots, may induce other changes in needles which retard chlorophyll loss or stimulate its synthesis. Reduced cytokinin contents in the winter may be related to long-term soil freezing inhibiting cytokinin synthesis in roots and mycorrhizas, or because temperature stress and solar radiation resulted in the consumption of cytokinins in needles.

Conclusions

Mycorrhiza formation could increase the survival potential of overwintering Korean pine seedlings by: (1) increasing the synthesis of endogenous protection substances so that the winter resistance of seedlings is enhanced and winter injury resulting from photooxidation is reduced; or (2) by promoting the recovery of seedlings after the period of cold stress is over. Further research will be required to examine the importance of mycorrhizal associations for the survival of pine seedlings in areas with harsh winter conditions.

Table 2. The cytokinin content in needles of potted bare-root seedlings (mg/100 g dry weight).

	Zeatin		IPA	
Time	Mycorrhizal	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal
October	2.32	2.03	0.29	0.13
December	0.19	0.05	0.30	0.16
April	2.34	0.93	0.88	0.42

Table 3. The cytokinin content of Korean pine needles in seedlings inoculated with *H. mesophaeum* in April during the recovery phase (mg/100 g dry weight).

Seedling	Zeatin	IPA
Inoculated	1.37	0.89
Uninoculated	0.65	0.19

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Auxin Accumulation by Homocaryotic and Dicaryotic Mycelia of the Ectomycorrhizal Fungus *Laccaria bicolor*

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Abstract

Homocaryotic mycelia obtained from spores of *Laccaria bicolor* (isolate S238) and dicaryotic strains obtained by crossing the compatible homocaryotic strains have been compared *in vitro* for their ability to synthesise the auxin IAA from tryptophane. IAA accumulation by homocaryotic or dicaryotic progeny was at least 8 times lower than that of the wild parent strains.

Key words: *Laccaria bicolor*, ectomycorrhizal fungus, IAA, auxin, homocaryon, dicaryon

THE capacity of ectomycorrhizal fungi to produce plant hormones has been reported by many authors (for a review see Gogala 1991). Based on comparative root morphology and root ramification following addition of the auxin IAA, or fungal colonisation, it has been suggested that fungal auxins play a key role in the establishment of ectomycorrhizal associations. Recent investigations using fluoroindole-resistant mutant of *Hebeloma cylindrosporum* indicated that fungal IAA controls major anatomical features of pine ectomycorrhiza (Gay et al. 1994). Overproduction of IAA induced an abnormal proliferation of the intercellular network of hyphae (Gea et al. 1994).

Massive secretion of fungal auxins toward the roots is unlikely. Auxin concentration in the different root tissues is presumably regulated by the fungal partner during the various stages of the mycorrhiza ontogeny. This regulation may imply targeted secretion of auxins and auxin conjugates, and localised regulation of root endogenous auxin metabolism (i.e. through a fungal auxin oxydase inhibitor). The role of other fungal indolic compounds should also be considered.

The purpose of this study was to investigate genetic variability among homocaryotic mycelia (obtained from spores) and dicaryotic progeny (obtained by crossing compatible homocaryons) of *Laccaria bicolor* isolate S238. The ability of homocaryotic or dicaryotic strains and the parent isolate to synthesise IAA from tryptophane was compared in sterile culture experiments.

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Materials and Methods

Fungal isolates

Strain S238 of *Laccaria bicolor* (Maire) P.D. Orton, isolated by Trappe and Molina in Oregon, USA was used in this study (Armstrong et al. 1989). This strain is known to be an efficient mycorrhizal fungus in nurseries (Le Tacon and Bouchard 1986). Carpophores of strain S238 were collected in an experimental nursery at Peyrat le Chateau, France, among one-year-old seedlings inoculated with this strain after soil fumigation with methyl bromide.

Spore germination and culture conditions

Spores collected in sterile petri dishes were germinated, using the method of Fries (1983). The homocaryotic strains growing from a single spore were isolated as soon as they could be observed under a dissecting microscope. The mycelia were maintained on malt agar (Difco), in petri dishes at 25°C. To obtain dicaryotic mycelia, two homocaryotic isolates were subcultured together on malt agar medium (Fries and Mueller 1984). When the strains were compatible, plasmogamy occurred within 2 weeks and clamp connections could be observed. For IAA production assessment, fungal strains were grown for 3 weeks on 20 mL of liquid Pashlewski medium

(Albrecht et al. 1994) supplemented with tryptophane (0.0, 0.1, 0.5, 1.0, 2.0 mM) in 100 mL flasks. Each result is the mean of 9 to 11 replicates.

IAA assays

IAA concentration in liquid media was assessed either colorimetrically using Salkowski reagent (Gay and Debaud 1987), or by liquid chromatography. HPLC was conducted (Graham 1991) on a System Gold instrument (Beckman) interfaced with programmable solvent system module 126 and programmable detector module 166 (UV detection at 280 nm). The column was a Lichrospher RP18 end capped (5 mm, 4x250 mm, Merck Chimie, Paris) using a gradient of 0 to 54% methanol in water at pH 3 over 36 min with a flow rate of 1.5 mL min^{-1}.

Results

Mycelium growth and IAA accumulation in the external medium of *Laccaria bicolor* S238 was dependent on tryptophane supply in culture media (Fig. 1). The optimum concentration of tryptophan for both fungal growth and IAA accumulation was 1 mM (Fig. 1B) and this concentration was used in subsequent experiments. Three weeks of growth were required before the nutrient medium could be sampled.

Comparison of *Laccaria bicolor* S238 with some homocaryotic or dicaryotic strains from its progeny indicates that the progeny grew at a faster or slower rate than the wild parent strain (Fig. 2A). However, the parent strain always accumulated more IAA in external medium (from 2.5–7.3 times more) than the homocaryotic or dicaryotic strains (Fig. 2B). These results were confirmed by comparing 87 homocaryotic strains and 15 dicaryotic strains. The homocaryotic strains tended to grow faster than the parent strain (Fig 3A), but the latter accumulated 8 times more IAA in the external medium than its progeny (Fig 3B).

The HPLC was used to confirm results from colorimetric assays. This technique confirmed that the IAA concentration in nutrient medium collected from the dicaryotic strain 152-123 was strongly reduced compared to the wild parent strain S238 (Fig. 4).

Discussion

Haploid homocaryotic strains obtained from germinated spores seem to have lost the capacity to excrete large amounts of IAA in their external medium. Dicaryotic strains obtained by crossing compatible homocaryotic strains also accumulated much less IAA than the wild parent strain. Such observations contrast with previously published results on IAA synthesis by *Hebeloma cylindrosporum* progeny

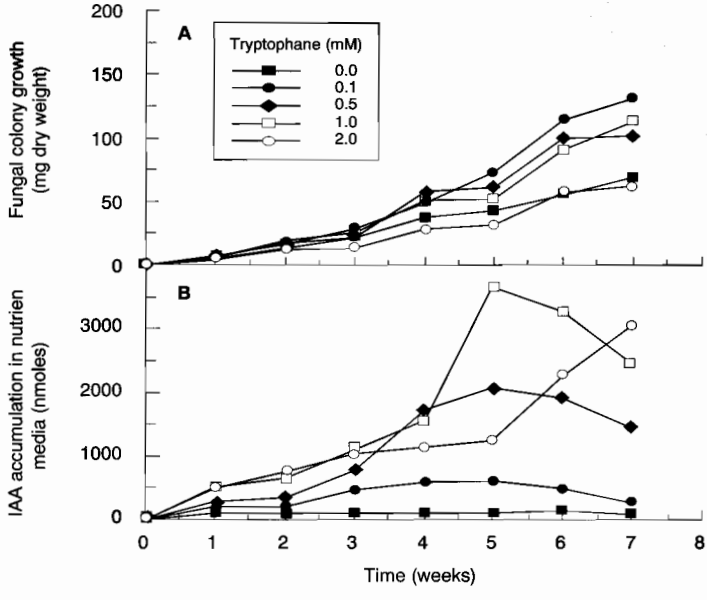

Figure 1. Mycelial growth (A) and IAA accumulation in culture media (B) by *Laccaria bicolor* S238 for 7 weeks on liquid nutrient media supplemented with tryptophane.

(Gay and Debaud 1987). There are numerous published reports, measuring various physiological parameters, which show that the activities of homocaryotic or dicaryotic progeny are similar to those of the wild parent strains (Meyselle et al. 1990; Wagner et al. 1989; Ducamp and Olivier 1989). Our results suggest that an unidentified factor controlling IAA synthesis may have been lost during meiosis in the fungal isolate we used. Further experiments are required to investigate the possible recovery of such factor either during mycorrhizal establishment or fruiting by the fungus.

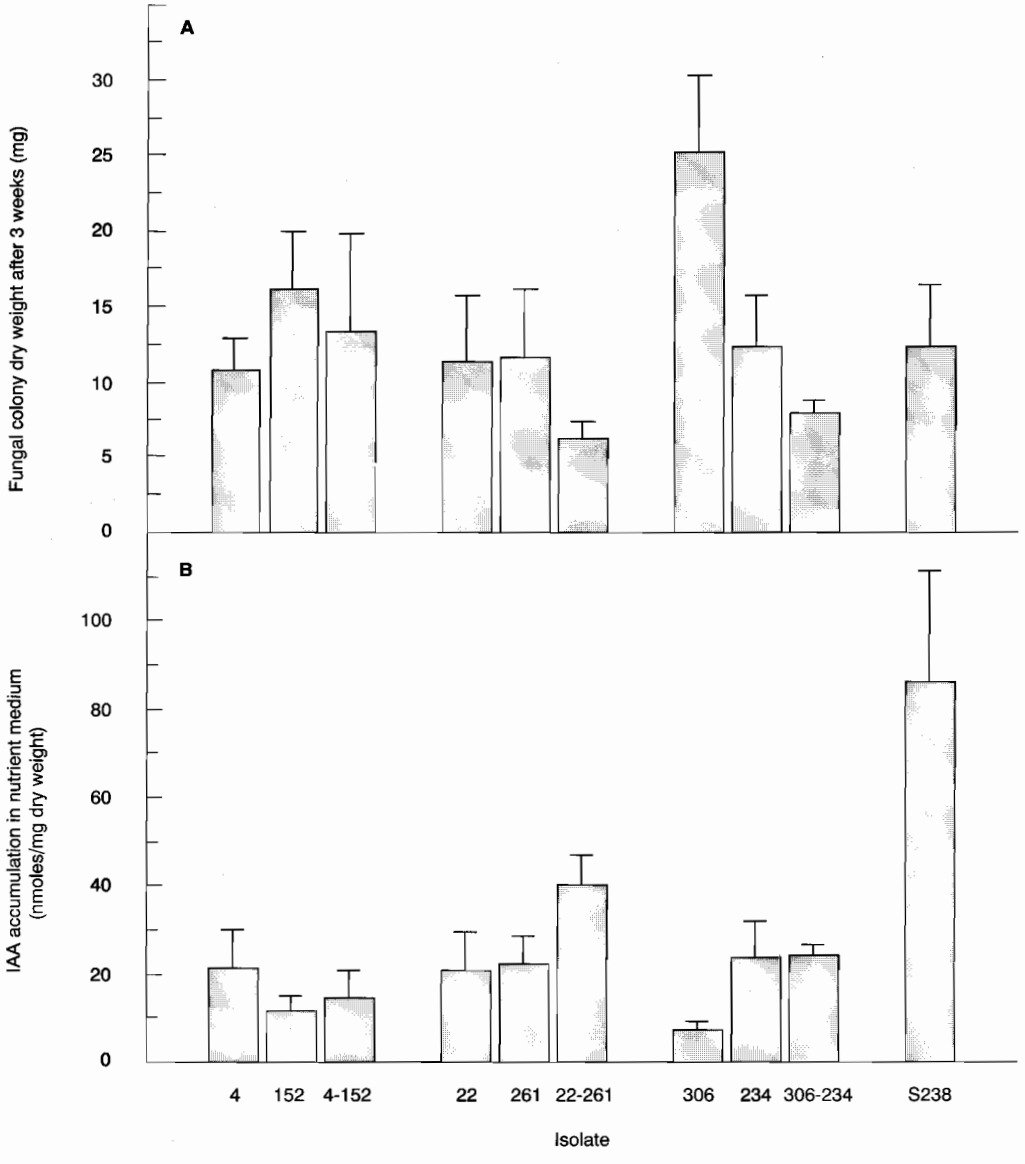

Figure 2. Mycelial growth (A) and IAA accumulation in media (B) by *Laccaria bicolor* S238, homocaryotic strains (4, 152, 22, 261, 306 and 234) and dicaryotic strains (4-152, 22-261, 306-234) on tryptophane supplemented nutrient medium. (Error bar confidence intervals are P=0.05).

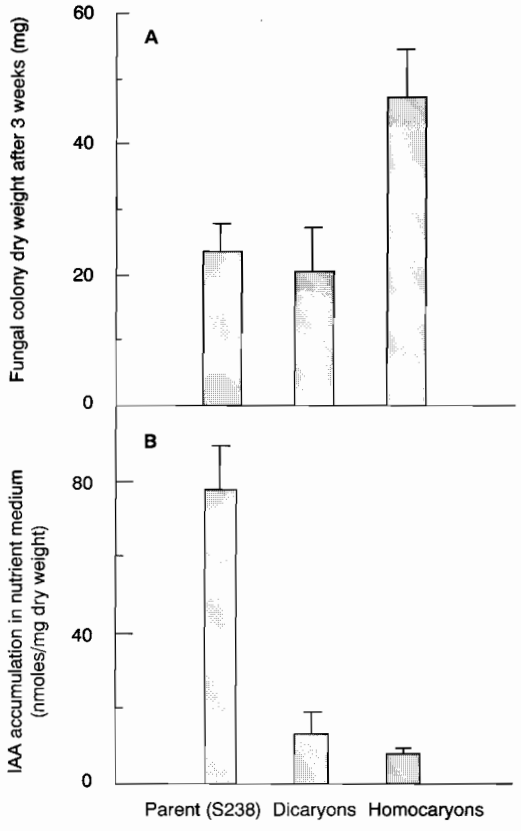

Figure 3. Mean mycelial growth (A) and IAA accumulation under fungal colonies (B) of 87 homocaryotic and 15 dicaryotic strains of *Laccaria bicolor*, compared to the parent strain (S238), on a tryptophane supplemented nutrient medium. (Error bar confidence intervals are P = 0.05).

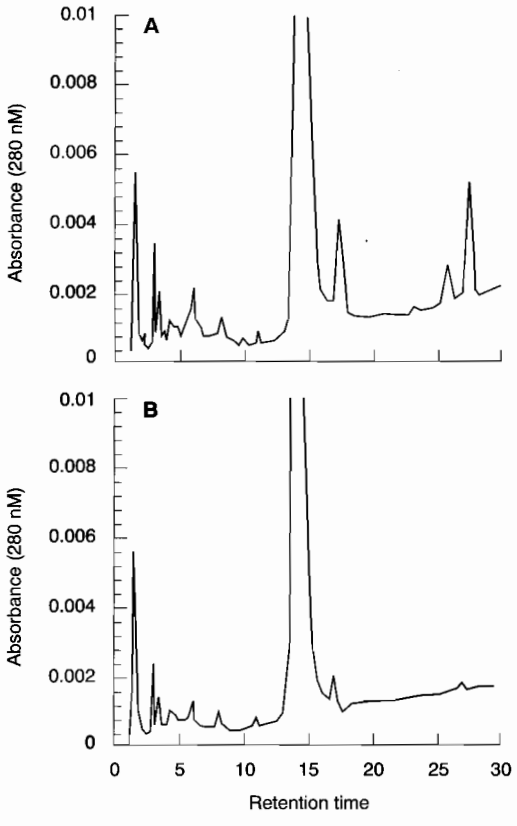

Figure 4. HPLC chromatograms of nutrient media collected 3 weeks after inoculation from cultures of *Laccaria bicolor* S238 (parent strain) (A) and 152–123 (dicaryotic strain) (B).

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Effects of Ectomycorrhiza Fungal Inoculation on Growth and Phosphorus Uptake of Four Provenances of *Eucalyptus urophylla*

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Abstract

Genetic variation in growth, ectomycorrhizal development and phosphorus (P) uptake by seedlings of four provenances of *Eucalyptus urophylla* were compared in a sand culture study at 5, 10 and 20 mg P kg^{-1} soil. There were marked variations in plant dry weight and P content between *E. urophylla* provenances at intermediate levels of P addition to soil (10 mg P kg^{-1}), but variations in these factors were less at either the higher or lower P levels. Most of the provenances inoculated with *Pisolithus tinctorius* and *Scleroderma cepa* showed increased biomass and P uptake at the low level (5 mg P kg^{-1}) and reduced or no effect on biomass and P uptake at adequate P supply to seedling growth (20 mg P kg^{-1} soil). Different combinations between provenances and fungi had different (positive and negative) effects on biomass and P uptake.

Key words: ectomycorrhizal fungi, phosphorus uptake, *Eucalyptus urophylla*

In Asia and South America *Eucalyptus urophylla* is extensively planted for firewood and pulpwood. Earlier research has shown that there are substantial differences in performance between provenances of this species (Mori and Kageyama 1988, Ngulube 1989; Zhou and Bai 1989; Lugo and Francis 1990; Bai 1994). Mori and Kageyama (1988) also reported that there was a strong genotype–locality interaction in their study. Part of this variation in plant growth may be attributed to the responses of different genotypes to the phosphorus (P) supply in the soil (Krannitz et al. 1991).

As *E. urophylla* is an exotic species to Southeast Asia, ectomycorrhizal fungal inoculation with eucalypt specific fungi can improve tree growth after planting (Gong et al. 1992). Inoculation of tree seedlings, leading to extensive development of ectomycorrhizas, has been shown to have widely varying effects on growth, ranging from growth depression (Marx et al. 1985; Gagnon et al. 1987) or no effect on growth (Malajczuk et al. 1975; Castellano and Trappe 1985), to increase in growth over uninoculated seedlings (Bougher et al. 1990). This variable effect of the mycobiont on growth can be attributed to a number of factors and the supply of nutrients, particularly P, is one of the most important of these (Bougher et al. 1990). High P concentration in soil can reduce mycorrhizal root length and growth responses (Bougher et al. 1990). Different species of mycorrhizal fungi can have varying effects on the growth of different eucalypt species (Burgess et al. 1993) or different half-sib families of *Eucalyptus globulus* (Burgess and Malajczuk 1989). As the provenances of *E. urophylla* are quite variable in their growth rates, inoculation with mycorrhizal fungi might be expected to have different effects on growth and P uptake. The aim of this experiment was to determine whether effects of soil P supply on growth responses to inoculation differ between four provenances of *E. urophylla*.

Materials and Methods

A P-deficient subsoil sand from the Spearwood dune system north of Perth, Western Australia (Bray extractable P < 2 mg kg^{-1} soil) was used for these experiments. Soil was steam/air sterilised at 80°C for 2 hours, oven dried at 75°C for 4 days and sieved through a 2.5 mm mesh screen. Pots lined with plastic bags were filled with 2.5 kg of soil. Nutrients were applied in solution to the soil surface at the following rates per kg (kg^{-1}) of soil: 50 mg K, 15 mg Ca, 4 mg Mg, 4 mg Mn, 2 mg Cu, 2 mg Zn, 0.25 mg Mo, 0.12

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Mg B and 0.1 mg Co. Soil was dried again before powdered $Ca(H_2PO_4)2.H_20$ was thoroughly incorporated at the following rates: 5, 10 and 20 mg kg^{-1} soil. N was added to pots each week at a rate of 5 mg kg^{-1}soil, increasing to 10 mg N kg^{-1} after 2 months.

Seeds of four *E. urophylla* genotypes (Table 1) were provided by the Australian Tree Seed Centre, CSIRO Division of Forestry. Seeds were rinsed in 70% ethanol for 10 seconds, then shaken in 5% $NaHClO_3$ for 10 minutes. After 5 rinses in sterile distilled water, seeds were transferred to petri dishes with tap water agar media and incubated in darkness at 24°C for 5 days. Germinated seedlings were then transferred aseptically in batches of 20 to dishes with actively growing 30-day-old colonies of *Scleroderma cepa* and *Pisolithus tinctorius* on modified Melin-Norkrans medium. Control seedlings did not receive inoculum. Dishes were incubated at 25°C. After 14 days incubation, seedlings were transplanted into pots. Four seedlings were planted in each pot. After 3 weeks, seedlings were thinned to 2 per pot. There were 3 replicate pots for each fungus/P level/provenance treatment combination. Pots were randomly arranged on glasshouse benches. Benches were rotated weekly. Soil moisture levels were maintained by regular watering to weight (8% soil moisture content). Average daily glasshouse temperatures ranged from 10°C to 18°C maximum.

A factorial design was used, with three fungal treatments (uninoculated and two fungal treatments) and three rates of applied P (5, 10 and 20 ppm P in soil). For statistical comparison of data the analysis of variance was transformed to a square root scale to ensure homogenity of variance.

Seedlings were harvested 120 days after planting. Roots were washed free of soil and divided into fine and coarse (>1.5 mm diameter) root fractions. After cutting up the fine roots, a subsample (1–1.5 g) was removed to estimate root length, and the remainder retained for nutrient analysis. Fine root subsamples were stored in 70% ethanol and their total length estimated by a line intercept method (Newman 1966). Short roots with complete development of a fungal mantle were scored as mycorrhizas. The remaining fine roots and other plant fractions were then oven-dried at 70°C and their P concentrations determined by automated colorimetric procedures following Kjeldahl digestion.

Results and Discussion

1. Biomass accumulation

The largest differences in seedling growth was caused by P treatments ($P < 0.001$). There was also an interaction ($P = 0.06$) between effects of P supply and mycorrhizal inoculation.This was due to some positive effects of mycorrhizal treatments on growth at 5 mg P kg^{-1} soil which were not evident at higher P rates (Fig. 1). The effect of both mycorrhizal isolates on dry matter production averaged over seedlots was similar at each P level. These results are consistent with those of Bougher et al., 1990 which showed the largest positive effects of inoculation occurred where soil P supply was most limiting.

There was also an interaction ($P < 0.05$) between seedlots and mycorrhizal effects. For provenances 17830 and 13828 (see Table 1), seedlings inoculated with *P. tinctorius* produced more dry matter (averaged over P treatments) than uninoculated or *S. cepa* inoculated seedlings. In contrast *P. tinctorius* inoculated seedlings had the lowest average dry matter for provenances 13827 and 14532. There was no strong evidence that inoculation reduced variation in growth among seedlots previously reported for *E. globulus* inoculated with a range of mycorrhizal isolates (Burgess and Malajczuk 1989). This is possibly due to the relatively small effect of mycorrhizal treatments on growth in this experiment.

2. Uptake of P

Phosphorus uptake by seedlings of the different provenances generally followed plant growth trends. Largest differences in P uptake were associated with P treatments ($P < 0.001$) and seedlots ($P < 0.001$). Inoculation with *P. tinctorius* increased P uptake for two provenances (17830 and 13828) and reduced P uptake for the other two provenances (13827 and 14532) relative to uninoculated and *S. cepa* inoculated seedlings (seedlot × mycorrhizal treatment interaction, $P = 0.05$; Fig. 2). The mycorrhizal treatments had a significant effect on the allocation of P between roots and shoots and this was most evident at the lowest P rate (P × mycorrhizal inoculation interaction; $P < 0.001$). At this low P rate the ratio of P in roots:shoots for *S. cepa* seedlings was 0.84, com-

Table 1. Provenances of *E. urophylla* seedlots used in experiments.

Seedlot no.	Parent trees	Locality	Latitude	Longitude	Altitude (m)
17830	4	NW of Ilwari, Wetar Is., Indonesia	7°54'	126°26'	490
13827	?	Mt. Egon, Flores Is., Indonesia	8°40'	122°30'	900
13828	?	Mt. Mutis, Timor Is., Indonesia	9°34'	124°17'	1200
14532	31	Mt. Lewotobi, Flores Is., Indonesia	8°31'	122°45'	393

pared with 0.50 for *P. tinctorius* and 0.53 for uninoculated seedlings. At the highest P rate (15 mg P kg^{-1} soil) the equivalent ratios were 0.25, 0.26 and 0.25 respectively.

3. Mycorrhizal root development

There was no evidence of mycorrhizal development on roots of uninoculated seedlings. For the two inoculation treatments mycorrhizal root development was highest ($P < 0.001$) for all provenances at the low level of P supply (> 10%) and lowest at the high rate (< 4%) where P was adequate for maximum seedling growth. The extent of mycorrhizal development differed among provenances for *P. tinctorius* but not for *S. cepa* ($P < 0.05$). Percentage of fine roots that were mycorrhizal for seedlings inoculated with *P. tinctorius* was less for provenances 13827 and 14532 (average 15% over P treatments) than for the other two provenances (7.5%) (Fig. 3). The two provenances (17830 and 13828) which were heavily colonised with *P. tinctorius* also had relatively higher growth and P uptake (Figs. 1 and 2).

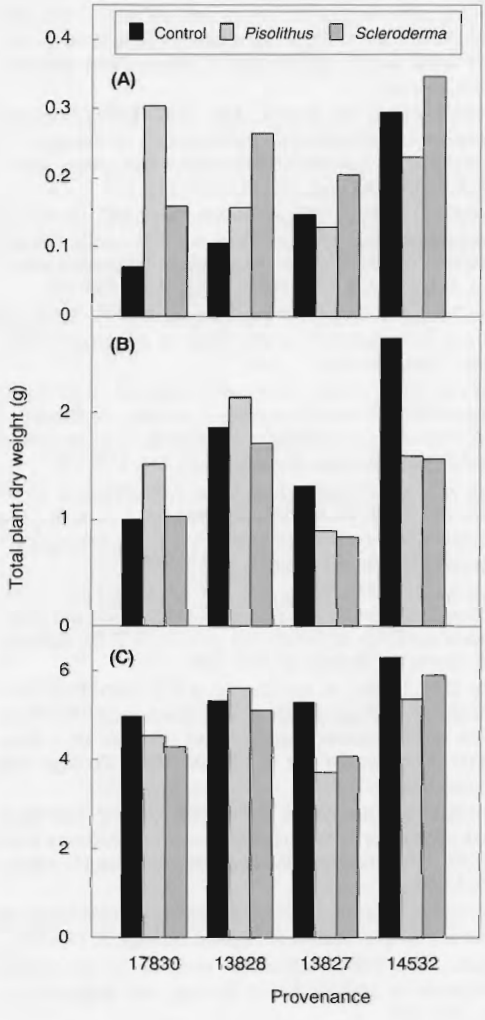

Figure 1. Growth response of four provenences of *E. urophylla* to mycorrhizal inoculation at three P levels: (A) 5, (B) 10 and (C) 20 mg P kg^{-1}.

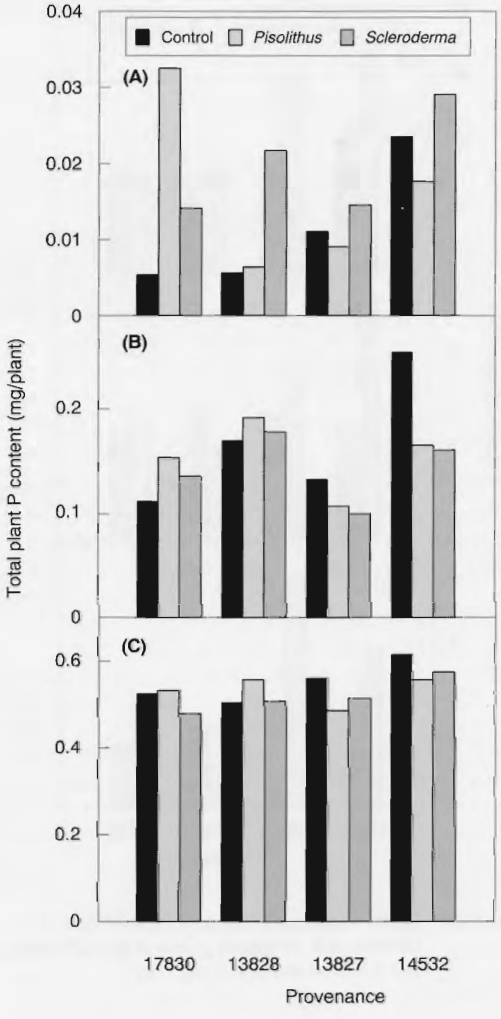

Figure 2. Phosphorus accumulation in four provences of *E. urophylla* to mycorrhizal inoculation at three P levels: (A) 5, (B) 10 and (C) 20 mg P kg^{-1}.

Conclusions

The results demonstrate that differences in growth of different provenances of *E. urophylla* can be attributed to their response to P supply in the soil and infection by different ectomycorrhizal fungi. This suggests that there is a need to separate the gains attributed to genetic differences from those mediated by site factors such as P supply and mycorrhizal infection. Differences between provenances would be expected to be most important if soils are infertile or small if large amounts of fertilisers are applied.

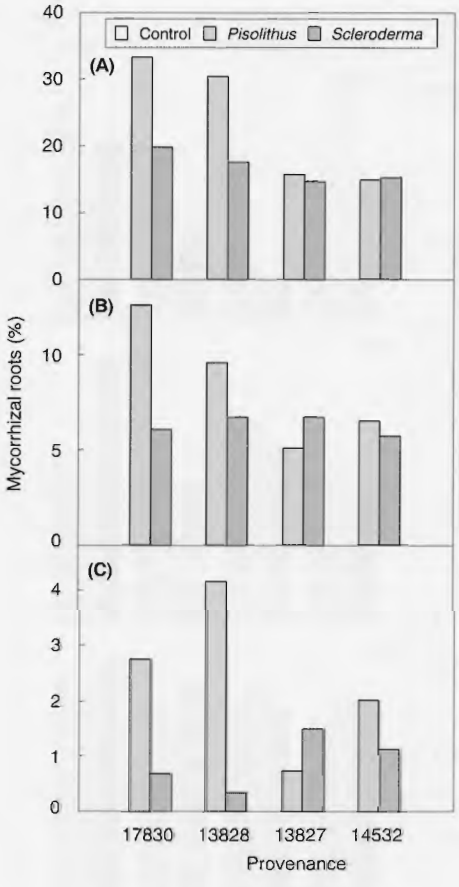

Figure 3. Mycorrhizal colonization of roots of four provenences of *E. urophylla* grown at three P levels: (A) 5, (B) 10 and (C) 20 mg P kg^{-1}.

Acknowledgments

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Management of Mycorrhizal Associations in the Nursery and Field

Fertiliser Requirements for Ectomycorrhizal Eucalypts in Forest Nurseries and Field Plantings in Southern China

B. Dell* and N. Malajczuk†

Abstract

The objective of inoculating trees in the nursery is to produce plants with abundant mycorrhizas at the time of transplanting to the field. Maximum mycorrhizal development is dependent on having a supply of nutrients that is conducive for both root and mycelial growth. Incorrect supply of mineral nutrients to seedlings is one of the key factors that can depress the development of ectomycorrhizas on eucalypts in forest nurseries. The most important pool of nutrients for the two partners is the available pool in the potting mix which has been supplemented through fertilisation. Both under- and over-fertilisation can affect hyphal colonisation of roots in nursery containers. Mycorrhization is favoured under mild nitrogen and phosphorus deficiency due to the enhanced availability of assimilates in roots. Hence, available nitrogen and phosphorus in the potting mix, or applied as a foliar spray, should be at a level that is limiting for the growth of non-mycorrhizal seedlings. Deficient supplies of potassium, calcium, magnesium, iron, manganese and boron are predicted to depress ectomycorrhizal development due to the reduced availability of assimilates in the rhizosphere, reduced dry matter partitioning to roots and reduced root growth.

Field application of fertilisers is essential for maximising the potential benefits of inoculation programs for eucalypts in southern China. Whilst further research is required to develop site specific fertiliser practices that optimise colonisation of roots by ectomycorrhizal fungi and plant growth, previous ACIAR ectomycorrhizal and nutrition trials in China have identified key elements which are deficient for the growth of eucalypts over wide areas of eucalypt plantations. Therefore, fertiliser regimes are proposed for providing nutrients at a level that will maximise ectomycorrhizal development in containerised forest nurseries as well as in field sites in southern China.

Key words: eucalypts, ectomycorrhizal fungi, fertiliser rates, field trials, nursery

EUCALYPTS which are being selected for plantations in China are fast-growing species with high internal nutrient demands in the early stages of growth. In the field, they experience soil nutrient imbalances that may be quite different from those encountered in natural stands. Further, most of the soil or soil-less mixes currently used in containerised forest nurseries in southern China do not have the capacity to deliver a balanced supply of inorganic nutrients to the roots of such fast-growing tree seedlings. Consequently, the addition of fertiliser is recommended in all nurseries. Ideally, fertiliser should be supplied in small amounts throughout the growing season to meet the plant's requirement for growth. The production of ectomycorrhizal eucalypts, however, requires extra care in formulating fertiliser regimes and fertiliser rates. This is because the overriding objective is to produce well inoculated seedlings, cuttings or tissue explants. Maximum growth is not necessary, and is often undesirable for eucalypts because, from our experience in Yunnan Province, plants with large shoots do not transplant well. Before considering fertiliser rates, it is appropriate to briefly examine the nutrient requirements of eucalypt ectomycorrhizas.

The mycelium that extends from ectomycorrhizas can penetrate soil for considerable distances (Skinner and Bowen 1974; Jones et al. 1990). Nutrient uptake sites near hyphal tips thus can occur at some distance from rootlets in field soils but are constrained in nursery containers. Even so, eucalypts in pots may have 50–350 m hyphae/m of colonised root (Thomson et al. 1994). The main sources of mineral nutrients for

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uptake by fungal hyphae would be those in solution and those on exchange surfaces, or weakly adsorbed to soil particles. With fast-growing eucalypts in nursery containers, there is insufficient time for ectomycorrhizal fungi to access nutrients such as nitrogen which are constituents of organic components of potting mixes. Indeed, two common inoculant fungi, *Laccaria laccata* and *Pisolithus tinctorius*, have little proteolytic potential and are therefore dependent on mineralisation processes for the release of nitrogen (Read 1993). Nutrients are thus best supplied as dilute solutions of mineral salts to nursery containers rather than relying on the uncomposted organic matter in the potting mix to supply some elements over time.

Both nutrient supply and composition can influence hyphal development in the nursery. In containers, mycorrhizal development is greatest where the phosphorus supply to roots is moderately to severely limiting and there is no response to inoculation where the phosphorus supply is adequate (Bougher et al. 1990; Jones et al. 1990). The form of nitogen (ammonium, nitrate) applied can influence the growth of eucalypt seedlings in pots (Shedley et al. 1993) and the mycorrhizal response to inoculation (Dell and Malajczuk, pers. comm.).

Reforestation programs with eucalypts usually require species of ectomycorrhizal fungi that are adapted to mineral rather than humic soils. This is particularly evident in southern China where extensive slopes available for eucalypt plantations are low in organic matter. These soils are generally deficient in nitrogen and phosphorus for tree growth. In addition, micronutrient deficiencies, such as boron in Yunnan (Dell and Malajczuk 1994), also cause widespread deficiency symptoms and reduce tree growth. The low nutrient supply in soils is associated with past depletion of organic matter and nutrient-rich surface soil, the lack of nutrient input to forest areas and to the strong reactions of soils with applied nutrients.

This paper presents fertiliser recommendations for routine production of ectomycorrhizal eucalypts in nurseries and for evaluating effective ectomycorrhizal fungi in eucalypt field trials in southern China. Issues considered to be important for understanding the fertiliser requirements of ectomycorrhizal eucalypts are discussed. Much additional information on fertiliser composition and usage for containerised tree nurseries is given in the manual by Landis et al. (1989).

Materials and Methods

The fertiliser strategies recommended in this paper arise from observations and experimental data acquired from nursery and field fertiliser and mycorrhizal trials in Guangdong and Yunnan Provinces in China (Malajczuk et al. 1994a; Dell and Malajczuk 1994). Additional knowledge on the internal and external nutrient requirements for non-mycorrhizal seedlings was obtained from numerous glasshouse trials in Australia (Dell 1994; Dell and Robinson 1993; Dell and Xu Daping 1995; Dell et al. 1995).

Results and Discussion

Nutrients for containerised eucalypt nurseries

We have found that fast-growing species of eucalypts are very sensitive to nutrient deficiency resulting in depressed growth and the appearance of deficiency symptoms, commonly in the foliage. Two examples showing the response of eucalypt seedlings to phosphorus (Fig. 1) and zinc (Fig. 2) are illustrated. In both cases, plants were supplied with a basal fertiliser containing adequate amounts of all the other essential plant nutrients.

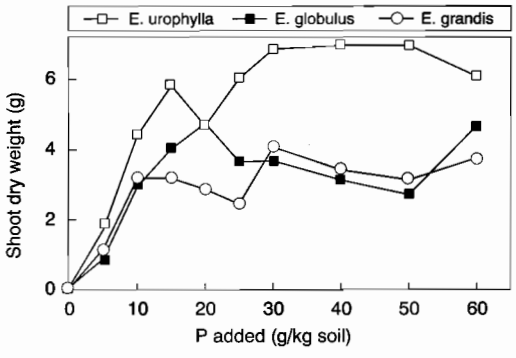

Figure 1. Effect of phosphorus (P) supply in a virgin soil from Lancelin, Western Australia, on shoot dry weight of 3-month-old seedlings of three eucalypt species in a glasshouse trial (Malajczuk et al. 1994b).

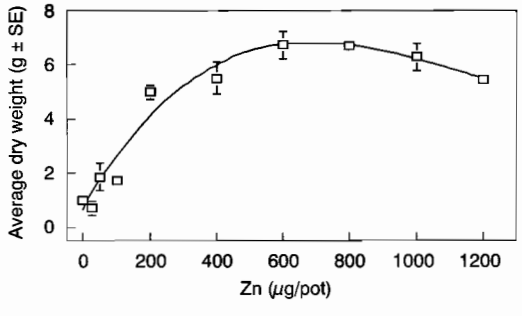

Figure 2. Effect of zinc (Zn) supply in a virgin soil from Lancelin, Western Australia, on shoot dry weight of 12-week-old *Eucalyptus urophylla* seedlings in a glasshouse trial. Standard error bars are shown where they exceed the size of the symbol. Each pot contained 3 kg soil (adapted from Dell and Xu Daping 1995).

Eucalypts and ectomycorrhizal fungi have largely similar requirements for essential macro- and micronutrients, the exceptions being the alleged ability of some fungi to grow without boron (Kendrick 1992). In general, nutrient concentrations in fungal tissues are of the same order of magnitude as tissues in young eucalypts. Further, the total inorganic nutrient demand for hyphal growth in pots is small. Typically, about half of the lateral roots on well-inoculated eucalypt seedlings are colonised by fungal hyphae and this hyphae can contribute up to 50% of the biomass of these rootlets. Assuming that hyphal penetration into the potting mix is about equivalent to hyphal growth on roots, then the nutritional requirement for the growth of the inoculant fungus in nursery containers is less than 15% of the total nutrient demand of the plant. Of greater consideration is the potential impact that inappropriate levels of fertiliser can have on ectomycorrhizal development (Table 1), through effects on the availability of assimilates to roots and on root growth.

Table 1. Likely impact of nutrient deficiencies on ectomycorrhizal development of eucalypts in nursery containers.

Element	Number of ectomycorrhizas	Plant effect*
Nitrogen	slightly increased	a, b
Phosphorus	strongly increased	c
Potassium	slightly decreased	d
Calcium	strongly decreased	e, f, g
Iron	slightly decreased	f
Manganese	slightly decreased	d, e
Boron	strongly decreased	d, f, g

*a = increased supply of sugars to roots; b = increased allocation of biomass to roots; c = increased root exudation; d = decreased supply of sugars to roots; e = decreased allocation of biomass to roots; f = decreased root growth; g = decreased number of lateral roots

Source: Based on unpublished observations by the authors and Hepper and O'Shea (1984); Beckford et al. (1985); Gagnon et al. (1988); Ericsson and Kähr (1993); Göransson (1993), (1994).

We have achieved good ectomycorrhizal development in containers using well-aerated, pasteurised potting mixes where the inorganic fertiliser is applied in solution in small doses during the growing season. We recommend this approach because the amount of fertiliser can be easily regulated according to the growth rate and appearance of the plants. Furthermore, there is less risk in having levels of phosphorus and nitrogen which may inhibit ectomycorrhizal development when the plants are very small. Liquid fertiliser can be applied either in watering cans by hand or it may be added in the irrigation water if appropriate systems are available in the nursery. A recommended schedule is proposed in Table 2.

Table 2. Recommended nutrient concentrations in liquid fertiliser for promoting development of eucalypt ectomycorrhizas in nursery containers.

Element	Concentration (μM) in application water	Recommended source of element
Nitrogen	2000	Urea, NH_4NO_3
Potassium	400	KCl
Calcium	250	$CaCl_2$
Magnesium	250	$MgSO_4.7H_2O$
Sulfur	250	$MgSO_4.7H_2O$
Phosphorus	20	KH_2PO_4, K_2HPO_4
Iron	10	FeEDTA, FeEDDHA, $FeSO_4$
Boron	10	H_3BO_3
Zinc	2	$ZnSO_4.7H_2O$
Manganese	2	$MnSO_4.5H_2O$
Copper	1	$CuSO_4.5H_2O$
Molybdenum	0.1	$NaMoO_4.2H_2O$

Notes: 1. Stock solutions, at about 1000 times the above dosages, should be made up separately for each of the above compounds and then diluted to produce the working solution
2. The working solution should be applied over the foliage of containerised plants at two to three weekly intervals
3. Apply fertiliser in late afternoon

Nutrients for field plantings of ectomycorrhizal eucalypts

Field trials in Yunnan and Guangdong Provinces have indicated that most soils are low in phosphorus (Fig. 3), boron, potassium and nitrogen (Dell and Malajczuk 1994; Dell et al. 1995). The high levels of P fertiliser required for the maximum growth of eucalypts, as illustrated in Fig. 3, are well in excess of that which the forest farmers can afford to apply. We have shown elsewhere (Ji Dagan et al., this volume) that growth rates of trees can be enhanced by inoculation of seedlings in the nursery with selected fungi. These ectomycorrhizal fungi perform well and significantly promote the growth of eucalypts in the field at the lower rates of P fertiliser that the farmers use. We recommend that in addition to P, a balanced supply of the other limiting major and minor nutrients be applied to ectomycorrhizal trees at planting (Table 3).

Table 3. Recommended nutrient additions during the first growing season for ectomycorrhizal screening trials on eucalypts in southern China. These rates are for spot applications of fertiliser at about 30 cm from the stem.

Element	Rate (g element/tree)	Recommended fertiliser
N	20[a]	urea
P	3–10[b]	superphosphate, NPK blend, $Ca(H_2PO_4)_2.H_2O$
K	10[c]	K_2SO_4, KCl
S	[d]	$CaSO_4$
B	1.5–2.5	H_3BO_3
Zn	2	$ZnSO_4.7H_2O$
Cu	1	$CuSO_4.5H_2O$

[a] Reapply 30–50 g N/tree after 3 months
[b] Use higher rate on severely P-deficient or high P-fixing soils
[c] Reapply 10–20 g K/tree after 3 months
[d] Apply only if S is not supplied in K fertiliser
Note: Commercial fertilisers may contain variable amounts of micronutrients as contaminants. Triple superphosphate can have 50–70 mg B/kg.

It is important that these rates are not exceeded as fertiliser practices are known to affect tree growth responses to mycorrhizal fungus inoculation. In particular, high rates of phosphorus and nitrogen fertilisers are well known to suppress mycorrhizal development (Menge et al. 1977; Newton and Pigott 1991) and high concentrations of soil nitrogen can also reduce the number and relative abundance of different ectomycorrhiza types (Alexander and Fairley 1983).

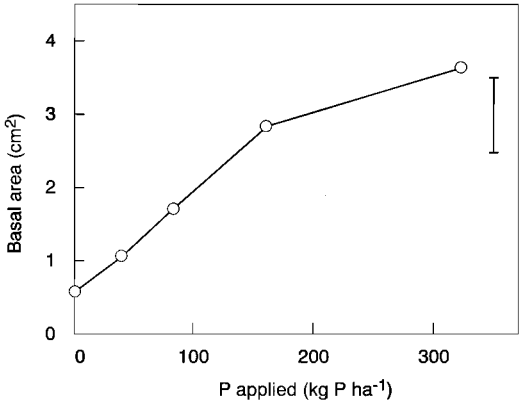

Figure 3. Effect of phosphorus (P) fertiliser on growth of 9-month-old non-mycorrhizal *E. globulus* in a red-yellow earth near Kunming, P.R. China (the bar is 2 x SEM).

Conclusions

Inoculation of eucalypt seedlings with ectomycorrhizal fungi should aim to ensure that seedlings have extensive infection at the time of transplanting from the nursery to the field. When inoculation procedures are introduced into forest nurseries in China, cultural practices may have to be modified to produce conditions which are optimal for the development of ectomycorrhiza in the nursery. Attention must be given to supplying inorganic nutrients that maximise ectomycorrhizal development rather than promoting maximum plant growth in the nursery.

In the field, the largest effects of inoculation on the host are expected at an early stage of tree growth when there is a high nutrient demand by the tree. Hence, it is very important that due consideration is given to fertiliser regimes at planting which optimise development and function of the symbioses and thus result in the maximum possible promotion of tree growth. The conservative fertiliser recommendations included in this paper should be followed until local experience requires their modification.

Acknowledgments

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Optimising Phosphorus Fertiliser Rates and Mycorrhiza Fungal Inoculation for Growth of *Eucalyptus grandis* × *E. urophylla* in Southern China

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Abstract

The effectiveness of specific ectomycorrhizal fungi in increasing the growth of *Eucalyptus grandis* × *E. urophylla* was examined in relation to the rate of phosphorus fertiliser added to a phosphorus deficient ex-pine site near Kaiping in Guangdong Province. Six phosphorus treatments (0–312 kg P ha^{-1}) were applied to the soil surface just before planting seedlings that were either uninoculated or had been inoculated with one of seven ectomycorrhizal fungal isolates. In the first year after planting, there were differences in tree heights and stem basal areas among fungal treatments, but these were less substantial than responses to phosphorus treatments. There was little or no interaction between effects of fungal and phosphorus treatments. The small effect of fungal treatments on early tree growth in this experiment was associated with poor colonisation of roots by inoculant fungi at the time of planting. Differences in tree growth among phosphorus treatments were large. The highest rate of phosphorus application resulted in a 10-fold increase in above-ground dry matter 19 months after planting, relative to unfertilised trees. The response functions obtained are providing the means to economically evaluate gains from fungal inoculation and P fertiliser treatments. Preliminary analysis of a function relating stemwood volume to rate of applied P suggests that significant improvements in plantation productivity and greater economic benefits can be attained on these soils through adoption of higher fertiliser rates.

Key words: Eucalypt, ectomycorrhizal fungi, inoculation, phosphorus nutrition, plantation, wood production

ECTOMYCORRHIZAL fungi enhance tree growth by increasing the uptake of phosphorus (P) by roots in P-deficient soils (Grove and Le Tacon 1993). For tree seedlings growing in controlled-environment conditions, ectomycorrhiza formation and rates of P absorption are strongly dependent on the P status of the plant and therefore on concentrations of available P in the soil (Bougher et al. 1990, Jones et al. 1990). The effectiveness of ectomycorrhizal fungi in enhancing tree growth can therefore vary markedly according to soil P supply. However, this mycorrhiza-P supply interaction has not been extensively examined in the field.

Eucalypt plantations are being established in Guangdong Province on hill soils that are naturally infertile and have been degraded through past land practices. As the eucalypts are introduced species, they lack the broad range of ectomycorrhizal fungal species that are associated with native eucalypt ecosystems in Australia (Malajczuk et al. 1994). The extent to which the inoculation of eucalypt seedlings with compatible fungal isolates, combined with optimum P fertiliser application, can improve the growth of plantations growing on these soils in southern China has not been investigated. The field experiment reported here was established to (i) measure the effectiveness of selected ectomycorrhiza fungi in increasing growth of a *E. grandis* × *E. urophylla* hybrid, (ii) determine the effectiveness of the fungi in relation to soil P supply, and (iii) quantify tree growth in relation to soil P supply as a basis for determining optimum P application rates on these soils.

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Materials and Methods

Site

The experiment was situated on the mid to lower slopes of a hill (25° slope, south to west aspect) in Kaiping County, Guangdong Province. The soils are deep red–yellow earths. Annual rainfall is about 1800 mm and mean monthly temperatures range from 28°C in July to 13°C in January. A *Pinus elliotii* plantation growing on the site was clear-felled and residues and remaining vegetation burnt about 6 months before establishment of the experiment. The site was prepared for planting using strip terracing with 3 m spacing between strips.

Four composite surface (0–10 cm) soil samples were taken from the site and returned to Australia for analysis. They were air-dried, sieved and the fine earth (< 2 mm) fraction analysed. The pH was determined using a glass electrode in 1:5 soil: water suspensions. Concentrations of total P and N were determined by automated colorimetric methods following digestion using a modified Kjeldahl (H_2SO_4/H_2O_2) procedure. Organic carbon was estimated by use of the dichromate oxidation procedure. An estimate of available P was obtained by 2 minute shaking of a 1:50 soil:Bray No.1 reagent and automated colorimetric determination of phosphate. Soil P adsorption isotherms were determined by equilibrating soils in 0.01M $CaCl_2$ solutions containing various concentrations of P (soil:solution ratio 1:100; 24 hr shaking) and measuring the P remaining in solution.

Experimental design

A randomised block split-plot design was used in which eight fungal treatments (uninoculated control and 7 inoculation treatments) were established in subplots randomised within main plots which represented different P treatments. The six P treatments applied to main plots were randomised within each of four replicate blocks. Each subplot consisted of a single row of 5 treatment trees. Subplots within main plots on the same terrace were separated by a single 'buffer' tree (*E. grandis* × *E. urophylla*). Main plots representing P treatments were separated by a terrace of 'buffer' trees on the lower and upper sides and by 3 'buffer' trees along the terraces.

The fungal inoculation treatments allocated to subplots are listed in Table 1. Phosphorus was applied as superphosphate at one of the following rates: 0, 13, 52, 104, 208 or 312 kg P ha^{-1}. The superphosphate was broadcast on the surface in a band 0.6 m wide along the terrace in each subplot just before planting. A basal dressing of other major and minor nutrients was also applied to the trees (see below).

Seedling establishment and fungal inoculation

Seedlings of *Eucalyptus grandis* × *E. urophylla* were grown from tissue cultures and inoculated with ectomycorrhizal fungi in the Jiangmen Forest Nursery. Micropropagated plantlets that had developed roots were transferred to growing cultures of the fungal isolates and incubated for a further two weeks. Plantlets for the control treatment were transferred to sterile agar plates. Plantlets were then transferred into standard 3 cm diameter paper tubes containing heat-sterilised field soil in the nursery. Seedlings were transported to the Zhenhai Forest Farm just before planting in the field and the roots of a random sample of seedlings were assessed for ectomycorrhiza development using a binocular microscope.

Field planting and site maintenance

Seedlings were planted in the field in early April, 1992. In addition to the P fertiliser treatments, a basal dressing of other nutrients was applied to treatment trees. A trace element mix (1.5% Cu, 0.26% Zn, 3.9% Mn, 6.0% Mg, 5.7% Fe, 0.19% Mo, 0.13% B and 0.03% Co) was applied (50 g/tree) once at planting. Urea and potassium sulfate were applied on three occasions in the first year after planting (total amounts 417 kg urea and 120 kg K_2SO_4 ha^{-1}). Trees planted in the buffer zones between the plots and subplots received a standard dressing of NPK fertiliser (130 g $tree^{-1}$).

Measurements and data analysis

Heights of treatment trees were measured approximately 3, 6, 9, 12, and 19 months after planting. Tree stem diameters were also measured on four occasions up to 24 months after planting. Effects of fungal and P fertiliser treatments on tree height and stem basal areas were statistically analysed using the GENSTAT statistical program (Rothamsted Experimental Station).

Economic analysis

An analysis using tree growth responses to P application only was carried out to estimate optimum P application rates and expected economic returns from harvesting when the stand was 19 and 25 months old. These estimates are preliminary due to the lack of precise information at this stage on product values and full production costs. Only woodchips are considered in this exercise. Woodchips are sold for 350 yuan per m^3 at Yangxi, but no stumpage value is available. In this analysis we assumed stumpage values of 80, 120 and 160 yuan per cubic metre. Costs of plantation establishment are 2250–3000 yuan/ha and this includes site preparation, seedlings, fertilisers and tending. Fertiliser

costs in yuan/kg are: superphosphate 1; urea 1.6; potassium sulfate 1.6–2. The analysis relied on derived functions that related stemwood volumes (estimated from stem heights and diameters) to P application rates. The optimum P application rate was calculated as the rate where marginal costs and marginal return were equal. The value of the tree crop was then calculated from the stemwood yield at this optimum P rate.

Results and Discussion

Colonisation of roots by inoculant fungi, as revealed by the presence of mycelia and ectomycorrhizas, was generally poor in all fungal treatments at the time seedlings were planted in the field. However, two years after planting fruiting bodies confirmed to belong to the inoculant strain of *Pisolithus* (H4111) were observed at the base of some inoculated trees (Malajczuk et al. unpublished data).

Both fungal and P fertiliser treatments affected tree growth from an early stage. Three months after planting, tree height differed ($P<0.05$) among fungal treatments and was increased ($P<0.001$) by P application. Six months after planting, mean stem basal areas differed ($P<0.01$) among fungal treatments and there was a small interaction ($P<0.05$) between the effects of P and fungal treatments. This interaction was not evident at later measurements. Marked interactions have been reported for seedlings grown in controlled environment conditions, with positive responses to inoculation being greatest in the P deficiency range (Bougher et al. 1990, Jones et al. 1990). There were also differences ($P<0.05$) in stem basal areas among fungal treatments at 9 months after planting but not thereafter. Stem basal areas for two fungal treatments and the average of all fungal treatments are shown in Figure 1 in relation to level of applied P at 6 and 12 months after planting.

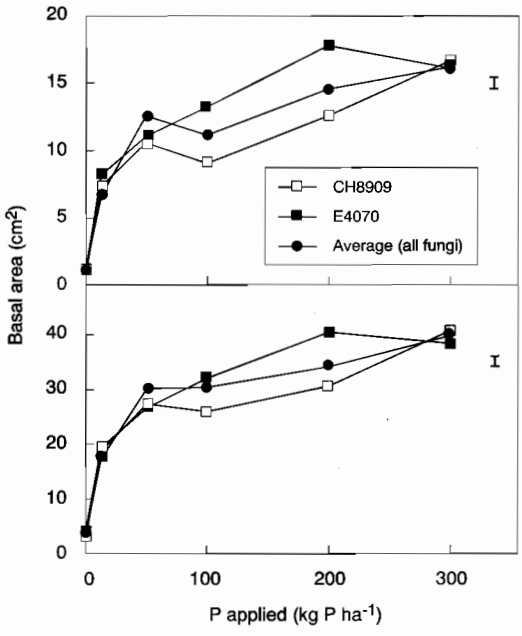

Figure 1. Stem basal area (per tree) of *Eucalyptus grandis* × *E. urophylla* inoculated with different ectomycorrhizal fungi, in relation to external P supply, at 6 months (A), and 12 months (B) after planting (CH8909 is a Chinese isolate of *Pisolithus* and E4070 is an Australian *Hebeloma* species. The error bars = 2xSEM).

In the first year after planting, trees inoculated with *Hebeloma* and uninoculated trees generally had the highest mean basal areas and trees inoculated with two *Pisolithus* isolates (H4937 and CH8909) the lowest (Fig. 2). The high growth rate of uninoculated seedlings relative to some other fungal treatments may be explained by the low level of colonisation of roots of

Table 1. Fungal isolates used in the trial.

Treatment	Code	Fungal species	Origin
1	Control	(uninoculated)	
2	H4937	*Pisolithus* sp.	Queensland
3	E4240	*Tylopilus* sp.	Queensland
4	E4014	*Tricholoma* sp.	Queensland
5	E4100	*Laccaria* sp.	Queensland
6	E4070	*Hebeloma westraliense*	Southwestern Australia
7	CH8909	*Pisolithus* sp.	China
8	H4111	*Pisolithus* sp.	Queensland

inoculated seedlings at the time of planting. If some inoculant fungi colonised roots rapidly following planting in the field, the greater carbon allocation to roots could initially suppress shoot growth, as has been observed in other studies (Bougher et al. 1990, Grove and Le Tacon 1993). This possibility is supported by later measurements where reduced effects of fungal treatments on growth resulted from more rapid growth in the two *Pisolithus* treatments relative to other treatments (Fig. 2). Further measurements are required to see whether this trend continues. The early differences among fungal treatments indicate that the fungi were active on the roots, but the lack of positive responses at this stage emphasises the need for roots to be well colonised in the nursery before the time of planting.

Where P was not applied, growth of *E. grandis* × *E. urophylla* was poor. There was a large response ($P<0.001$) to P application from 3 months after planting. Absolute differences in tree heights between P treatments increased with time, and although relative differences were generally constant over the first year, they had decreased in the second year (Fig. 3). Application of 312 kg P ha^{-1} resulted in 13-fold increases in stem basal area (averaged over fungal treatments) at 6 and 9 months, a 10-fold increase at 12 months (Fig. 2, Fig. 4) and an 8-fold increase at 19 months. Growth in the higher P fertiliser treatments also greatly exceeded growth in the surrounding plantation which had received conventional fertiliser applications (Fig. 4).

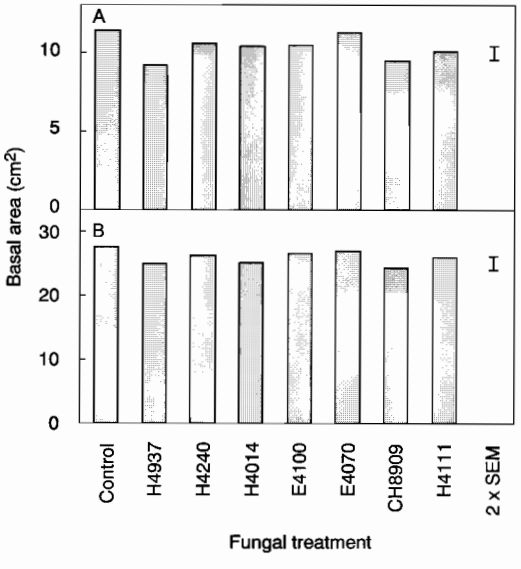

Figure 2. Variation between fungal treatments in the stem basal area (per tree) of *Eucalyptus grandis* × *E. urophylla* averaged over all P treatments, at 6 months (A), and 12 months (B) after planting (see Table 1 for fungal treatments).

The large response to applied P is consistent with data on the chemical properties of surface soils at the site (Table 2). Concentrations of total and Bray-extractable P in the soil are low in comparison with a range of P-deficient eucalypt forest soils in Tasmania (Grove and Malajczuk, unpublished report) and south-western Australia (Grove and Malajczuk 1985, Grove et al. 1986). The P adsorption capacity (Table 2) indicates that these soils will strongly react with, and immobilise applied P. The surface soil also has a low pH and low concentrations of organic C and total N (Table 2). Basal dressings of N and other nutrients were applied to this experiment. Further research is required to determine requirements of these nutrients to sustain high growth rates on these soils.

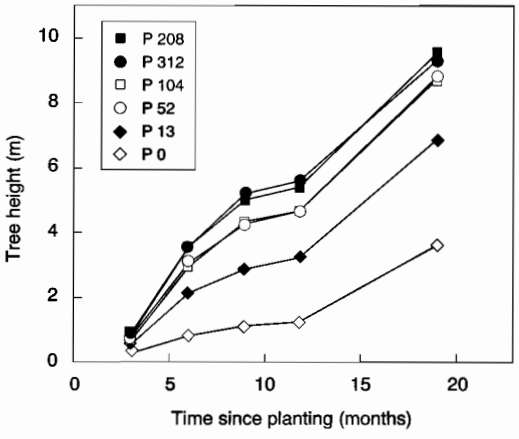

Figure 3. Changes in heights (averaged over fungal treatments) of *Eucalyptus grandis* × *E. urophylla* in the six P treatments with time after planting.

Preliminary estimates of optimum P fertiliser rates to maximise economic returns, and the value of the tree crop for woodchips increases markedly with age (Table 3). These estimates were based on the relationship between predicted stemwood volume and P fertiliser rate which was well defined by a quadratic equation with the P rate on a square root scale (Fig. 5). More complete cost and price data, risk analysis and growth information from a full rotation will be required to properly evaluate P fertiliser requirements and the gains from optimising fertiliser practices. However, the preliminary figures in Table 3 indicate that gains from optimising fertiliser practices are potentially large. For example, at a P rate of 98 kg P/ha and value at the stump of 120 yuan, value of stemwood for woodchips would exceed costs by about 1000 yuan/ha after 2 years. With standard silvicultural practices, costs still exceed wood value at this time (based on growth equivalent to that at the second P rate).

Table 2. Chemical properties of surface soils (0–10cm). Data are means of composite samples from each replicate block with the standard deviation in parenthesis.

Total P (%)	Bray P (μg/g)	Maximum P adsorption (μgP/g)	Total N (%)	Organic C (%)	C/N ratio	pH (in water)
0.015	4.11	672	0.134	2.49	18.6	4.26
(0.001)	(0.32)	(50)	(0.006)	(0.19)	(1.0)	(0.15)

Table 3. Estimates of optimum P requirements (rate where marginal costs = marginal returns) and value of the tree crop at different stumpage values.

		Stumpage (yuan m^{-3})		
Parameter	Tree age	80	120	160
Optimum P	19 months	32	53	72
rate (kg/ha)	25 months	70	98	119
Value of crop	19 months	1236	2204	3238
at optimum P (yuan/ha)	25 months	3305	5427	7568

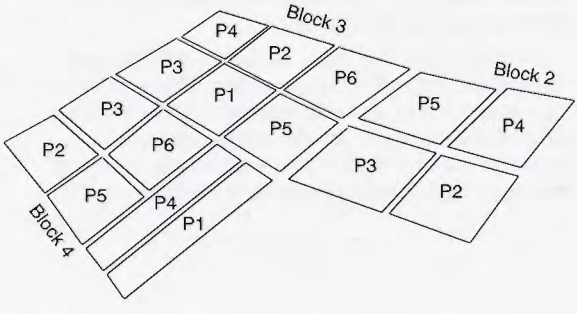

Figure 4. View of blocks 2, 3 and 4 of the ectomycorrhiza P nutrition experiment 12 months after planting in Kaiping County. Fungal treatments were randomly arranged in 4 split rows within each block.

Stemwood volume is likely to have doubled at 4 years of age (see Cameron et al. 1989) and value of the crop (stumpage of 120 yuan) could exceed costs by at least 5000 yuan/ha at the higher fertiliser rates compared with about 1000 yuan with standard practices producing half the stem volume. These figures suggest that large economic benefits may be obtained through improved predictions of fertiliser requirements or where improved inoculation procedures can increase productivity. Furthermore, additional economic gains through residual effects of treatments on future plantation production are still to be evaluated.

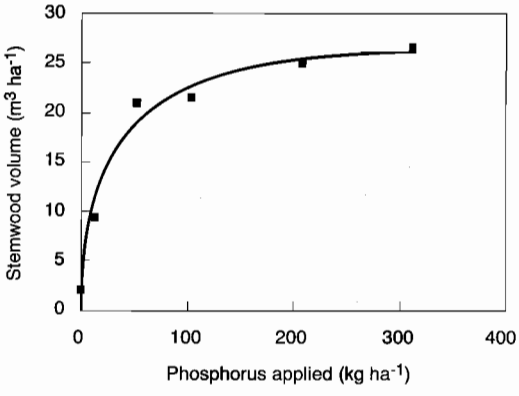

Figure 5. Estimated stemwood volume (averaged over fungal treatments) of *Eucalyptus grandis* × *E. urophylla* in relation to rate of applied P 19 months after planting.

Conclusions

Inoculation of seedlings with the selected ectomycorrhizal fungi had a small effect on tree growth only in the first year after planting. This was partly attributed to the low levels of colonisation of roots by inoculant fungi at the time of planting, which precluded a proper evaluation of the effectiveness of these fungal isolates and their interactive effects with P fertiliser application in this experiment. Improved inoculation and nursery procedures that produce seedlings with well colonised roots are being developed before further field testing of fungal isolates.

The experiment has led to the complete P response function for this soil being quantified. This is providing a sound economic basis to predict optimum P fertiliser rates to apply to *Eucalyptus grandis* × *E. urophylla* plantations. Preliminary analysis indicates that significant gains in plantation productivity and in economic returns can be attained on these soils through adoption of higher fertiliser rates. Furthermore, the high P fertiliser requirement and the immobility of P in these soils suggests that introduction of effective ectomycorrhizal fungi may also improve the productivity of plantations in this region.

Acknowledgments

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Inoculation of Larch *(Larix gmelini)* Seedlings with the Ectomycorrhizal Fungus *Suillus grevillei:* Growth Responses in a Nursery and an Afforestation Site in Inner Mongolia

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Abstract

Larch seedlings, including container and bare-root seedlings, were inoculated with pure inoculum of the mycorrhizal fungus *Suillus grevillei* in nursery and field trials. When compared with uninoculated control seedlings, all growth parameters of inoculated seedlings were greatly increased (seedling emergence, height, diameter, lateral root numbers, mycorrhizal formation, survival and nutrient concentrations). These results suggest that *Suillus grevillei* is a suitable mycorrhizal fungus for use to inoculate larch seedlings, which are to be grown in both cold mountain areas, and more favourable lowland sites. We suggest that inoculum of this fungus should be applied when larch seedlings are grown in the nursery, or planted in the field.

Key words: *Suillus grevillei*, ectomycorrhizal inoculation, *Larix gmelini*, tree propagation, afforestation

INOCULATING seedlings with mycorrhizas is widely acknowledged as a key process in the production of fast growing forest trees in plantations (Trappe 1977) and is being practiced in many forest nursery operations throughout the world (Hu Hongdao 1979; Guo Xiuzhen and Bi Guochang 1989). In China many pine nurseries have only recently implemented techniques to introduce effective ectomycorrhizas with their seedlings (Trappe and Stand 1969; Hu Hongdao 1979). In northern China *Larix* is one of the major tree species used in the afforestation of mountain regions. It is an obligately ectomycorrhizal species, requiring effective mycorrhizas for its survival and growth in the field (Mikola 1970). This study reports on the potential of mycorrhizal technology to enhance the growth and survival of *Larix gmelini* seedlings produced in the Central Nursery of Chaoyuan Forest Bureau, Inner Mongolia for use in plantations.

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Materials and Methods

Study site

The Central Nursery is located in Chaoyuan Forest Bureau, in the southern part of the Hulunbeier Region, in the central-south section of the Great Xingan Mountain Range in Inner Mongolia (120° 14' E, 48° 10' N), 97 km from the Bolin Railway. This area belongs to the cold temperature zone of China, with an annual mean temperature of –3.3° C, a frost-free growing season of 90 days, annual precipitation of 470 mm, and annual evaporation of 1048.4 mm. The soil type is loamy with a pH of 6.4.

The Chaoyuan Forest Bureau nursery is located between two mountain ranges and is exposed to harsh winds, mostly from the northwest, throughout the year. At this site plant growth often suffers from frost and hailstone damage resulting in failures in seedling establishment. Further, the soils are extremely infertile and the level of natural populations of ectomycorrhizal fungi are low. Most of the seedlings raised in the nursery are larch, but some spruce and poplar are also grown.

Production of seedlings

Container-grown seedlings of Dahurian larch, grown in a plastic greenhouse, were used in inoculation trials. The plastic greenhouse had 88 seed beds (0.07 ha in area), four of which were used as control areas. There were 2000 plastic cells, 8 x 12 cm in size, on each of the seed beds. There were 176 000 cells in total. Before seeding, a fixed amount of *Suillus grevillei* inoculum was mixed evenly with the growth media, which had been sterilised. Seeds were planted into each cell after seven days.

Larch seeds were processed by cold soaking, washing and placed in sterilised sand to hasten germination. Germinated seeds were sown into cells (3–4 seeds per cell) 4 days after germination began and then covered with growth medium and watered. The control area was treated as above.

To measure seedling growth and emergence in mycorrhizal experiments, 5 plots of 1 m^2 in size were chosen randomly for measurements. Seedling growth and emergence rate was investigated every 2 days.

Inoculation of bare-root larch seedlings

The seeding area was 5 ha, 0.1 ha of which was used as a control area. The size of seed beds was 1.1 x 20 x 0.25 m. Germinating seeds were added 5 days after the soil was sterilised with 1% $FeSO_4$. Before seeding, a fixed amount (4.5–6 g/m^2) of *Suillus grevillei* inoculum was applied to seed beds at 10 cm depth and mixed with the soil. This occurred at the same time as seed treating processes. After seeding, beds were levelled by press rolling.

Inoculation of transplanted larch seedlings

Larix gmelini, cultivar S1-0, seedlings were used. A fixed amount (250 g/m^2) of *Suillus grevillei* inoculum was spread in furrows at 10 cm depth, before transplanting seedlings, covering and press rolling. These treatments were also applied to uninoculated seedlings in the control area.

Inoculation of larch planting stock

Two methods were used: (1) a fixed amount of *Suillus grevillei* inoculum was spread into mounds (10 g/mound) where seedlings were planted; or (2) inoculum was mixed with water (500 g/L) to form a slurry into which seedling roots were dipped, before seedlings were planted in hills.

Chemical analysis of seedlings

After the appropriate digestions, the N and P contents of plant material was determined spectophotometrically and other elements were measured by atomic absorption spectroscopy.

Results and Discussion

Ectomycorrhizal influences on seedling growth and survival

The inoculated container seedlings grew much better than those in uninoculated control seedbed areas (Table 1). Mycorrhizal inoculation resulted in faster growth, thicker stems and a lush green colour. The emergence rate of inoculated seeds was 144.9% greater than the control (Table 1).

Table 1. The rate of emergence and growth of container-grown seedlings.

Treatment of seedlings	Inoculated	Control
A. Container-grown seedlings		
Germination[a] (%)	73.1	36.9
Height[b](cm)	3.8	2.9
B. Seedbed seedlings[c]		
Germination[a] (stems/m)	436.5	301.2
Height[b] (cm)	12.2	4.8
Collar diameter (mm)	2.4	1.4
Tap root length (cm)	11.4	11.1
Lateral root length (cm)	15.5	9.3
Dry weight (g)	0.3	0.2
Mycorrhizal formation (%)	51.2	27.5

[a] Only one seedling was counted for each of 320 cells
[b] Seedlings were measured after 40 days
[c] 100 days old

Seedlings in the inoculated and control seed beds were measured in autumn (after 100 days of growth) to compare growth effects. The results (Table 1) show that growth of inoculated seedlings (in height, collar diameter, tap root length, the length of lateral roots and dry weight) was greater than that of the controls. Comparisons of seedling height and growth rates by T-tests confirmed that the inoculation effects were statistically significant ($P < 0.05$). Microscopic examinations of sectioned roots showed that a Hartig net and mantle were present and confirmed that inoculation was successful.

Concentration of nutrients in ectomycorrhizal seedlings

Concentrations of N, P, K and Zn in inoculated seedlings were higher than those in control seedlings (Table 2). The concentrations of Cu and Mn in seedlings were not substantially affected by mycorrhizal inoculation (Table 2).

Table 2. The concentrations of mineral nutrients in inoculated and uninoculated seedlings in the nursery after 3 months.

Mineral nutrient (μg/g)	Inoculated	Control
N	13540	11630
P	3340	2920
K	7831	5763
Cu	27	27
Zn	88	36
Mn	121	115

Note: Values are averages, $n = 100$

Inoculation effects on transplanted seedlings

To assess survival rates, seedlings were measured in the inoculated and control seed plots by random sampling one month after inoculation. The survival of inoculated seedlings was substantially greater than that of the uninoculated controls (Table 3).

Table 3. Comparison of the survival and growth of inoculated and uninoculated transplanted seedlings.

Treatment of seedlings[a]	Inoculated	Control
Survival (%)	96.6	86.1
Height (cm)	32.2	23.9
Collar diameter (mm)	5.4	3.8
Tap root length (cm)	16.7	12.7
Lateral root length (cm)	20.8	13.1
Dry weight (g)	3.8	2.1

[a] Averages for 175–226 seedlings in 5 plots 1 m^2 in size after 1 year

At the end of the first growing season, results showed that the growth of inoculated seedlings was better than that of control seedlings, when their height, diameter, root production, biomass and mycorrhizal formation was compared (Table 3). T-tests confirmed that the inoculation effects were statistically significant ($P < 0.05$).

Inoculation effects on larch seedlings used for afforestation

The survival of transplanted seedlings in field sites was investigated in the middle of, and at the end of, their first year of growth (Table 4). The results of measurements at these two times showed that the survival rate of inoculated seedlings was greater in an afforestation site (Table 4). At the end of the first growing season, the height of inoculated seedlings was significantly greater than that of control seedlings ($P < 0.05$).

Table 4. Comparison of the survival and growth of inoculated and uninoculated transplanted seedlings in an afforestation site.

Treatment of seedlings	Inoculated	Control
Survival in mid-season[a] (%)	94.2	83.0
Survival after 1 season[a] (%)	96.2	84.8
Height[b] (cm)	16.7	9.9

[a] $n = 104–358$
[b] Growth after 1 year, $n = 104–112$

Conclusions

Seedlings inoculated with pure cultured inoculum of the mycorrhizal fungus *Suillus grevillei* grew faster and were superior in quality to uninoculated seedlings. Nursery inoculation with a mycorrhizal fungus can resolve technical problems by increasing seedling survival and increasing their drought-tolerance after planting. These technological advances in seedling cultivation have prospects for wider utilisation in forest nurseries in China.

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Use of Vesicular-Arbuscular Mycorrhizal Fungi to Promote Tree Growth in China

Zhang Meiqing*

Abstract

In this paper, research on vesicular-arbuscular mycorrhizal (VAM) associations in China since the 1980s is reviewed. This research includes investigations of the occurrence of VAM associations with tree crops such as *Citrus* and *Malus* as well as documentation of the presence of particular mycorrhizal fungi. The results of this survey suggest that many important tree crops in China have associations with VAM fungi and there is a great potential for the future utilisation of these associations.

Key words: Vesicular-arbuscular mycorrhiza, tree crops, *Citrus*, *Malus*, China

VESICULAR-arbuscular mycorrhizal (VAM) associations are symbiotic associations between fungi in the Zygomycete order Glomales and the roots of plants. In China, research on VAM associations is still in its infancy having started later than ectomycorrhizal research. The study of VAM associations is a major component of this research. Scientists working with VAM fungi still have many problems to resolve. For instance, fungal identification is often very difficult and methods for the axenic culture of these fungi have not been found, which limits options for the production of inoculum for practical use.

In general there have been two types of surveys of VAM resources in China, describing (1) the occurrence of associations; and (2) the occurrence of fungi. There has been additional research on fungal propagation and utilisation in the field. The purpose of this paper is to provide a brief review on our current knowledge of the occurrence and utilisation of VAM fungi associated with tree crops in China.

The Occurrence of VAM Associations

Scientific surveys of the occurrence of VAM associations have documented the colonisation of plant roots by these fungi, the morphology of mycorrhizal associations in roots, or the abundance of spores produced by these fungi in the rhizosphere. Results of surveys are summarised below.

Chen Xiangxing (1980) investigated 285 tree species from seven provinces in China. He found that 67 tree species had ectomycorrhizal roots, while 107 tree species had VAM associations. Su Liying and Liang Xiutang (1985) observed mycorrhizal associations in the roots of 58 *Eucalyptus* tree species and found that all of them had VAM. Yi Weimin (1991) investigated 125 plant species in two artificial forests in subtropical hills and tropical eroded land and found that 105 plant species had VAM. Zhang Mongchang (1985) observed the morphology of VAM roots and spores in the rhizosphere of *Actinidia* sp. and Cao Yuqing and Guo Zhigang (1989) examined VAM of trees of *Ammodendron argenteum* and Russian olive.

Zhang Qinghua of the Citrus Institute, Chinese Agricultural Academy (pers. comm.) investigated trees in 112 orange orchards and nurseries from 14 cities in 3 provinces in south China. He found that most citrus trees were mycorrhizal, but many had roots that were weakly colonised by these fungi. He believed that it was feasible to inoculate citrus with VAM fungi to increase mycorrhizal formation. Liu Runjing et al. (1987) of Shandong Agricultural College examined mycorrhizal roots and spore numbers in soil in the rhizosphere of 14 fruit plant species in 16 cities in Shandong Province. They found that VAM associations were very common in this region and that different species of fruit trees had different levels of root colonisation by these fungi.

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VAM Fungi Associated with Trees

Knowledge of the diversity of VAM fungi, identified from spores from the rhizosphere of Chinese trees is summarised in Table 1. We are currently investigating VAM fungus resources in 6 provinces in the east and south coast of China (Table 2). Many of the host plants in this survey were trees. We found that different VAM fungi were found in southern and northern China. In northern China, the main genus encountered was *Glomus*, and *G. mosseae* was the most common species in this area, but we never found *Sclerocystis*. However, in southern China there were species, such as *G. mosseae*, which were uncommon, while *Sclerocystis* species and other *Glomus* species, including *G. formosanum* and *G. aggregatum*, were common.

Information about which species of VAM fungi are dominant in the rhizosphere of particular trees is of value for selecting fungi for use with that tree in the future. From 1996 we plan to continue our survey to include southwest and central China. We hope this work, will provide us with a general idea about the distribution of VAM fungi in China, and will help to select fungal species for practical use.

Applied Research with VAM Fungi and Trees

Applied VAM research has occurred in many parts of China. Among the host plants used for this research, the main tree species have been *Citrus* and *Malus* species, with which there has been much progress.

Citrus

The earliest VAM research in China was on *Citrus* species. Tang Zhenyao and others at the Citrus Institute, Chinese Agricultural Academy started the research of VAM in 1978. They have isolated fungi from the rhizosphere of citrus, studied the influence of VAM on phosphorus, calcium, and iron uptake with radioisotope techniques, and conducted many applied experiments (Tang Zhenyao and Hao Kailan 1984; Tang Zhenyao and Zang Mu 1984; Tang Zhenyao and Chen Anning 1986; Tang Zhenyao et al. 1989; Tang Zhenyao and Zang Mu 1990). For instance, in one of their experiments, they used trifoliate orange and *Citrus aurantium* as host plants in seedbeds without sterilisation. Two months after

Table 1. Some reports on the occurrence of VAM fungi with trees in China.

VAM fungi	Host plants	Location	Source of information
Glomus citricola	*Citrus sinensis* Osbeck	Dao Country, Hunan Province	Tang Zhenyao and Zang Mu 1984
Gigaspora sp.	*Camellia oleifera* Abel (oil tea camellia)	Zejiang Province	Zhou Chonglian (pers. comm.)
VAM fungi	*Calamus* sp.	Guangdong Province	Gong Mingqin et al. 1989
VAM fungi	*Dimocarpus longan* Lour. (Longan)	Fujiang Agricultural Academy	He Guangze, Fujiang Agricultural Academy
Sclerocystis sinuosa	*Acacia* sp.	Taiwan	Wang Honggang et al. 1992
15 species of VAM fungi	Species of *Acer, Ailanthus, Citrus, Crategus, Fraxinus, Juglans, Leptodermis, Malus, Poncirus, Prunus, Rosa, Salix Sorbus* and *Ulmus*	Xingjiang and Jiling Provinces and Beijing	Zhang Meiqing et al. 1992, 1994
VAM fungi	*Camellia sinensis* Kuntze (tea tree)	Anhui Province	Ji Ruiying (pers. comm.)

Table 2. The occurrence of some species of *Glomus* and *Sclerocystis* in soil samples from six provinces in China.

VAM fungus	Incidence (% of soil samples)					
	Xinjiang	Beijing	Jiling	Shandong	Fujiang	Guangdong
Glomus mosseae	27	45	23	38	10	6
G. aggregatum & *G. microcarpum*	0	0	0	23	29	32
Sclerocystis spp.	0	0	0	0.8	33	40

inoculation with VAM fungi they found big differences in plant growth relative to uninoculated controls. Later they applied *G. citricola* to container-grown citrus and successfully increased the growth of trifoliate orange seedlings.

Wan Shuiling (1992) in Jiangxi Province used the donor plant transplantation method for efficient inoculation. Vigorous mycelial growth in seedbeds resulted in rapid infection of trifoliate orange seedlings and faster production of seedlings than the standard methods. Mycorrhizal seedlings were found to have a higher survival rate without a recovery period when transplanted into the field. More than 100 000 mycorrhizal citrus seedlings have since been produced by this method. In China, research on VAM associations of citrus has occurred in Sichuan, Jiangxi, Hunan, Hubei and Guangdong Provinces (Tang Zhenyao and Hao Kailan 1984; Tang Zhenyao and Zang Mu 1984; Su Liying and Liang Xiutang 1985; Tang Zhenyao and Chen Anning 1986; Tang Zhenyao et al. 1989; Bi Guochang et al. 1990; Shen Tinghou 1990; Tang Zhenyao and Zang Mu 1990; Wan Shuiling 1990, 1992).

Other trees

The research on VAM associations of *Malus* species has occurred at Shandong Agricultural University and Laiyang Agricultural College in Shandong Province (Liu Runjing et al. 1987; Liu Runjing and Luo Xinshu 1988a,b; Xue Bingye and Luo Xinshu 1990, 1991; Liu Runjing and Hao Wenying 1994). This research measured mineral nutrient and water uptake and plant growth responses. In one of the experiments by Liu Runjing and Luo Xinshu (1988a), *Prunus pseudocerasus* was inoculated with *G. mosseae* and *G. versiforme* and substantial growth increases were observed after two months. Research on apple replant disease, which started in 1989, has suggested that growth decreases due to this disorder can be eliminated by soil fumigation with formalin followed by inoculation with VAM fungi (Liu Runjing et al., unpublished data).

Other experiments on watermelon and peach have also produced good results (Xue Bingye and Luo Xinshu 1990, 1991). In another study, Wang Yuanzhen (1991) in Fujiang Agricultural College examined the influence of VAM on rosary-pea tree (*Abrus precatorius* L.). Zhang Meifang (1989) suggested that hedge acacia should be inoculated with VAM fungi and rhizobium for fast growth, nutrient-deficiency tolerance, and to produce more biomass for use as forage, fertiliser, and fuel. Gong Mingqin et al. (1989) studied the growth effect of *Calamus* sp. and *Casuarina* after inoculation with VAM fungi. He found that in some cases responses to VAM fungi were better than responses to ectomycorrhizal fungi. There has also been research on the VAM associations of *Dimocarpus longan* Lour. (Longan) by He Guangze (Fujiang Agricultural College, pers. comm.) and Liang Xioutang (this volume).

Conclusions

From the information summarised above, we can see that there has been a good start to research on the VAM associations of trees in the laboratory, greenhouse or field in China. Since tree crops are of great value to the national economy and many are associated with VAM fungi, further research on these associations, is desirable, both as worthwhile scientific accomplishments and to explore the potential for economic benefit. It is clear that the VAM fungi associated with trees in China provide a very valuable biological resource, with great potential for future use.

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Inoculation of Forest and Fruit Trees with Vesicular-Arbuscular Mycorrhizal Fungi in Guangxi Province, China

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Abstract

Vesicular-arbuscular mycorrhizal (VAM) fungi were used to inoculate one-year-old trees of *Mytilarea laosemsis*, *Eucalyptus exserta*, *Michelia macclurei* var. *sublanea*, *Mangifera indica*, *Litichi chinensis* and *Dimocarpus longan*. Inoculation significantly increased plant height, stem diameter and biomass, compared with uninoculated plants. VAM fungus inoculation also increased the chlorophyll contents and photosynthetic rates in leaves and enhanced nutrient uptake by these species. These effects were particularly significant in *L. chinensis* plants grown in red soil. The concentrations of P, K, Ca and Mg in leaves and roots of inoculated *L. chinensis* plants were increased relative to those in the uninoculated plants.

Key words: vesicular-arbuscular mycorrhizal fungi, inoculation, tree crops, growth responses, nutrient uptake

MYCORRHIZAS are one the most common types of symbiotic associations between plants and microorganisms and have been the focus of research by scientists in many countries. Some results from this research have been applied in agriculture, forestry and horticulture (Liu Lunjing 1988; Gou Xiouzhen 1989; Lin Xiangui 1989; Liang Xiutang 1992), but much of this potential remains unexploited. Vesicular-arbuscular mycorrhizal (VAM) associations are the most prevalent type of mycorrhizal association. Research on the VAM associations of tree crops in China is summarised elsewhere (Zhang Meiqing, this volume). The main objective of this study was to demonstrate the value of VAM associations to important tree crops in China and to select effective fungi for use in different environmental conditions.

Materials and Methods

The soils used were red soil and sediment mud — pond sediments with high organic matter content (Table 1). The VAM fungi used were *Glomus mosseae*, *G. caledonium*, and *G. citricola*. These pot-culture isolates were obtained from the microbiological group of the Horticulture Department, Inner-Mongolia Agriculture and Husbandry College and the Nanjing Soil Science Institute of Chinese Academy. Tree species used in experiments were *Mytilarea laosemsis* H. Lec., *Eucalyptus exserta* F. V. Muell., *Michelia macclurei* Dandy var. *sublanea* Dandy, *Mangifera indica* Linn., *Litichi chinensis* Sonn. and *Dimocarpus longan* Lour.

Pot-culture and field trial experiments

In pot-culture experiments there were two treatments with inoculated or uninoculated plants. Each treatment was replicated 3–20 times with 1–2 plants in each replicate. Pots were randomly arranged. Sterilised soil (2 kg) was placed in each pot, which was then inoculated with 20 VAM fungus spores which were placed with the seeds 3 cm below the soil surface. No inoculum was applied to the control treatments. In each pot, 4 seeds were sown (only 1 seed was sown for *Mangifera indica*) and plants were thinned to 1–2 per pot after emergence.

In field trials there were two treatments with uninoculated and inoculated plants, with 3 replicates in each treatment. The area of each field plot was 1 m^2 and there were 5–10 plants per plot. Each of the inoculated plots received 1 kg of pot-culture inoculum soil containing VAM fungus propagules. This inoculum was placed 6 cm deep around tree seedlings. Plants were not fertilised during the experimental period.

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Table 1. Background levels of mineral nutrients in the soils used in experiments (ppm).

Soil type	Total	Available		Exchangeable	
	N	P	K	Ca	Mg
Red soil	45.5	3.5	31.2	19.4	5.6
Mud	62.2	7.4	33.1	22.3	4.3

Measurements

Mycorrhizal formation in roots (infection ratio) was measured 3–4 months after inoculation. The roots were fixed, cleared, acidified and stained, using standard procedures (Phillips and Hayman 1970) and then examined microscopically. Two plants of various sizes were sampled in each treatment and 30–100 root pieces examined to measure the proportion of mycorrhizal roots.

The height, stem diameter and fresh and dry weights of shoots and roots was measured. Plant dependency on VAM associations was calculated as the percentage ratio of the dry matter of mycorrhizal plants to that of non-mycorrhizal plants. Nitrogen was determined by semi-micro Kehldahl digestion. Total P was determined by Mo-Sb-ascorbate colorimetry. The concentrations of K, Ca and Mg were determined by AAS (Forest Soil Institute of China 1988).

Results and Discussion

The degree of VAM formation in roots of the inoculated tree seedlings varied with tree species (Table 2). The effects of mycorrhiza on plant biomass increased with the infection rates (Table 3). The positive effect of mycorrhizal inoculation on plant biomass was particularly significant in *M. laosemsis* seedlings of which the inoculated plants were as much as 4-times larger than uninoculated plants. The plants inoculated with VAM had greater height and fresh weight than the uninoculated ones (Table 3). These increases in plant growth were likely due to increases in the uptake of water and nutrients by mycorrhizal plants.

There was a significant effect of VAM fungi on the growth of *M. indica* plants, in terms of the growth parameters examined (Table 4). The height, stem diameter and shoot dry weight of inoculated plants were 17%, 280% and 64% higher respectively than that of uninoculated plants.

As shown in Table 5, all 3 species of plants tested showed the greatest response to *G. mosseae*. The inoculation of the plants with this fungus increased plant height and biomass by 1.7 and 3.9 times in *L. chinensis* plants, 1.3 and 1.7 times in *M. indica* plants, and 1.3 and 1.3 times in *D. longan* plants respectively, in comparison to the uninoculated plants. In contrast, the other 2 species of mycorrhiza were not so suitable. Inoculation with the other two fungi produced less substantial responses (Table 5), indicating that they were less effective under these conditions. It is necessary to select the most suitable combination of VAM fungus and symbiont plant species to make full use of these associations. From the experimental results collected by the author over many years, *G. mosseae* can form mycorrhizal associations with many plant species with significant effects. There is a considerable potential for the development of inoculum of *G. mosseae* for use with tree crops.

The results indicated that different species of plants have different dependency on VAM fungi (Table 6). Among the plant species tested in the present experiment, *L. chinensis* plants had the highest mycorrhizal dependency (330%), *M. indica* plants the second and *D. longan* plants the lowest. As a result, *L. chinensis* plants inoculated with VAM had greatest differences in heights and biomass compared to uninoculated plants (Table 5). The effect of VAM inoculation on *M. indica* and *D. longan* was less substantial than that with *L. chinensis*, but these species still benefited from the association.

Many factors may influence the dependency on mycorrhiza, such as the species of mycorrhiza, root morphology, plant growth rate, and soil type. In the present experiment, plants were grown in red soil and mud. The response to mycorrhizas varied with soil type (Table 7). The mycorrhizal dependency of *L. chinensis* grown in mud on mycorrhiza was lowest (129%), but was higher in red soil (330%). This may be related to the effect of soil properties on mycorrhizal fungi. In order to make full use of the effects of mycorrhiza, it is important to apply mycorrhiza in suitable soils.

Plants inoculated with VAM fungi had significantly higher P contents in their roots and leaves than uninoculated plants (Table 8). This effect was particularly significant in *L. chinensis* grown in red soil, where inoculation increased P levels by 2.4 and 2.9 times and K by 1.04 and 1.3 times in roots and leaves respectively, relative to uninoculated plants. VAM fungi are known to help plants obtain P from soil, because their hyphae obtain nutrients a considerable distance from roots (Harley and Smith 1983). The effects of VAM on the uptake and utilisation of P were more significant in inoculated plants grown in red soil than those grown in mud.

Table 2. VAM colonisation of roots of different species of trees in pot and field experiments (% root length).

Tree species	Fungus		
	G. mosseae	*G. caledonium*	*G. citricola*
A. Pot culture experiment			
Mangifera indica	28	22	21
Litichi chinensis	28	17	19
Dimocarpus longan	20	13	17
Mytilarea laosemsis	14		
Eucalyptus exserta	9		
Michelia macclurei	12		
B. Field plot experiment			
Mytilarea laosemsis	30		
Eucalyptus exserta	19		
Michelia macclurei	20		

Note: n = 35–100 roots

Table 3. Effect of inoculation with VAM on growth of different species of trees.

	Mytilarea laosemsis			*Eucalyptus exserta*			*Michelia macclurei*		
	Height (m)	Shoot (g)	Root (g)	Height (m)	Shoot (g)	Root (g)	Height (m)	Shoot (g)	Root (g)
A. Pot culture experiment									
Inoculated	1.53	9.6	2.8	1.65	5.5	3.3	1.45	7.1	5.1
Uninoculated	1.15	6.1	1.7	1.44	3.6	1.0	1.21	4.26	2.3
B. Field plot experiment									
Inoculated		372.9	75.2	4.6	905.7	501.2			
Uninoculated		74.9	20.7	3.3	633.7	390.3			

Notes: Shoot and root measurements are fresh weight.

Table 4. Plant biomass and shoot to root (S/R) ratio of mycorrhizal and non-mycorrhizal *Mangifera indica.*

Treatment	Height (m)	Diameter (m)	Shoot (g)	Root (g)	S/R ratio
Inoculated (*G. mosseae*)	63.1	1.5**	46.8**	17.1**	2.9
Uninoculated (control)	54.0	0.4	24.8	10.3	2.4

* = $P < 0.05$, ** = $P < 0.01$ (F tests)
Note: Shoot and root measurements are dry weight

Table 5. Effects of 3 species of VAM fungus on the growth of three fruit trees.

Tree species	Mycorrhizal fungus	Height (cm)	Diameter (cm)	Shoot (g)	Root (g)	Total (g)
Litichi chinensis	*G. mosseae*	31.6	0.61	7.67	4.55	12.22*
	G. caledonium	19.1	0.40	3.27	1.86	5.13
	G. citricola	23.4	0.41	4.92	2.23	7.15
	Control	17.7	0.34	2.30	1.42	3.71
Mangifera indica	*G. mosseae*	52.2	1.04	16.70	4.84	21.81*
	G. caledonium	48.5	1.05	15.60	3.74	19.34
	G. citricola	52.0	0.90	12.41	4.43	16.97
	Control	39.7	1.02	7.84	4.41	12.25*
Dimocarpus longan	*G. mosseae*	65.5	0.27	15.23	4.75	19.98
	G. caledonium	52.4	0.26	12.80	2.36	15.15
	G. citricola	54.1	0.26	13.16	2.70	15.86
	Control	46.1	0.25	10.86	3.46	14.32

* = $P < 0.05$ (F test)
Notes: The data are all means. Root, shoot and total are dry weights

Table 6. The dependency of different species of trees on VAM fungi.

Soil type		Mycorrhizal dependency (%)		
		G. mosseae	*G. caledonium*	*G. citricola*
Red soil	*Mangifera indica*	329	151	140
	Litichi chinensis	188	250	150
	Dimocarpus longan	139	106	106
Mud	*Mangifera indica*	129	88	28
	Litichi chinensis	73		
	Dimocarpus longan	112	110	106

Table 7. Effects of mycorrhizal inoculation on the growth of different trees in two soils.

Tree	Soil	VAM	Height (cm)	Fresh (g)	Shoot (g)	Root (g)	Total (g)
Litichi chinensis	red soil	+	31.6	24.9	7.67	4.55	12.2*
		–	17.7	5.8	2.29	1.42	3.71
	mud	+	26.5	17.8	8.69	2.99	11.7
		–	23.4	14.0	7.28	2.21	9.50
Dimocarpus longan	red soil	+	52.2	58.0	17.0	4.84	21.8*
		–	39.7	22.7	12.4	3.74	12.5
	mud	+	57.5	53.9	14.0	7.10	20.4
		–	64.0	66.4	22.7	4.74	27.8

* = $P < 0.05$ (F test)

Table 8. Effect of mycorrhizal inoculation on nutrient concentrations in the leaves and roots of 3 trees grown in 2 soils with or without VAM inoculation (%).

Tree	Part	VAM	Soil	N	P	K	Ca	Mg
Litichi chinensis	leaves	+	red soil	0.86	0.10	0.52	0.36	0.23
		–	red soil	1.01	0.03	0.21	0.12	0.17
		+	mud	0.93	0.13	0.61	0.77	0.30
		–	mud	1.21	0.04	0.27	0.32	0.22
	roots	+	red soil	0.52	0.08	0.43	0.23	0.21
		–	red soil	0.55	0.03	0.38	0.21	0.21
		+	mud	0.51	0.12	0.43	0.17	0.19
		–	mud	0.54	0.05	0.32	0.15	0.20
Dimocarpus longan	leaves	+	red soil	1.00	0.17	0.54	1.93	0.15
		–	red soil	1.02	0.16	0.60	1.80	0.24
		+	mud	0.97	0.16	0.49	1.82	0.24
		–	mud	0.95	0.14	0.48	1.98	0.26
	roots	+	red soil	0.21	0.09	0.37	0.40	0.10
		–	red soil	0.31	0.06	0.30	0.38	0.10
		+	mud	0.32	0.11	0.37	0.38	0.11
		–	mud	0.34	0.10	0.29	0.30	0.10
Mangifera indica	leaves	+	red soil	1.15	0.09	0.92	1.50	0.19
		–	red soil	1.28	0.10	0.88	1.12	0.19
	roots	+	mud	0.44	0.05	0.39	0.09	0.11
		–	mud	0.63	0.07	0.48	0.15	0.19

Conclusions

Many tree crops, including those included in the present study, can benefit from inoculation with VAM fungi. These mycorrhizal associations improve plant growth and nutrient uptake. The response of plants to VAM associations depends on the mycorrhizal fungus, host plant and soil used. Consequently, it is important to select mycorrhizal fungi for practical use that are compatible with host plants and soil conditions.

Acknowledgment

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Dual Inoculation of Mimosaceae Seedlings with Vesicular-Arbuscular Mycorrhizal Fungi and Rhizobium

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Abstract

Seedlings of the Mimosaceae plants *Leucaena leucocephala* and *Acacia mearnsii* were inoculated with vesicular-arbuscular mycorrhizal (VAM) fungi and rhizobium nodule bacteria. The experiment showed that seedlings with dual VAM/rhizobium associations had greatly improved numbers of nodules and growth compared with the seedlings in control plots. Not only was the height and weight of seedlings increased, but nitrogen fixation and phosphorus absorption were also increased substantially by inoculation with VAM fungi.

Key words: vesicular-arbuscular mycorrhiza, rhizobium, Mimosaceae, dual associations

MYCORRHIZAL fungi are universally present in soil and associations occur with most plants (Deng Shuqun 1963). These associations augment plant mineral nutrient uptake by increasing the absorptive capacity of their roots. Mycorrhizal fungi acquire nutrients by developing a hyphal network in soil. Mycorrhizal formation by plants can be inhibited by topsoil disturbance, the misuse of chemical fertilisers and pesticides, or by pollution.

Mycorrhizal fungi are an important key in the production of nursery stock of some tree species, and are a necessary measure for enhancing the biomass of transplanted seedlings (Guo Xiuzhen 1989). Members of the family Mimosaceae typically form symbiotic associations with vesicular-arbuscular mycorrhizal (VAM) fungi and nodule bacteria (NB) from the genera *Rhizobium* or *Bradyrhizobium*. In recent years, experiments with woody plants in the family Mimosaceae have investigated the role, and potential for manipulation, of both VAM and NB associations. This paper reports research concerning the effects of inoculation with these organisms using *Leucaena leucocephala* cv. 'Salvador.', and *Acacia mearnsii*.

Materials and Methods

Glomus mosseae (VA9), *Glomus* spp. (VA12, VA15) and Rhizobium were obtained from the Chinese Academy of Agriculture. *Setaria glauca*, *Zea mays* and *Trifolium repens* were used as hosts for producing bulk inoculum in pot cultures. After verifying infection by inspecting roots and quantifying spores, the mixed rhizosphere soil and roots from pot-grown plants was used as inoculum for the following experiments.

1. VAM inoculation of *Leucaena leucocephala*

Seeds were treated with concentrated sulfuric acid (for 15–20 sec) then germinated in soil at a constant temperature (25°C). Seeds were sown in rows in infertile nursery soil, with 20 seeds per row and received the following inoculation treatments. Plants inoculated with VAM fungus received 50 g of soil inoculum, 10 g of phosphate (P_2O_5) fertiliser (P) and 50 g of sawdust. Plants inoculated with nodule bacteria (NB) received 5 g NB, 10 g P and 50 g of sawdust. Plants inoculated with both VAM and NB received 50 g VAM inoculum, 5 g NB, 10 g P and 50 g sawdust. Control plants recieved 10 g P and 50 g of sawdust only.

Plants were grown in nonsterilised soil, with supplemental watering, but without additional fertilisers. Each plot was 1 m^2 and had 5 rows of plants with 10 seedlings per row, resulting in 12 plots that were randomly arranged and there were 3 replications per treatment. There was a 0.5 m^2 buffer area between experimental plots. The inoculum and fertiliser quantities described above were mixed with sawdust and broadcasted using seed drills. Seeds were sown and covered to 1 cm depth. At 3 months after emergence, 15 young seedlings were selected at random and sampled. The basal diameter, height, and shoot and root biomass of plants were measured. Mycorrhizal formation was measured and data presented as a percentage of total root length.

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2. VAM inoculation of *Acacia mearnsii*

Seeds were germinated in 8 × 12 cm nursery containers in sterilised (105°C) or nonsterilised soil with the following treatments: 5 g of VAM fungus inoculum per container; 5 g VAM inoculum and 40 ppm P_2O_5 per container; or controls without inoculation or extra fertilisers. There were 3 replicates per treatment.

3. VAM and NB inoculation of *Acacia mearnsii.*

Seedlings were prepared as described for experiment 2 above using nonsterilised soil. Experimental treatments were as follows: 5 g VAM inoculum per container; 5 g VAM and 2 g NB inoculum in each container; 2 g NB in each container; 5 g VAM and 40 ppm P_2O_5 per container; and the control with 5 g of VAM inoculum, which was killed by autoclaving.

Results and Discussion

1. VAM inoculation of *Leucaena leucocephala*

In VAM inoculated plots, only 12% of seedlings were nodulated and the average nodule number for each seedling was 0.15 nodules per plant. In NB inoculated plots, all seedlings were nodulated and the average nodule number for each seedling was 2.2 nodules per plant. Dual inoculation with both VAM and NB resulted in all seedlings becoming nodulated at an average nodule number of 3.6 nodules per plant. There was no nodulation in control plots.

The results demonstrated that VAM and NB inoculation can improve the growth and development of seedlings of *Leucaena leucocephala.* Growth and dry weight were increased substantially, especially with dual inoculation. The dual inoculated seedlings were taller (71%), with thicker stems (20%) and larger shoot (125%) and root (135%) fresh weights and dry weights (shoot 144%, root 163%) than the control plants (Table 1). The increase in biomass of the seedlings was correlated with the extent of VAM infection.

2. VAM inoculation of *Acacia mearnsii*

VAM inoculation could promote growth of *Acacia mearnsii* whether the soil was sterilised or not. However, its effect was greater in sterilised soil (Table 2). Inoculation resulted in height increases of 181%, while the fresh and dry weight of above-ground and below-ground parts were also substantially increased, when compared to uninoculated control plants. Mycorrhizal inoculation resulted in a 147% increase in mycorrhizal colonisation, but this effect may not have occurred in non-sterile soil.

3. Dual VAM and NB inoculation of *Acacia mearnsii*

Statistical analysis of the height of seedlings showed that there was a substantial difference among treatments (Table 3).The best results were obtained by dual inoculation with VAM and NB. The height of seedlings was increased by 204% by the dual inoculation treatment, 128% by VAM alone (Fig. 1). The number of nodules increased 75% in the dual inoculated treatment, as compared with the control. There also were differences in the performance of the two isolates of VAM fungi (Fig. 1). There was evidence of differences in the rate of root colonisation between these VAM isolates. These colonisation rates could be used as an index for selecting isolates of these fungi.

Conclusions

Although either VAM or NB inoculation can improve the growth of seedlings of *Leucaena* and *Acacia*, the most effective treatment was dual inoculation with both organisms. Inoculation with VAM fungi may have improved nodulation by increasing the ability of seedlings to absorb P, Mo, Fe, S and Zn, which are necessary for the formation of root nodules. The effect of inoculation with these microbes was greatest in soil which had been sterilised to remove indigenous symbiotic microorganisms. Further research is required to investigate interactions between mycorrhizal and nitrogen-fixing associations for plants which have both.

Table 1. Effect of inoculating *Leucaena* with VAM and/or nodule bacteria (NB) on mycorrhizal formation and plant growth.

Inoculation treatment	Basal diameter (cm)	Height (cm)	Shoot weight (g)	Root weight (g)	VAM (%)
VAM	0.53	33.6	17.0	36.8	10.3
NB	0.52	31.3	12.1	23.7	12.5
VAM + NB	0.59	45.3	19.8	52.6	20.4
Control (uninoculated)	0.49	26.6	8.1	20.0	4.0

Weight = dry weight; VAM = % root length colonised

Table 2. Results of *Acacia mearnsii* inoculation with VAM fungi.

Inoculation treatment	Basal diameter (cm)	Height (cm)	Shoot weight (g)	Root weight (g)	VAM (%)	Number of leaves
Sterile soil control	0.20	12.4	0.44	0.28	30.7	5.5
Non-sterile soil control	0.19	10.7	0.14	0.10	25.5	6.4
Sterile soil +VAM	0.33	34.9	1.4	0.64	75.7	11.1
Non-sterile soil +VAM	0.31	29.6	1.16	0.60	16.5	8.5
Sterile soil +P	0.25	17.5	0.36	0.48	24.0	7.0
Non-sterile soil +P	0.28	25.1	1.20	0.62	22.8	–

Weight = dry weight; VAM = % root length colonised; P = 40 ppm P_2O_5

Table 3. Analysis of variance (ANOVA) of results of VAM and NB inoculation trials with *Acacia mearnsii.*

	DF	SS	MS	F
Treatment	7	785.50	112.21	17.16**
Replication	2	21.74	10.87	1.66
Error	14	91.57	6.54	
Total	23	898.81		

** = $P < 0.01$

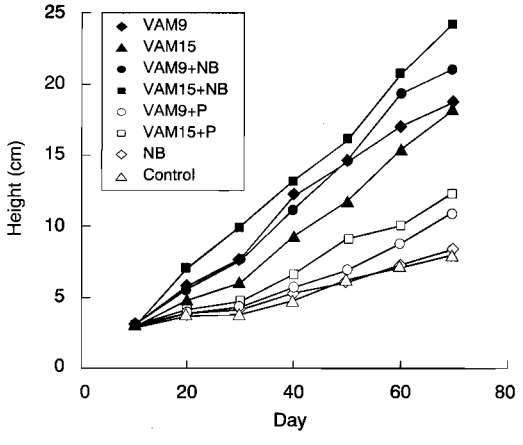

Figure 1. The effect of inoculation with rhizobium (NB) and/or different isolates of VAM fungi (9 and 15) and phosphorus addition (P) on the growth of *Acacia mearnsii.*

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Inoculation of *Casuarina* with Ectomycorrhizal Fungi, Vesicular-Arbuscular Mycorrhizal Fungi and *Frankia*

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Abstract

Experiments were conducted in the glasshouse, nursery or field to study the effect of ectomycorrhizal fungi, vesicular-arbuscular mycorrhizal (VAM) fungi and *Frankia* on the growth of young plants or trees of *Casuarina*. These experiments compared growth of *Casuarina cunninghamiana* and *C. equisetifolia* inoculated with a mycorrhizal fungus alone, a *Frankia* isolate with a mycorrhizal fungus and with *Frankia* alone. Height growth of these two species of *Casuarina* were compared to test the effect of different inoculation treatments, or fertilisation with different phosphorus application rates. Mycorrhizal fungal species were found to be important for improving the growth of casuarina seedlings, when *Pisolithus* spore or agar inoculum was applied. There was an interaction between casuarina seedling genotypes and mycorrhizal inoculation results. The height of *Casuarina* seedlings and trees were significantly increased by dual inoculation with a mycorrhizal fungus and a *Frankia* isolate. Phosphorus application rates influenced the effect of mycorrhizal inoculation. When 3–18 g P plant^{-1} was applied at planting, the heights of two casuarina clones were significantly greater with dual mycorrhizal fungus–*Frankia* inoculation than the uninoculated controls. For one clone (CE907), there was also a significant effect due to inoculation with the ectomycorrhizal fungus *Pisolithus* alone.

Key words: *Casuarina*, ectomycorrhiza, vesicular-arbuscular mycorrhiza, *Frankia*, dual inoculation

THE roots of *Casuarina* plants can have symbiotic associations with ectomycorrhizal fungi, vesicular-arbuscular mycorrhizal (VAM) fungi and nodule-forming *Frankia* bacteria. Both mycorrhizal associations help *Casuarina* plants absorb soil nutrients, while *Frankia* form a nitrogen-fixing association. There has been some research on the benefits of mycorrhizal associations to *Casuarina* in other countries, but these associations have not been studied in China.

This paper summarises 4 experiments in the glasshouse, nursery or field, where *Casuarina* species were inoculated with ectomycorrhizal fungi or the nitrogen-fixing symbiont *Frankia* sp. The experimental aims were to study the effect of these beneficial associations, alone or together, on the growth of seedlings, or young trees of *Casuarina* and to understand the effect of phosphorus application on inoculation with these organisms.

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Materials and Methods

Experiment 1. The effect of different mycorrhizal fungi on growth of *Casuarina cunninghamiana* in the glasshouse

This experiment was carried out in a glasshouse where the average monthly temperature was 22.6°C. Mycorrhizal fungi used in the experiments were isolates of *Pisolithus tinctorius* (9216) and *Scleroderma* (9215), spores of *Pisolithus* and *Scleroderma* and a VAM fungus *Glomus* sp. (isolate 9004 — isolated by Zhang Meiqing in Northern China). Seeds of *Casuarina cunninghamiana* were sterilised by immersion in 3% NaOCl for 3 minutes, then were sown in a sterilised growth medium. Seedlings were transplanted into pots when they were about 4.5 cm in height. The growth medium consisted of sand, peat and vermiculite (equal parts by volume) and was sterilised at 120°C for 2 hours.

Inoculum of the VAM fungus *Glomus* sp. was propagated by a pot-culture method with *Trifolium*

sp. as the host plant grown in sand mixed with peat. Spores of *Pisolithus* and *Scleroderma* were collected from *Casuarina* plantations in southern China. *Pisolithus* 9216 and *Scleroderma* 9215 isolates were cultivated in Melan Norkans media (Marx 1969) with agar. One day after transplanting, all seedlings were inoculated using agar-culture inoculum of ectomycorrhizal fungi and VAM pot-culture inoculum (50 g of soil), or left as controls. Agar inoculum (about 2 cm^2 of mycelium on agar) was placed in the soil near seedling roots. Spore slurry inoculum was made by mixing 0.5 g of spores with 100 mL of water to make a solution, which was injected into the soil near seedling roots at a rate of 10 mL of spore solution per seedling.

At 9 months after inoculation, the heights and diameter at ground level of seedlings were measured and all seedlings were harvested for biomass measurements. Assessment of VAM formation occurred after Trypan blue staining (Phillips and Hayman 1970) by observing 30 root sections 1 cm in length and calculating the average infection rate. Observations of root colonisation in ectomycorrhizal fungus treatments was by microscopic examinations and reported as the percentage of mycorrhizal seedlings. All seedlings were dried at 80°C for 48 hours and then weighed to determine root biomass, aboveground biomass, and total biomass. The experiment was implemented and analysed as a completely randomised block design with 6 fungal treatments, 3 seedlings per treatment and 3 replicates.

Experiment 2. Effect of mycobead inoculum and *Frankia* inoculation on the growth of *Casuarina cunninghamiana*

This experiment was carried out in the field at Yangxi, Guangdong Province, in a site with a latosol with a pH of 4.5 and readily available N, P, K of 72.4, 1.8 and 37.4 mg kg^{-1} soil, respectively. Mean annual temperature at the site was 22.2°C, and annual rainfall was 2250 mm. Fungal inoculum was applied as mycobeads of *Hebeloma westraliense*, provided by the CSIRO Forestry Research Group in Western Australia. *Frankia* inoculum was collected and isolated in south China. Mycobeads were placed in the soil near seedling roots during transplanting. *Frankia* was applied in P-media (liquid) by injection with a pipette into soil near seedling roots. Plant spacing was 2 × 1 m. Seedlings were grown for 5 months in the nursery before planting in the field. At 65 months after planting, tree heights and diameter at 1.3 m were measured. This experiment was a completely randomised block design with four treatments, 10 trees per treatment and 2 replicates of all treatments.

Experiment 3. The Effect of *Pisolithus tinctorius* spore inoculum and *Frankia* on height growth of 2 *Casuarina equisetifolia* clones

This experiment was carried out in a field nursery at Gangkou, Huidong County, Guangdong Province. It was implemented and analysed as a completely randomised split-plot design. In each subplot there were 2 clones of *Casuarina equisetifolia* (CE601 and CE907) and 4 inoculation treatments, with 4 replicates and 5 seedlings per treatment. Inoculation treatments were CK = control (uninoculated), M = inoculum of *Pisolithus tinctorius* spores, F = *Frankia* isolate and MF = M + F. The height of seedlings was measured 5 months after they were inoculated.

Experiment 4. The effect of P application rates on different *Casuarina* genotypes inoculated with ectomycorrhizal fungi and *Frankia*.

This experiment was carried out in the field at Gangkou, Huidong, China. The site has a sandy soil of pH 4.6, with readily available N, P, K values of 44.1, 3.1, 24.0 mg kg^{-1} respectively. It was implemented as a factorial design with 2 clones of *Casuarina equisetifolia* (CE601 and CE907), 4 inoculation treatments (the same as experiment 3) and 6 phosphorous application rates (0, 25, 50, 100, 200 and 300 g superphosphate per tree, which was equivalent to 0, 1.5, 3.0, 6.0, 12.0 and 18.0 g P per tree). Five trees were used for each P rate for inoculation treatment and the experiment was replicated 4 times. Tree heights were measured 19 months after planting.

Results and Discussion

Experiment 1

Inoculation of *Casuarina* plants with agar culture material, spores of *Pisolithus tinctorius* or spores of *Scleroderma* sp.; or the application VAM inoculum of *Glomus* sp. were all highly successful (Table 1).

Table 1. Infection rates (% of roots with mycorrhizas) of *Casuarina* plants after inoculation with different mycorrhizal fungi preparations.

Treatment[a]	S1	S2	P1	P2	VA	CK
Infection (%)	100	88.9	100	100	80.3	0.0

[a] Culture material (S1) or spores of *Scleroderma* (S2); agar material (P1) or spores of *Pisolithus tinctorius* (P2); VAM inoculum of *Glomus* (VA), or without inoculum (CK)

Inoculation with mycorrhizal fungi resulted in substantial increases in the growth (measured by height, diameter, or biomass) for *Casuarina* plants (Table 2).

Table 2. Mean height, diameter, and dry weight of *Casuarina* seedlings 9 months after the application of 6 inoculation treatments.

			Dry weight (g/plant)		
Treatment*	Height (m)	Diameter (cm)	Above ground	Below ground	Total
CK	13.3a	0.11a	0.34a	0.10a	0.45a
S1	17.6ab	0.15ab	0.59ab	0.17ab	0.76a
S2	15.4a	0.15ab	0.64b	0.17ab	0.79ab
P1	21.6bc	0.18bc	0.85bc	0.31cd	1.15bc
P2	23.4c	0.22cd	0.95c	0.23bc	1.18c
VA	28.8d	0.24d	1.39d	0.38d	1.76d

*See Table 1
Note: Means within a column sharing a common letter are not significantly different by least significant difference test (P=0.05)

Seedling height, diameter and dry weight were all significantly increased after inoculation with *Pisolithus tinctorius* and *Glomus* 9004. The height and diameter of the VAM treatment were more than 2 times greater than those of the control plants, while seedling dry weight was increased by a factor of 3 by this treatment. Total dry weight of both *Pisolithus* inoculation treatments was increased by more than 2 times, in comparison with control plants. However, when inoculum of *Scleroderma* was applied, only the spore treatment significantly increased seedling dry weight.

Experiment 2

Frankia inoculation was very effective when introduced onto *Casuarina* plants colonised by an ectomycorrhizal fungus (Table 3).

Table 3. Mean height and diameter (breast height, 1.3 m) of *Casuarina* 65 months after planting with 4 treatments.

Treatment[a]	Height (m)	Diameter (cm)
CK	4.09a	2.07a
M	4.36ab	2.51ab
F	5.13bc	2.99b
MF	6.70c	3.83c

[a] CK = control; M = ectomycorrhizal; F = *Frankia*; MF = M+F
Note: Statistical comparisons are the same as in Table 2

Experiment 3

Analysis of variance of experiment 3 results showed that there were highly significant differences ($P<0.01$) in seedling heights between two *Casuarina* clones and among 4 inoculation treatments, and that there were significant interactions ($P<0.05$) between clones and treatments (Table 4). Tree genotypes had a substantial influence on the effectiveness of the inoculum. For example, in comparison with the control treatment, the mycorrhizal fungal treatment was significantly taller for clone CE601, but not for clone CE907 (Table 5).

Experiment 4

Variance analysis showed that there were significant differences in tree heights between two *Casuarina* clones and among P application rates (Table 6). There were also differences between inoculating treatments and significant interactions between clones and P rates (Table 6). When tree genotypes were compared, CE907 tree heights were significantly higher than those of CE601. Comparing growth to the control, only the MF inoculation treatment significantly increased tree heights, but P application always improved tree heights (Table 7).

Comparing results between the no-phosphorous application treatments and P rates ranging from 3.0 (or 6.0) to 18.0 g/tree, the heights of CE907 trees inoculated with mycorrhizal fungus (or *Frankia*) were significantly increased by higher P levels, but not in the heights of clone CE601 trees. At P levels from 3.0 to 18.0 g/tree, dual mycorrhizal fungus–*Frankia* treatments (MF) improved the height growth of both clones, although the two single inoculation treatments (M or F) did not affect the heights of clone CE601 (Table 8).

Conclusions

Mycorrhizal fungi can improve the growth of *Casuarina* seedlings. Dual inoculation with both a mycorrhizal fungus and a *Frankia* isolate provides further benefits to these plants. Both agar and spore inoculum forms of the ectomycorrhizal fungus *Pisolithus*, and soil inoculum of a VAM fungus, were effective in promoting the growth of *Casuarina*.

Table 4. Analysis of variance (ANOVA) comparing seedling heights for 2 clones of *Casuarina equisetifolia* and 4 inoculation treatments.

Source	df	SS	MS	F
Clones	1	12727.06	12727.06	481.75***
Inoculation (inoc.)	3	4721.02	1573.67	59.57***
Replicate (rep.)	3	50.57	16.86	0.64NS
Clones × inoc.	3	394.62	131.54	4.98**
Clones × rep.	3	114.67	38.22	1.45NS
Inoc × rep.	9	170.16	18.91	0.72NS
Residual	137	3619.36	26.42	
Total	159	21797.44		

***P = 0.01, **P = 0.05, *P = 0.10, NS = not significant.

Table 5. Mean heights (cm) of 2 clones of *Casuarina equisetifolia* (CE 601 and CE 907) 5 months after the application of 4 inoculation treatments.

Treatment[a]	CE601	CE907
CK	11.10a	20.57a
M	14.85b	25.95ab
F	21.65c	28.47b
MF	27.40d	35.67c

[a] See Table 3
Note: Statistical comparisons are the same as Table 2

Table 6. Analysis of variance (ANOVA) of tree height of two poplar clones and their responses to mycorrhizal inoculation and P fertilisation19 months after plantings.

Source	df	SS	MS	F
Clone	1	19.918	19.918	397.09***
Inoculation (inoc.)	3	0.381	0.127	2.53*
P rates	5	2.356	0.471	9.39***
Clone × inoc.	3	0.246	0.082	1.64NS
Clone × P rates	5	0.748	0.150	2.98**
Inoc × P rates	15	1.131	0.075	1.50NS
Error	159	7.975	0.050	
Total	191	32.755		

*** P = 0.01, **P = 0.05, *P = 0.10, NS = not significant

Table 7. Multiple range analysis comparing the average height growth of *Casuarina* clones, inoculation treatments and P application rates in Experiment 4.

Clone	Height (m)	Treatment[a]	Height (m)	P-rates (g/tree)	Height (m)
CE601	1.46a	CK	1.77ab	0	1.56a
CE907	2.11b	M	1.72a	1.5	1.75b
		F	1.80ab	3.0	1.82b
		MF	1.84b	6.0	1.85b
				12.0	1.88b
				18.0	1.86b
P level	P=0.05		P=0.05		P=0.10

[a] Treatments are the same as Table 3
Note: Statistical comparisons are the same as Table 2 (P = probability)

Table 8. Mean height of 2 *Casuarina* clones (CE601 and CE907) 19 months after planting after 4 inoculation treatments and grown at 6 P rates.

	CE601				CE907			
P rates	CK	M	F	MF	CK	M	F	MF
0	1.15a	1.26a	1.35a	1.28a	1.75a	1.71a	1.89a	1.84a
1.5	1.51ab	1.52a	1.42a	1.58ab	2.03ab	1.96ab	1.91ab	1.89ab
3.0	1.49ab	1.33a	1.35a	1.62b	2.18b	2.12b	2.19abc	2.22c
6.0	1.49ab	1.62a	1.38a	1.68b	2.10ab	2.16b	2.23bc	2.16bc
12.0	1.47ab	1.36a	1.47a	1.63b	2.21b	2.16b	2.36c	2.26c
18.0	1.56b	1.24a	1.45a	1.74b	2.27b	2.23b	2.44c	2.30c

Note: Treatments are the same as Table 3 and statistical comparisons are the same as Table 2

Two *Casuarina* genotypes responded differently to inoculation treatments and phosphorus application rates had a great effect on the growth of plants. Further work is necessary to select more mycorrhizal fungal species which can be used to promote the growth of *Casuarina* plants and examine interactions between different types of mycorrhizal associations, tree genotypes and soil fertility.

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The Occurrence and Use of Ectomycorrhizal Fungi in Eucalypt Plantations in Thailand

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Abstract

This paper provides a summary of information about the occurrence of ectomycorrhizal fungi associated with eucalypt plantations in Thailand. Fruiting bodies of ectomycorrhizal fungi associated with eucalypt tree roots were observed, collected and identified during the rainy seasons over a seven-year period. Common ectomycorrhizal fungi included species of *Tylopilus, Amanita, Pisolithus, Russula, Scleroderma, Thelephora* and others. Plantation eucalypts raised from uninoculated stocks naturally established ectomycorrhizas, as evidenced by fungal fruiting, within 2–4 years. Current research and development is focused on the development and comparison of mycorrhizal inoculum forms, which include natural soils, crushed fruiting bodies, pure mycelia, spore inoculum and spore tablets, for use to promote the growth of *E. camaldulensis* trees in plantations in Thailand. In one experiment reported here, inoculation of *Eucalyptus camaldulensis* seedlings with a peat/vermiculite inoculum containing *Pisolithus tinctorius* mycelia, substantially improved plant growth. This method is considered to be superior to natural soil inoculum which was used in the past.

Key words: eucalypts, ectomycorrhizal fungi, forest plantations, Thailand

EUCALYPTS are mainly indigenous to Australia and closely adjacent islands, such as Papua New Guinea, the Indonesian archipelago and the island of Mindanao in the Philippines. Over 500 species of *Eucalyptus* are known (Jacobs 1979). Eucalypts have been introduced as plantation trees in many countries, including South America, Africa and Asia. Eucalypts are used in plantations because of their wide range of adaptations to site conditions and their fast growth in exotic locations. Eucalypts were originally introduced to Thailand from Australia for species trials in 1850 (Thirawat 1952), but the first large trials were in 1964. In these trials, more than 20 species of eucalypts were grown, but *E. camaldulensis* had the best growth and survival performance in fertile and infertile sites in Thailand (Pinyopusarerk 1989; Williams and Luangviriyasaeng 1989).

Currently, *E. camaldulensis* is used on a large scale in Thailand, with over 224 000 ha of plantations, established to produce wood chips, pulp and paper, fibreboard, posts, poles, drywood, fuel, charcoal and construction materials. Eucalypts are also used to rehabilitate degraded woodlands. Five years after planting, *E. camaldulensis* can be harvested and plantations can produce 2–3 crops (with 5-year rotations). Typical yields for these plantations after 5 years are estimated to be: total above-ground biomass of 35.5 tons ha^{-1}, diameter growth (DBH) of 12.77 cm and height growth of 14.2 m (Pengpreecha et al. 1982).

Like many trees, eucalypt roots have symbiotic mycorrhizal associations that are essential for their establishment and healthy growth. Introduction of eucalypts into new regions might be expected to require their inoculation with appropriate mycorrhizal fungi, but this problem has seldom been reported. However, there is evidence that inoculation of eucalypts with mycorrhizal fungi can improve plant growth in some countries (Bowen 1980; Janos 1983; Marx 1991). Various studies have been made of the occurrence of mycorrhizas in eucalypts grown outside their natural habitats. The morphology of associations, inoculation procedures, nutrient uptake and growth promotion have been studied by many scientists (Malajczuk 1987; Bailey and Peterson 1987; Bougher et al. 1987; Neumann 1959; Pryor 1956a,b). The specific objectives of this

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research were to document the occurrence of ectomycorrhizal fungi in eucalypt plantations in Thailand and to investigate the most effective methods of inoculation to introduce these fungi into new locations in the field.

Materials and Methods

Fungal surveys were made to investigate the symbiotic relationships between ectomycorrhizal fungi and tree roots of *Eucalyptus camaldulensis* Dehn. and some other eucalypt species in forest plantations of Thailand during seven years in the period from 1977 to 1994. Fruiting bodies of ectomycorrhizal fungi, that were assumed to be symbiotically associated with roots of particular trees, were collected, identified and isolated during the rainy reasons (May to October). Study locations included eucalypt plantations throughout Thailand.

Important features of fungi that were collected were recorded, including size, colour, smell, taste, exudation, spore print features, morphology of pileus, warts, stalk, gill, ring, volva, cystidia. Fungi were identified to the genus or species level if possible. Axenic culture isolations from collected sporocarps were attempted when possible to provide isolates for further studies. Fungi were grown on solid or in liquid culture solutions of Melin-Norkans (MN) or potato dextrose (PD) media, depending on the requirements of particular fungi (Marx and Kenney 1982).

Various forms of inoculum of fungi were tested to establish their capacity to form symbiotic relationships by inoculating containers sown with seeds of *E. camaldulensis*. The production of different forms of inoculum is described below. For sterile treatments, the soil was fumigated with methyl bromide.

1. Soil inoculum

Topsoil collected from eucalypt plantations was used as inoculum. In nursery practice, 10–20% (by volume) of soil inoculum is incorporated into the nursery soils (top 10 cm of beds). The local soil and the mycorrhizal soil inoculum are mixed in a heap, then raked and placed in polythene bags before sowing the seeds.

2. Spore inoculum and crushed fruiting bodies

Spores of *P. tinctorius* were collected by homogenising sporophores with ruptured peridia with a blender, then sieving them through a 25–30 mesh screen. Screened basidiospores are air dried for several days at low humidity, then stored at 5°C in a refrigerator before use. Spores stored in this manner can be kept for several years. In most tests 1–2 mg of spores per seedling were applied. There is about 1 million basidiospores of *P. tinctorius* per mg of spore preparation.

Basidiospores of *P. tinctorius* have also been mixed with clay to produce tablets. One tablet is applied to each seedling pot or polythene bag. About 1 or 2 mg of basidiospores is compressed per tablet is sufficient for each tablet.

3. Mycelial inoculum

Modified Melin-Norkans (MMN) agar medium contains 0.05 g $CaCl_2$, 0.025 g NaCl, 0.5 g KH_2PO_4, 0.15 g $MgSO_4.7H_2O$, 1.2 mL of 1% $FeCl_3$, 100 mg thiamine HCl, 3 g malt extract, 10 g glucose and 15–20 g agar in 1 litre of distilled water. This media has proven to be as good or better than most other media for growing *P. tinctorius* and many other ectomycorrhizal fungi (Marx and Kenney 1982). After autoclaving, the appropriate pH of both liquid and solid agar is adjusted to 5.5–5.7.

A vermiculite-peat moss substrate (2.8 : 1 v/v) saturated with MMN liquid medium (without agar) at approximately 50% v/v was the best substrate for inoculum production (Marx and Kenney 1982). *Pisolithus tinctorius* inoculum was used after 4 months of growth, by mixing about 10 % (v/v) inoculum with nursery soil.

Results and Discussion

Fungal diversity

Information on ectomycorrhizal fungus diversity was based on observations made over seven years. During this period, 19 species of fungi were recorded, mainly in *E. camaldulensis* plantations (Table 1). Most species were Basidiomycetes. The first sporocarps to be produced after rain (May–June) were mainly puffballs such as *Astraeus hygrometricus*, *Pisolithus tinctorius* and *Scleroderma* spp. and their production continued into July. Phenological patterns varied among species. The most widely distributed and abundant species were *Amanita* sp., *Tylopilus rubrobrunneus* and *Thelephora ramarioides*. The first two species are edible, and are economically important as food sources for local people. Multiplication of these fungi was difficult due to their slow growth in culture.

There is a need for the production of ectomycorrhizal inocula tailored with *E. camaldulensis*. The mycorrhizal fungus *Pisolithus tinctorius* is commonly found in association with *E. camaldulensis*, and is considered to be of high importance in any inoculation program in Thailand. Eucalypt seedlings, inoculated with this species, transplanted on field sites have been observed to have enhanced growth (data not presented).

Table 1. Putative ectomycorrhizal fungi observed in eucalypt plantations in Thailand.

Fungus	Family	Frequency
Amanita coccora	Amanitaceae	common
Amanita sp.	Amanitaceae	very common
Astraeus hygrometricus	Amanitaceae	uncommon
Clavulina cristata	Clavulinaceae	uncommon
Pisolithus tinctorius	Sclerodermataceae	common
Russula delica	Russulaceae	uncommon
R. foetens	Russulaceae	uncommon
R. lepida	Russulaceae	uncommon
R. nigricans	Russulaceae	uncommon
R. sanguineus	Russulaceae	uncommon
Thelephora ramarioides	Thelephoraceae	very common
Tylopilus rubrobrunneus	Boletaceae	very common
Scleroderma aurantium	Sclerodermataceae	uncommon
S. geastrum	Sclerodermataceae	uncommon
S. lycoperdioides	Sclerodermataceae	uncommon
S. cepa	Sclerodermataceae	common
S. verrucosum	Sclerodermataceae	uncommon

Note: Fungi were identified by comparison with descriptions and illustrations in books, many of which concern northern hemisphere fungi that associate with other hosts. Consequently, the identity of fungal species may change when more is known about the fungus flora of Thailand.

Comparison of inoculation methods

The use of soil from eucalypt stands, spores from sporocarps, or mycelia grown in sterile culture to inoculate seedlings of *E. camaldulensis* in the nursery were compared. Initial growth responses to inoculation in the nursery were not large (Table 2), but greater responses might be expected to occur after seedlings are planted out into less fertile field soils. The advantages and disadvantages of each of these inoculum forms are considered below.

1. Soil inoculum

Soil inoculum taken from under ectomycorrhizal host trees has been used extensively in Thailand in the past. While this method is relatively effective, there are serious disadvantages because weed seeds, rhizomes, and potential pathogens can easily be introduced to the nursery, and soil is bulky. There are also problems with inconsistency due to variations in inoculum quality of soil collected at different times or locations. The main advantage of this method is that it is simple and inexpensive.

Table 2. Comparisons among treatments of *Eucalyptus camaldulensis* seedlings after 8 months growth experiment with *Pisolithus tinctorius* and soil inoculations.

Treatments	Control (uninoculated)	Spore tablet	Soil inoculum	Crushed fruiting bodies	Mycelial inoculum
Total height (cm)	32.7	34.8	35.8	35.8	35.7
Collar diameter (cm)	0.56	0.57	0.58	0.57	0.57
Shoot biomass (g)	2.37	2.42	2.23	2.29	2.25
Root biomass (g)	2.72	4.01	4.10	3.26	3.69
Shoot/root ratio (g/g)	0.87	0.60	0.54	0.70	0.61
Total biomass (g)	5.09	6.43	6.33	5.55	5.94

Source: Modified from Boonthanvikoon et al. (1992) and Thaiusa et al. (1991)

2. Spore inoculum and crushed fruiting bodies

Spores or crushed fruiting bodies of ectomycorrhizal *Pisolithus tinctorius* provide good inoculum and produce numerous basidiospores that are easier to collect in large quantities than those of many other mycorrhizal fungi. Marx and Kenney (1982) has successfully used basidiospore inoculum of *P. tinctorius* to form ectomycorrhizas on pines, oaks and other seedlings. The major disadvantage of spore inoculum is the lack of standard laboratory tests to determine spore viability. A variety of physical, chemical and biological factors have been tried to stimulate the germination of basidiospores of *P. tinctorius* without success. Another problem in using spore inoculum is that basidiospores of *P. tinctorius* collected from different sporophores and locations may have different genetic traits. Genetic variation would be greater if basidiospores from sporophores collected from many geographical areas and different tree hosts were combined into a single inoculum (Marx and Kenney 1982).

The main advantage of using spore inoculum is that it is very efficient. One gram of *P. tinctorius* basidiospores contains more than 1 billion spores. Thus 450 kg of mature and dry spores of *P. tinctorius* are enough for production of more than 225 million seedlings (at 1 mg of spores per seedling). The formation of ectomycorrhizas by *P. tinctorius* basidiospores usually takes 3–4 weeks longer than the use of mycelial inoculum of the same fungus. This is a disadvantage because during this period pathogenic fungi and foreign ectomycorrhizal fungi often colonise the root and reduce the effectiveness of the required spore inoculum.

3. Mycelial inoculum

Pure mycelium and/or cultured inoculum of ectomycorrhizal fungi have often been recommended as the most biologically sound method of inoculation (Bowen 1965; Marx and Kenney 1982; Mikola 1973). Unfortunately many ectomycorrhizal fungi cannot be grown in pure culture, grow slowly, or die after a few months. Many of the fungi require specific growth substances such as thiamine and biotin, in addition to simple carbohydrates. The main advantage of using mycelial inoculum is that a superior strain of *P. tinctorius* can be used and rapid mycorrhizal formation helps avoid problems with pathogenic organisms in nursery soils. The disadvantage of this method is that special techniques of preparation are needed, it is time consuming and relatively expensive.

Results of *P. tinctorius* inoculation experiments, using mycelia grown in peat-vermiculite, applied to *E. camaldulensis* seedlings are shown in Tables 3 and 4. Statistically significant differences in plant biomass between uninoculated and inoculated seedlings (ANOVA $P < 0.01$) were observed in sterile and non-sterile soil, without manure application (Table 3). Ectomycorrhizas did not form in treatments given manure. Mycorrhizal *E. camaldulensis* seedlings had significantly higher concentrations of P and Mg than their non-mycorrhizal counterparts (Table 4).

Table 3. Biomass of *Eucalyptus camaldulensis* seedlings inoculated with *Pisolithus tinctorius*, compared with uninoculated controls, after 6 months of growth.

Soil media	Total dry mass (g/plant)	
	Uninoculated	Inoculated
Non-sterile soil	3.76	9.12
Sterile soil	4.38	9.94
Non-sterile soil + manure	11.28	9.84
Sterile soil + manure	9.56	8.60

Table 4. The effect of inoculation with *Pisolithus tinctorius* on nutrients in shoots of *Eucalyptus camaldulensis* seedlings after 6 months of growth.

	Nutrient concentration (%)				
Treatment	N	P	K	Ca	Mg
Uninoculated	1.82	0.19	1.39	0.90	0.26
Inoculated	1.88	0.21	1.42	1.19	0.36
ANOVA	NS	$P < 0.05$	NS	NS	$P < 0.01$

Conclusions

The diversity of ectomycorrhizal fungi in eucalypt plantations in Thailand is relatively low compared to the wide range of fungi associating with these trees in Australia (Bougher, this volume). Further research should therefore be aimed at selecting appropriate fungi for use in lowland regions in central and southern Thailand and the drier plateau areas in the north. These fungi would have to be introduced from Australia or other regions where eucalypts are grown.

The work presented here demonstrates the efficacy of using vegetatively-grown ectomycorrhizal fungus mycelium for the inoculation of planting stocks in the nursery. Substantial performance gains should be obtained by the routine use of ectomycorrhizal fungus inoculation. Soil, spores and vegetative mycelium are the three primary sources of ectomycorrhizal inoculum available in Thailand for containerised seedlings of *E. camaldulensis* and other eucalypts. Each has advan-

tages and disadvantages in relation to the objectives and economics of the inoculation program. Historically, soil inocula, taken from beneath ectomycorrhizal host trees and incorporated into the nursery soil before sowing, have been used extensively, especially in developing countries. However this method produced inconsistent results and may introduce weeds and potential pathogens. We recommend that other methods, such as the sterile inoculum production procedure used in this study, should be used in place of soil inoculum in the future. Clonally propagated lines of *E. camaldulensis* are now being planted in Thailand, so procedures for the inoculation of tissue cultured plants (e.g. Gong et al., this volume) with ectomycorrhizal fungi should be developed for use in Thailand.

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Ectomycorrhizal Research on Eucalypts in Yunnan Province of China

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Abstract

Vegetative mycelium of Australian and Chinese ectomycorrhizal fungi were produced in vermiculite moistened with liquid culture medium and used as the primary form of inoculum for *Eucalyptus globulus* seedlings in nurseries in China. Three other forms of experimental inoculum (mycelium on agar, spores and mycelium in alginate beads) were also used in the nursery. The results indicated that most fungi formed ectomycorrhizas on the fine roots and many promoted their growth. Mycorrhizal seedlings were transplanted at different sites throughout Yunnan Province. Substantial growth responses were observed for some isolates in the field.

Key words: eucalypts, ectomycorrhizal fungi, inoculation technology, field trials

In Yunnan Province, eucalypts are being planted on many degraded sites for timber production and for soil stabilisation. However, many of these stands show poor growth and this can be attributed to the low nutrient status of the soil (Dell and Malajczuk 1994). Increasing the growth of *Eucalyptus* in plantations by inoculating seedlings with specific ectomycorrhizal fungi has recently been reported. Improvements in the early growth of tropical eucalypts following inoculation of seedlings have been shown in studies in the Congo (Garbaye et al. 1988) and in the Philippines (De la Cruz et al. 1988). In southwestern Australia, growth responses to inoculation with specific fungal isolates were obtained for two temperate eucalypts planted on cleared eucalypt forest sites (Grove et al. 1991). Increases in aboveground biomass of up to 88% for *Eucalyptus diversicolor* and up to 46% for *E. globulus* relative to the biomass of uninoculated trees were recorded one year after planting.

This paper summarises research in Yunnan Province associated with ACIAR Project 9044 (Increasing Productivity of Eucalypt Plantations in China by Inoculation with Ectomycorrhizas and Nutrient Application). During this project, 100 ha of experimental plantations were established in many sites at different elevations in Yunnan Province. The objectives of these experiments were to develop and evaluate ectomycorrhizal inoculation technology for use with eucalypts in the nursery and field in China.

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Materials and Methods

Fungal inoculum

Inoculum of isolates of Australian and Chinese ectomycorrhizal fungi (Table 1) were produced by the following methods:

1. Mycelium in peat/vermiculite was produced using methods described by Song Meijin (1991), Ji Dagan and Gu Zhenrong (1988), Bi Guochang et al. (1989), Guo Xiuzhen and Bi Guochang (1989) and Schenck (1982). Medium (1 kg) containing vermiculite, peat and wood chips (11:1:2 by volume) moistened with MMN liquid culture medium was autoclaved in cellophane bags, inoculated with fungi and incubated at 20–30°C for 30–45 days.
2. Fungal spores obtained from dry sporocarps kept in Australia at room temperature (21°C), or fresh sporocarps, were macerated with sterile water and stored at 4°C before use.
3. Alginate beads were provided by C. Kuek, University of Western Sydney.
4. Mycelium was produced on MMN agar.

Table 1. Source of fungi and their inoculum form used in field trials in Yunnan Province.

Fungus	Code	Origin	Inoculum form	Field site[a]
Cortinarius lavendulensis	E4062	W. Australia	*in vitro* mycelium	3
Descomyces albellus	H584	W. Australia	wet spores	1
Descolea maculata	E4071	W. Australia	*in vitro* mycelium	3
Hebeloma westraliense	E4070	W. Australia	*in vitro* mycelium	3
			peat/vermiculite	2
Hydnangium	Hyd 1	W. Australia	dry spores	1
Hydnangium	Hyd 2	W. Australia	wet spores	1
Hysterangium nephriticum	K247	China	peat/vermiculite	2
Laccaria	E1045	Victoria	alginate bead	1
Laccaria	E4100	Queensland	*in vitro* mycelium	3
Pisolithus	H445	W. Australia	peat/vermiculite	1, 2, 4, 5
			in vitro mycelium	3
Pisolithus	K137	China	peat/vermiculite	1, 2, 4, 5, 6, 7
Pisolithus	H636	W. Australia	dry spores	1
Pisolithus	H4111	Queensland	peat/vermiculite	2
			in vitro mycelium	3
Pisolithus	H4937	Queensland	peat/vermiculite	2
Rhizopogon luteolus	K80	China	peat/vermiculite	1, 2, 4, 5
			wet spores	
Setchelliogaster	H51023	Tasmania	*in vitro* mycelium	3
Suillus granulatus	K159	China	peat/vermiculite	2
Scleroderma	H587	W. Australia	wet spores	1
Scleroderma	H5109	Tasmania	*in vitro* mycelium	3
Thaxterogaster	H5141	Tasmania	*in vitro* mycelium	3
Tylopilus	E4240	Queensland	*in vitro* mycelium	3

[a] Field sites: 1 = Jin Dian; 2 = Tai Ping trial 1; 3 = Tai Ping trial 2; 4 = Bao Shan; 5 = Yu Xi; 6 = Fu Min

Inoculation of seedlings

Seedlings of *Eucalyptus globulus* were inoculated with one of the following methods at the Jin Dian Forest Farm Nursery.

1. The inoculum was added to nursery soil in small plastic bags (approx. 500 g) and seeds, or 8 cm high seedlings from fumigated nursery beds, planted above the inoculum.
2. Small sterile germinated seedlings (approx. 1 cm high) were placed on mycelial lawns over agar in polycarbonate tubs for three weeks and then transplanted into autoclaved sphagnum peat/vermiculite (1:1 by volume) in 64-cell plastic nursery trays.

Nursery soil (3 parts soil and 1 part humus) was fumigated by drenching with 0.5% formalin under a plastic sheet for 10 days. The sheet was then removed and the formalin allowed to volatilise. Alternatively, forest soil (red/yellow earth) was heated by burning timber residues to provide partial sterilisation.

Seedling maintenance

Seedlings were watered once weekly with a weak complete nutrient solution (3 mL Wuxol™). After 2–3 months, seedlings were transplanted into field trials.

Nursery assessment

Development of ectomycorrhizas on nursery stock was not thoroughly assessed, but selected plants were examined under a binocular microscope to provide evidence that ectomycorrhizal plants were transplanted in the field.

Field experiments

Over a three-year period, field trials (approx. 100 ha) were established at five locations: Jin Dian, Tai Ping, Bao Shan, Yu Xi and Fu Min on the Yunnan Plateau at an average elevation of 1900 m. Planting took place in July. The region experiences a monsoon climate with a wet season from June to September. Soils for commercial forestry in the region (Land Resource Map of China 1:100 000 1989) are red–yellow earths. Generally, they are acidic (pH 4–5, 1:5 soil:water suspension) very low in total P, N, K, Na, Ca, Mg and organic matter (Table 2). These soils are high in extractable Mn and hot water extractable B concentrations are typically low (< 0.2 mg B g^{-1} soil; Dell and Malajczuk 1994). The remnant vegetation being replaced by eucalypt plantations was dominated by conifers (*Pinus yunnanensis*, *P. armandii*, *Cupressus* sp. and *Juniperus* sp.). The dominant vegetation of the plateau consists of stunted shrubs of *Pinus yunnanensis*.

Experimental design

The Jin Dian and Tai Ping experiments were established as fully randomised complete block designs, consisting of four blocks with single row plots containing 10 trees at 1 metre spacing. Planting holes were prepared by excavating individual holes or by trenching (Tai Ping). At the other sites, treatments were set up as large blocks for demonstration purposes.

Fertiliser application

Basal fertiliser, low in P, was applied at planting. Generally, from 100–130 g of NPK, consisting of 7.7% N, 8.2% P (6.5% water soluble P, 1.65% citrate-soluble P and 0.05% citrate-insoluble P), 22.9% K and 12.9% S, was applied in the bottom of the planting hole or on the surface at approx. 30 cm from the stem. At both Jin Dian and Tai Ping, additional basal fertiliser was applied: 40–45 g urea/tree, 25–40 g of micronutrient mix/tree (1.5% Cu, 0.26% Zn, 3.9% Mn, 6.0% Mg, 5.7% Fe, 0.19% Mo, 0.13% B and 0.03% Co).

Plant measurements

Stem diameters were measured at periods ranging from 4 to 36 months after planting. The height above ground level at which diameters were recorded varied with tree age (3 cm at 4 months, 10 cm at 9 and 16 months). Effects of fungal treatments on stem basal areas were statistically analysed using the GENSTAT statistical program (Rothamsted Experimental Station).

Results and Discussion

Jin Dian

The Jin Dian trial was established in 1992 to evaluate the use of a range of inoculum forms and to establish a protocol for mycorrhizal field trials in Yunnan. There was no significant effect of fungal treatments on stem basal area 4 months after planting. However, differences between treatments were apparent at 9 months ($P<0.10$) and these became more significant ($P<0.05$) 16 months after planting (Fig. 1). At 9 months, seedlings inoculated with the three *Pisolithus* treatments had the highest stem basal area. At 16 months, the largest trees occurred in the *Pisolithus* treatments and a *Hydnangium* treatment (Fig. 4). At 36 months, the trees in the *Hydnangium* treatment had stem basal areas nearly twice those of the uninoculated treatment.

Tai Ping

Two trials were set up in 1993, the first to compare Australian and Chinese fungal isolates and the second to evaluate a wider range of Australian fungi. In the first trial, seeds were inoculated in the Kunming Institute of Botany Nursery using peat/vermiculite inoculum. In the second trial, seedlings were inoculated *in vitro* with mycelium on agar. Three fungi (K80, K137 and H445), in peat/vermiculite inoculum, significantly increased growth of seedlings before transplanting to the field (data not presented). This initial growth stimulation could not be attributed entirely to the formation of ectomycorrhizas since this isolate of *Rhizopogon* from pines

Table 2. Chemical properties of surface soils (0–10 cm) at the Jin Dian and Tai Ping experimental sites.

Site	Total P (%)	Bray P (μg/g)	Total N (%)	Organic C (%)	Exchangeable cations (me/100g)			
					Na	K	Ca	Mg
Jin Dian	0.038	7.08	0.094	1.67	0.001	0.265	1.204	0.328
Tai Ping	0.015	3.13	0.025	0.45	0.002	0.079	0.595	0.184

Notes: Four surface (0–10 cm) soil samples were taken from each site. Each sample was obtained by bulking six cores taken at regular intervals within a replicate block of the experiment at the time of planting. Bulked soil samples were air-dried and sieved and the fine earth (< 2 mm) fraction analysed using the following methods: Total N and P (Murphy and Riley 1962); Bray P (Bray and Kurtz 1945); Organic C (Walkley 1947); Exchangeable cations (NH_4Cl at pH 8.2).

(K80) does not form normal ectomycorrhizas with eucalypts (Malajczuk and Dell, pers. comm.). At four months after transplanting (Fig. 2), the growth stimulation obtained with K80 and K137 in the nursery was still apparent. However, plants inoculated with *Hebeloma* (E4079), which showed no growth promotion in the nursery, also had significantly greater basal area than the uninoculated treatment in the field. Observations on older trees (data not presented) indicated that the initial growth stimulation obtained with K80 was not sustained in the field and trees inoculated with some Australian isolates had enhanced growth relative to the uninoculated trees.

In the second trial (Fig. 3), five inoculation treatments showed significantly superior growth at four months after transplanting relative to the uninoculated control.

The mycorrhizal response was stronger than that observed in the other mycorrhizal trial at this site. Reasons for this could include: (i) the use of a sterile potting medium allowed more extensive development of the inoculum on the roots before planting out; (ii) the use of a wider selection of Australian fungal isolates; (iii) better control over the level of basal fertiliser used in this experiment compared to the other trials in the region; and (iv) use of smaller plants (5 cm tall) at transplanting.

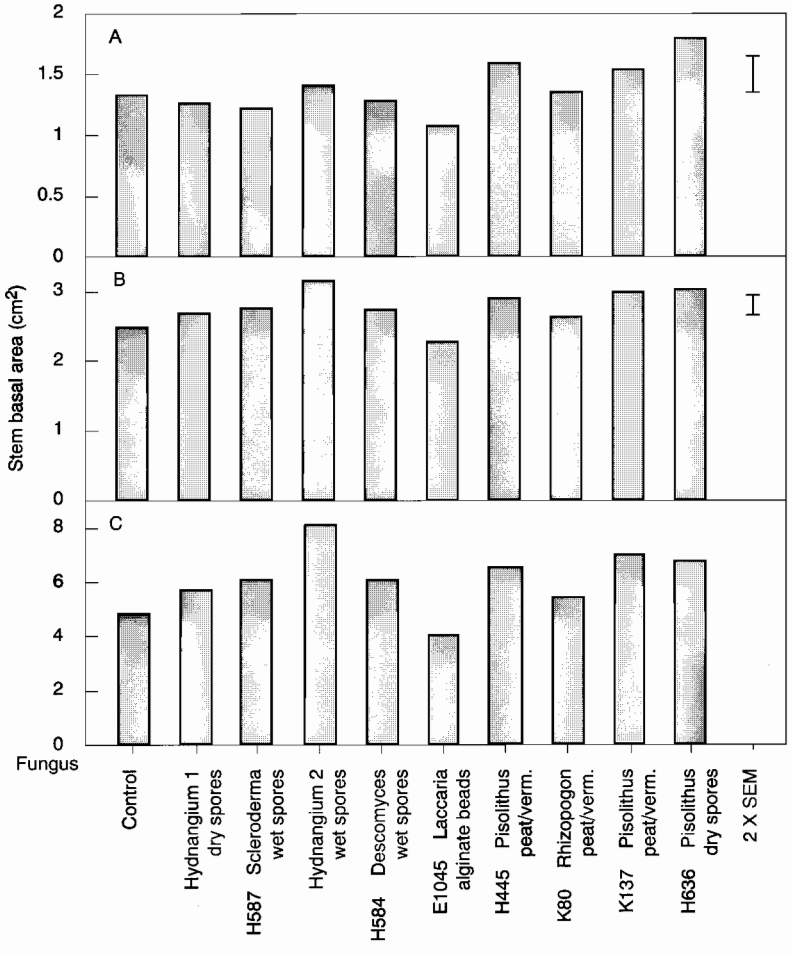

Figure 1. Variation between fungal treatments in the stem basal area of *Eucalyptus globulus* at 9 months (A), 16 months (B) and 3 years (C) after planting at the Yunnan Forest Nature Centre (peat/verm = peat vermiculite culture substrate; see Table 1 for key to fungal codes [H587, etc]).

Other sites

At Bao Shan, Yu Xi and Fu Min, seedlings were inoculated with peat/vermiculite inocula and plants set out in large blocks as part of commercial operations (Tables 3, 4). Most fungi chosen for these large-scale inoculations represented a range of fungi available in Kunming rather than species selected for their ability to promote the growth of eucalypts in the field. Nevertheless, most inoculation treatments resulted in initial growth responses.

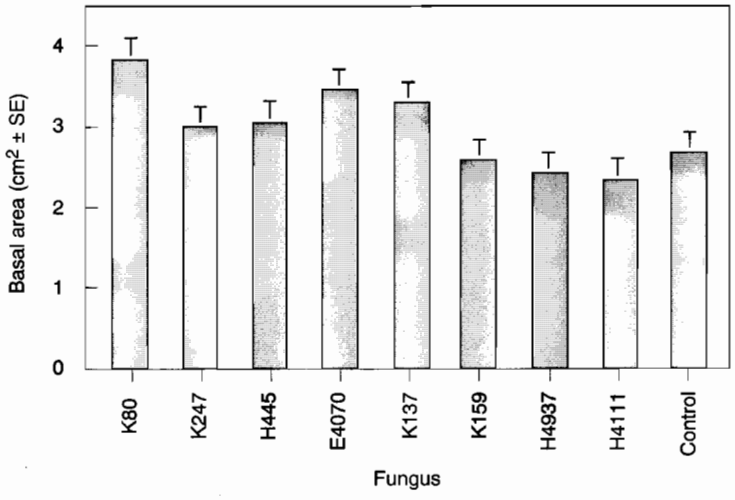

Figure 2. Effect of inoculation with different fungi on the basal area of *E. globulus* 4 months after transplanting in the field. The standard error bars (SE) are derived from the group mean square (see Table 1 for key to fungal codes).

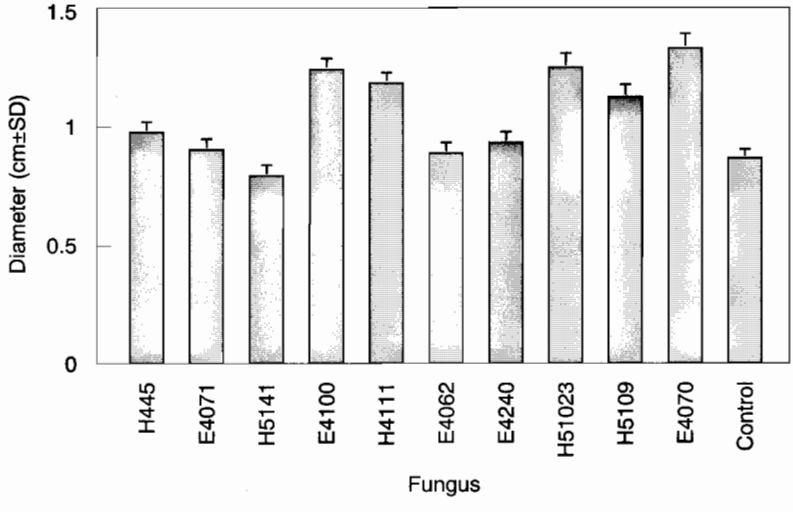

Figure 3. Effect of fungal treatments on the stem diameter of *Eucalyptus globulus* four months after transplanting. Error bars are standard deviations of the individual means (see Table 1 for key to fungal codes).

Table 3. Effect of inoculation on the growth of *E. globulus* six months after planting out in the field at Bao Shan.

Fungal treatments[a]	Mean height (cm)	Mean stem diameter (cm)	Increase in height (%)	Increase in diameter (%)
Control	140	2.3		
K80	166	2.8	18.6	18.7
K137	166	2.5	18.6	6.8
H445	152	2.5	8.6	7.6

[a] See Table 1 for fungal codes

Table 4. Effect of inoculation on the growth of *E. globulus* with seven months after planting out in the field at Yu Xi and Fu Min (with percentage increases).

Fungal	Fu Min site		Yu Xi site	
treatments[a]	Mean height (cm)	Mean stem diameter (cm)	Mean height (cm)	Mean stem diameter (cm)
Control	84.6	1.3	71.6	1.3
K137	145.2 (+72%)	2.0 (+50%)	93.2 (+30%)	1.8 (+ 34%)

[a] See Table 1 for fungal codes

Conclusions

A range of inoculum forms were used in experiments in China for the inoculation of *Eucalyptus globulus* in nurseries. Whilst each method has particular advantages, the *in vitro* inoculation resulted in plants with the best mycorrhizal development in the nursery. In the future, more attention must be given to optimising nursery conditions for maximising ectomycorrhizal development before planting out. Although infection was generally low in the nursery, when trees were planted out they showed a range of responses to inoculation with the different fungal species. Both Australian and Chinese fungal isolates stimulated growth of *E. globulus*. However, this effect did not persist with the Chinese isolates. Many of the Chinese isolates were obtained from fruiting bodies collected under pines. In the absence of specific eucalypt fungi, these may provide an initial growth response due to a rhizosphere effect. Further research is required to compare the effectiveness of Chinese and Australian isolates of ectomycorrhizal fungi for promoting the growth of plantation-grown eucalypts in China.

Acknowledgments

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Development of Technology for the Ectomycorrhizal Inoculation of *Eucalyptus* Cuttings and Tissue Culture Plantlets in Southern China

Gong Mingqin*, Wang Fengzhen Chenyu* and Chen Xia†

Abstract

Eucalyptus urophylla and *E. grandis* × *E. urophylla* plantlets and cuttings were inoculated using spore slurries and a tissue-culture inoculation procedure developed by Malajczuk and Hartney (1986). Three months after inoculation, mycorrhizal infection of eucalypt cuttings ranged from 32–60% for the various mycorrhizal isolates and cutting height was increased between 60–73% compared to uninoculated plants. *Eucalyptus* tissue culture plantlets were all pre-infected with mycelium during the root initiation stage of production and 80–100% of these seedlings were mycorrhizal when assessed in the nursery 4 months later. The average height of mycorrhizal seedlings was 28–57% greater than uninoculated plants. In preliminary field trials, 1 year after planting out *E. grandis* × *E. urophylla* cuttings inoculated with mycorrhizal spores were 20–24% taller and 14–21% greater in diameter at breast height (DBH), than controls. After 14 months of growth in the field, inoculated *E. urophylla* cuttings were 16–22% taller and had a DBH 14–21% greater, than uninoculated plants. The inoculation of eucalypt plantlets in culture, or cuttings, with spore slurries were both effective in establishing ectomycorrhizal associations.

Key words: *Eucalyptus*, ectomycorrhizal fungi, plant tissue culture, mycorrhizal inoculation

In southern China *Eucalyptus* species are the favoured plantation tree, providing timber and wood products for the local farmers (Davis et al. 1994). Significant improvements in tree productivity have been achieved in the past decade through the introduction of superior species, provenances and families of eucalypts from Australia (ACIAR Proceedings No. 48, 1994). Further gains in productivity have been made in China through interspecific hybridisation, assisted by vegetative propagation of this introduced genetic material (Bai Jiayu 1994).

Mycorrhizal inoculation of *Eucalyptus* seedlings can improve the productivity of plantations in China (Malajczuk et al. 1994). Several hundred hectares of mycorrhizal eucalypt plantations have now been established in southern China where spores have been used as a form of inoculum. These have been applied to seedlings in the seed bed or at the time of transplant. Although this method is simple and practical for use in all types of nurseries and sites throughout southern China, the success of inoculation is dependent on the availability of ectomycorrhizal fungi which produce abundant spores with a high capacity to germinate and initiate mycorrhizas. The use of spores as inoculum also introduces genetic variability, which may counter productivity gains made through the selection and propagation of superior strains of fungi.

Vegetative inoculum can be obtained from many ectomycorrhizal fungi, but the protocols for isolation, production and bulking of inoculum are limited to only a few laboratories in China, which have facilities for sterile manipulation. However, there has been an expansion of micropropagation laboratories in southern China for mass production of superior clonal eucalypt material. These facilitates are similar to those required in the adoption of ectomycorrhizal fungal inoculation protocols developed by Malajczuk and Hartney (1986) for the mass production of inoculated eucalypts.

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This paper presents the results of some studies undertaken during ACIAR project no. 9044, which aimed to transfer ectomycorrhizal fungus technologies to China. Results presented here concern the development of inoculation protocols for vegetatively propagated *Eucalyptus* cuttings and plantlets to tropical China and the performance of these plants in the field.

Materials and Methods

Plant materials

Cuttings of *Eucalyptus grandis* × *E. urophylla* (Qinzhou No.1) were provided by Jiangmen Forest Research Institute, Jiangmen, Guangdong Province. Cuttings of *E. urophylla* clones U4 and U5 were provided by the Vegetative Propagation Research Unit, Research Institute of Tropical Forestry (RITF), Longdong, Guangdong Province. Micropropagated tissue culture plantlets of *E. urophylla* × *E. grandis* (Qinzhou No. 1) were also provided by the Jiangmen Forest Research Institute.

Spore inoculum production

Spore suspensions (TM1 media) containing 6 g of dried spores from fruiting bodies, 1.5 g of P_2O_5, 1.5 g of a micronutrient preparation, and 0.6 g of a surfactant, in 1000 mL of water, were used to inoculate fungi. Inoculum for experiments was produced by the Mycorrhizal Research Group, RITF, Longdong and included *Pisolithus* (batches 9102 and 9218) and *Scleroderma* (batch number 9217).

Inoculation of cuttings

Eucalypt cuttings were transplanted into nursery soil (a latosol) in 500 mL polybags, immediately after they were obtained from mother trees. Spore (5 mL) inoculum in TM1 liquid was injected to a depth of 3–5 cm at the base of the cuttings. The ectomycorrhizal fungi *Pisolithus* (9102) and *Scleroderma* (9217) were used.

Vegetative culture inoculation of tissue cultured plantlets

Following shoot initiation, plantlets were placed onto modified *Eucalyptus* MS (Marashige and Skoog 1962) root initiation medium in 500 mL jars (Hartney 1982). This medium was found to be suitable for the growth of both the plantlets and mycorrhizal fungi. At the same time, the root initiating medium was inoculated with 2–3 agar slices (0.5 cm^2) of actively growing fungi. The jars were incubated for 15–20 days at 25–28°C in growth rooms with artificial light. Following root initiation, plantlets were transplanted, together with actively growing fungal hyphae, into nursery soil in 500 mL polybags (see above). Australian ectomycorrhizal fungal isolates used in tissue culture experiments were provided by Dr Malajczuk, CSIRO Division of Forestry, Australia, and were first proved to promote growth of eucalypts in glasshouse experiments (Table 1). An additional isolate (CH8909) was obtained from a eucalypt plantation in China.

Table 1. Fungal isolates used in tissue culture inoculation trials.

Code	Fungal species	Origin
Control	uninoculated	
H4937	*Pisolithus* sp.	Queensland
E4240	*Tylopilus* sp.	Queensland
E4014	*Tricholoma* sp.	Queensland
E4100	*Laccaria* sp.	Queensland
E4070	*Hebeloma westraliense*	Southwestern Australia
H4111	*Pisolithus* sp.	Queensland
CH8909	*Pisolithus* sp.	Guangdong Province, China

Effect of nursery soil mixes on mycorrhizal development by tissue culture plantlets

The aim of this experiment was to determine the appropriate soil mix to optimise development of mycorrhizal roots by tissue-culture plantlets following planting into nursery soil. Three soil mixes were included in the experiment: 100% yellow loamy subsoil; 100% river sand and a mix of 50% yellow subsoil; and 50% river sand. Half of the prepared soil mixes were sterilised by autoclaving for 2 h at 1.5 kg/cm^2. Inoculated plantlets were transplanted into 1L black polybags containing these soil mixes and there were 10 replicates per treatment. Seedlings were grown at the RITF nursery for three months, ensuring adequate watering and low nutrient inputs (a 3% solution of a liquid fertiliser containing all essential plant nutrients was applied every week — see Dell and Malajczuk, this volume).

Field trials

Mycorrhizal eucalypt plants, produced as described above, were planted out during the wet season. Experiments were arranged as random blocks with each of the mycorrhizal treatments originally applied to the cuttings. The field trials occupied approximately 2.0 ha of the Zhenhai Forest Farm, near Kaiping, Guangdong Province (see Grove et al., this volume). Other field trials occurred at Dianbai Forest Farm in Guangdong Province.

Nursery and field measurements

Mycorrhizal infection of all treatments was carried out before transplanting of cuttings and plantlets. Between 5–30 seedlings from each treatment were randomly selected and roots systems were examined under a dissecting microscope. Mycorrhizal infection was rated as a percentage of total roots examined visually. Measurements were taken of seedling height and DGH (diameter at ground surface) 3–12 months after planting out.

Results

Mycorrhiza formation by eucalypt cuttings

After 11 weeks, abundant hyphae were observed on 60% of fine roots of *E. grandis* × *E. urophylla* cuttings inoculated with spores of two ectomycorrhizal fungi and many plants had initiated mycorrhizas (Fig. 1). More than 80% of these plants were mycorrhizal after 4 months, forming dense clusters of roots and hyphae. Inoculation of *E. grandis* × *E. urophylla* cuttings resulted in substantial increases in plant height, compared to the uninoculated controls (Fig. 1). Mycorrhizal seedling were suitable for transplanting 7–10 days earlier than the controls and showed increased tolerance to low temperatures in spring or winter.

Mycorrhizal development by tissue-cultured plantlets

Roots of *E. urophylla* × *E. grandis* plantlets inoculated in tissue culture were examined for mycorrhizal development after 16 weeks of growth in different soils in the nursery. Mycorrhizal root forms included unbranched (*Laccaria*), clustered (*Pisolithus*, *Scleroderma*) and pinnate (*Hebeloma*). Mycorrhizas and mycelium of *Pisolithus tinctorius* were typically yellow–brown whereas all other isolates were white or hyaline. There was little difference in formation between plantlets raised in sterilised or non-sterilised soil but large differences in development among seedlings growing in different soil types (Table 2). Fungus Isolates E4240, H4937, H4111, E4100 and CH8909 were the most effective in increasing seedling height compared with the controls (Table 2). Mycorrhizal infection was greater than 80% for most treatments.

Field trials with mycorrhizal cuttings

Examination of one-year-old mycorrhizal *E. urophylla* × *E. grandis* cuttings showed that seedling height and diameter increased, when compared with the uninoculated controls (Fig. 2). Cuttings inoculated with a *Scleroderma* spore suspension showed the largest increase in growth. Mycorrhizal *E. urophylla* cuttings inoculated with *Pisolithus* spores in TM1 media, were also substantially larger than uninoculated cuttings after 14 months of growth in the field (Fig. 3).

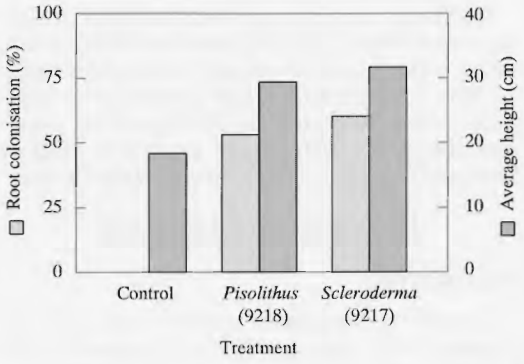

Figure 1. Mycorrhiza formation (proportion of plants) and growth of *E. grandis* × *E. urophylla* cuttings 11 weeks after inoculation with spore slurries of two fungi.

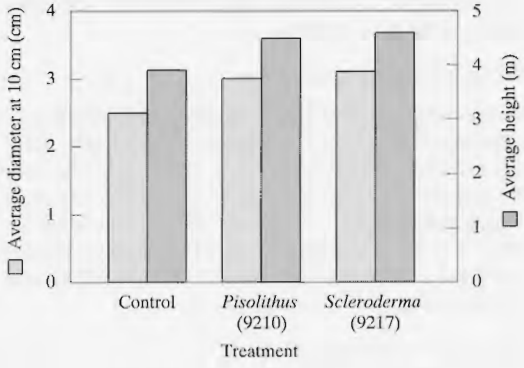

Figure 2. Growth of mycorrhizal inoculated and uninoculated *E. grandis* × *E. urophylla* cuttings at Kaiping, Guangdong Province 1 year after planting out.

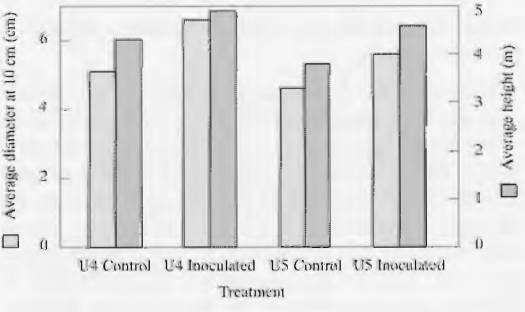

Figure 3. Growth of mycorrhizal inoculated and uninoculated *E. urophylla* cuttings inoculated with *Pisolithus* (9218) spores at Dianbai in Guangdong Province, 14 months after planting out (see Table 1 for key to fungal species).

Table 2. Average height (mm) of mycorrhizal *E. grandis* × *E. urophylla* plantlets inoculated in culture with ectomycorrhizal fungi (Table 1) growing in different soil media in the nursery.

Fungal isolate[a]	H4937	E4070	E4240	H4111	E4014	E4100	CH8909	Control
Sterilised soils								
100% soil	78.2	60.1	69.1	70.1	43.1	66.9	65.3	50.2
100% sand	69.6	69.7	90.4	101.1	71.3	80.5	61.3	49.1
50% soil + 50% sand	101.8	86.4	104.1	75.1	74.7	91.3	88.8	61.5
Non-sterilised soils								
100% soil	73.6	42.3	74.2	71.1	65.9	74.7	50.3	37.0
100% sand	81.7	91.7	77.8	81.6	80.9	82.1	84.1	68.2
50% soil + 50% sand	99.7	68.9	104.1	108.2	92.7	91.9	98.6	78.4
Average height (cm)	84.1	71.0	87.0	83.4	71.5	81.0	74.5	55.5
% Increase over control	51.5	27.9	56.7	50.3	28.7	46.0	34.7	–

[a] See Table 1 for key to fungal species

Field trial with mycorrhizal tube-grown cuttings

One year after transplanting, the average size of mycorrhizal *E. urophylla* plants was increased substantially, when compared with uninoculated plants (Fig. 4). Root examination demonstrated that there was extensive ectomycorrhizal development of the inoculated plantlets as well as abundant vesicular-arbuscular mycorrhizal (VAM) formation in roots of both uninoculated and inoculated plants.

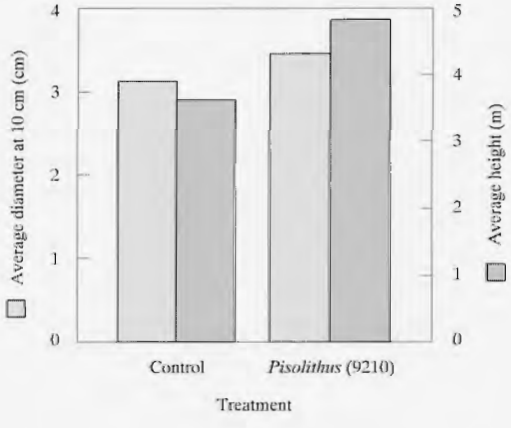

Figure 4. Growth of mycorrhizal and uninoculated *E. urophylla* × *E. grandis* plantlets at Kaiping, Guangdong Province after one year.

Conclusions

As shown above, the Australian ectomycorrhizal fungi currently available in China include isolates that can significantly enhance the growth of eucalypts. Further research is needed to determine the persistence of these Australian isolates in China and the interaction with indigenous fungi, including those forming VAM. Field studies have indicated that, where there was a high incidence of VAM in eucalypt roots, the response of plants to ectomycorrhizal inoculation can be significantly reduced. These factors require further investigation.

Clonal tree production represents the future for eucalypt plantation establishment in southern China. Many of the tropical and subtropical eucalypts can be readily propagated vegetatively in nurseries, thus providing a means for mass production of superior clones for plantations. Appropriate protocols for the ectomycorrhizal inoculation of cuttings and plantlets are therefore needed if significant gains from mycorrhizal inoculation are to be achieved. In this study, inoculation methods were developed for both cuttings and tissue culture plantlets in the nursery based on inoculation protocols reported by Malajczuk and Hartney (1986). Cuttings of *E. urophylla* and *E. grandis* × *E. urophylla* were inoculated with slurry inoculum while plantlets of *E. grandis* × *E. urophylla* were pre-inoculated during root formation in sterile containers. Both methods were successful and are relatively simple to implement, so can be adopted at most tissue culture laboratories and nurseries in southern China. Large-scale use of these technologies however would be dependent on the availability of suitable bulked inoculum of superior ectomycorrhizal fungal isolates. Currently, the Research Institute of Tropical Forestry is capable of large-scale production of inoculum and future collaboration will be developed with industrial partners to allow commercial inoculum production.

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Concluding Remarks and Future Activities

UNDERSTANDING tree growth requires knowledge of the relationship between their roots and symbiotic fungi. It has been 110 years since Frank first described the 'fungus-root', yet our understanding of the basic functioning of mycorrhizas, and how we can effectively manipulate these essential microorganisms, remains incomplete. More information is required to fully develop management options which can be used to enhance plant productivity and efficient use of fertiliser in forestry, agriculture and horticulture.

Mycorrhizal research and development programs have only recently been seriously attempted in China and Asia. This increased commitment has been in part attributed to the ever-decreasing natural forest resource and the need to establish 'new forests' on degraded sites where mycorrhizas are essential for nutrient acquisition. In China, for example, there are over 700 000 hectares of plantation forests and it is expected that 15 million hectares of new plantations will be established by the year 2000 (Chinese Academy of Forestry, miscellaneous publication). The establishment of high quality tree plantations is the paramount goal of any forestry program, and the Chinese National Reafforestation program is no exception. It is therefore imperative that some of the basic questions concerning seedling growth and tree establishment, which include knowledge of the form, function and manipulation of mycorrhizas, are addressed by well-focused research programs.

A program on mycorrhizas was established by ACIAR in 1988 with the goal of increasing the productivity of eucalypt plantations. Generous funding over a six-year period allowed rapid development of knowledge on eucalypt mycorrhizas and the setting up of mycorrhizal trials for long-term monitoring. The approach developed in this work is a model for future research programs in China and Australasia. The flowchart below illustrates the approach that would be necessary to develop a program for the use of mycorrhizal inoculation to enhance the productivity of plantations in different environmental situations. The program would have four major research components: (a) the acquisition and selection of superior fungal isolates; (b) development of procedures for the successful inoculation of seedlings in the glasshouse and nursery; (c) examination of the impact of soil fertility on mycorrhizal benefits; and (d) field testing of superior fungal isolates.

There are no short cuts in developing an understanding of the form, function and manipulation of mycorrhizas. However, lessons from past work should allow rapid application of new mycorrhizal knowledge. These proceedings document exciting and innovative work in the Asian region. It is also apparent that there are still major gaps in knowledge and areas where research is urgently required are listed below.

1. *Taxonomic knowledge of mycorrhizal fungi.* The training of more scientists in fungal taxonomy will allow fungi associated with important host trees to be identified. Skills and resources for the storage, culture and maintenance of fungal isolates and herbarium specimens must also be developed to harness this biodiversity.
2. *Identification of beneficial fungi that are compatible with key host trees.* There is a need to collect fungi from under target trees in their native habitats to ensure they are compatible. The diversity of ectomycorrhizal and decomposer fungi are the key to resilience of ecosystems and plantations, so more than one fungal species should be targeted for potential use. Selection criteria should also focus on fungi that produce edible sporocarps. Wild fungi are a traditional and important source of food and medicine in China and other Asian countries. Thus, the introduction of edible fungi to plantations would serve ecological, economic and social purposes, while enhancing ecological diversity. Moreover, plantations that produced edible fungi would be valued by local people for that reason and thus protected from illicit tree cutting or excessive harvest of humus and litter. Rural communities

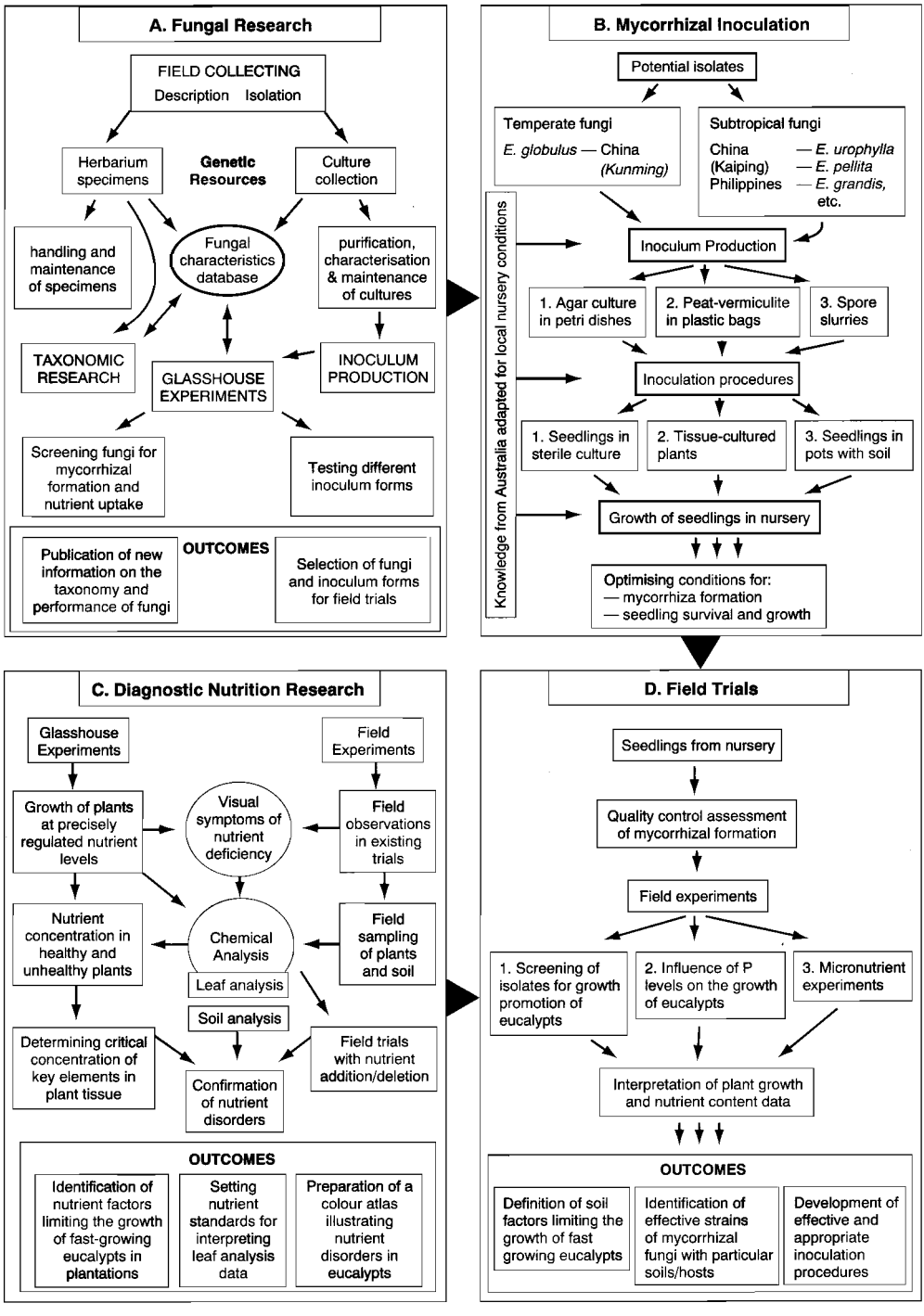
could supplement their diet with a prized wild food and enhance their income by selling wild mushrooms in local markets. Given enough of the wild mushroom resource, dried or canned wild mushrooms could be processed for urban or export markets. A traditional activity that might otherwise decline would therefore be preserved and enhanced.

3. *Effective inoculation procedures for ectomycorrhizal fungi.* Trials with different forms of inoculum are required to optimise mycorrhizal formation. Once isolates have been chosen for use in field trials, additional information about their culture requirements should be obtained by experiments with media formulations to allow large-scale production of fungal inoculum. For some fungi, mycelium from liquid cultures can be produced and used in macerated or encapsulated form. Other fungi will not grow in culture but produce numerous spores which can be used to make spore pellets or slurries. It may not be possible to use some fungi, due to practical limitations resulting from difficulty with their culture or spore production. Existing information will have to be supplemented by experiments investigating the capacity of cultured mycelia to withstand various treatments, or factors influencing the germination of spores. This will allow cost-effective inoculation protocols for particular fungi to be developed.
4. *Criteria with which to select fungal isolates for use in particular soils/habitats.* Often inoculation programs use fungi that are inappropriate for the soil conditions that prevail where seedlings are to be planted out. It is important to screen fungi in the laboratory and/or glasshouse to obtain isolates with desirable qualities (e.g. growth promotion, salt tolerance, persistence in soil) to be confident that mycorrhizal fungal candidates will perform well in the field.
5. *Practical and effective mycorrhizal inoculation protocols for use in forest nurseries.* There is a need to optimise nursery management to ensure abundant mycorrhizal development on seedlings before they are planted out in the field, if valid results are to be obtained from field experiments. Our experience suggests that simply adding inoculum to seedlings in the nursery will not ensure adequate mycorrhizal development. It is essential that mycorrhizal development is monitored in the nursery. Adjustments to nursery practices may be necessary (such as adding less nutrients or using target fungicides) to maximise mycorrhizal development before the seedlings are planted out.
6. *The persistence, survival and performance of mycorrhizal fungi in soils.* To validate the results of nursery and field inoculation trials, it is necessary to establish that fungi applied as inoculum form mycorrhizas in the nursery and continue to spread through the root system of trees in the field. The presence of ectomycorrhizal fungi can sometimes be established by recognition of their fruiting bodies, and fungal identification systems (such as mycorrhizal anatomy, molecular probes) can be used to follow inoculated fungi in the field.
7. *The nutrient status of soils in plantations and formulation of corrective fertiliser protocols.* Many of the soils allocated for establishment of eucalypt plantations in China are infertile or have imbalances in nutrients essential for tree growth. There is a need to undertake fertiliser trials in conjunction with soil and plant analyses at key sites in different regions. This information can then be used to formulate appropriate fertiliser recommendations for plantations. Failure to do this can result in either:

   (i) a potential mycorrhizal response being completely overridden by a nutrient disorder, such as a deficiency in boron; or

   (ii) the full potential of growth responses to inoculation not being expressed in the field.

In general, current fertiliser practices will require some modification to allow the maximum benefit to be derived from selected fungal isolates in the field. It is essential that fertilisers are used that provide a correct balance of nutrients and fertiliser rates are conducive to mycorrhizal development and function.



Flowchart outlining a mycorrhizal research program from an ACIAR-funded project in China, Australia and the Philippines. This approach could also provide a framework for future research activities in the Asian region.

8. *Communication.* It is essential that mycorrhizal scientists and land managers are aware of the successes and failures of mycorrhizal work being conducted throughout China and Australasia. Establishing a network of mycorrhizal scientists is a prerequisite for an exchange of ideas and for focusing research objectives. The goal of increasing productivity through mycorrhizal manipulation presents an exciting yet challenging area of scientific endeavor. The largest hurdle for mycorrhizal scientists is to demonstrate to other scientists and land mangers that mycorrhizal fungi are an integral and essential component of the root system of trees and plants and are required for the health of the crop. This can be achieved through field demonstration trials, workshops, conferences and accessible publications, such as those sponsored by ACIAR.

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