Symbiosis, 3 (1987) 179–206
Balaban Publishers, Philadelphia/Rehovot

Review Article

The Use of Mycorrhizas in Temperate and Tropical Forests

FRANÇOIS LE TACON, JEAN GARBAYE and GEOFF CARR

I.N.R.A.-C.R.F. De Nancy
Laboratoire de Microbiologie Forestière
Champenoux — 54280 Seichamps, France
Tel. 83 31 7080 Telex INRANCY 960 458 F

Received April 8, 1987; Accepted May 30, 1987

Abstract

A brief introduction concerning the beneficial effects of mycorrhizas on plant growth and development is followed by a review of the methods used for inoculum production and application. The main part of the article brings together information on the types of trees used in reforestation and re-afforestation in temperate and tropical regions of the world, and identifies why these trees might need to be inoculated with mycorrhizal fungi. Where possible, experimental results are given to demonstrate the potential benefits of inoculation.

1. Introduction

Mycorrhizal fungi benefit woody plants in a variety of ways, though probably the most important is in aiding mineral acquisition. The extramatrical growth of hyphae from the mycorrhizal root systems increases the volume of soil from which nutrients can be absorbed. These are taken up either directly or after mobilization by active metabolic processes, and are translocated as assimilated metabolites to the host root. Mycorrhizas also benefit woody plants by enhancing water uptake and regulation (Levy and Krikun, 1980), increasing tolerance to adverse soil conditions (low and high soil pH, presence

of heavy metals) (Berry and Marx, 1976), and by reducing the effects of soil borne pathogens on host roots (Marx, 1972). It can be appreciated therefore that mycorrhizas are very important in natural ecosystems. Many scientists and land managers, however, are unaware of this, which has certainly limited the utilization of mycorrhizal fungi to improve tree growth. Even if aware, mycorrhizal inoculation cannot become a routine practice if inoculation techniques are not available. In this paper we discuss the need for inoculating forest nursery soils with mycorrhizal fungi, the different techniques used for inoculation, the mycorrhizal status of tropical and temperate forests, and the main results of mycorrhizal inoculation in the world.

2. Why There is a Need for Inoculation

In natural ecosystems, trees cannot survive without mycorrhizas. In afforestation sites devoid of mycorrhizal propagules (e.g. cutover lands, treeless areas, mining spoils), non-mycorrhizal seedlings never fully develop and die. In these conditions, the success of the plantation depends totally on the mycorrhizas associated with the seedlings coming from the nursery. Fungi forming mycorrhizas on tree seedlings in nurseries must be ecologically adapted to the planting sites. If not, the mycorrhizas disappear and the seedlings cannot survive. In reforestation sites with natural mycorrhizal propagules, non-mycorrhizal seedlings fail to develop during the first one or two years after transplanting, their growth only normalizing after mycorrhizal establishment. So it is essential for afforestation to produce tree seedlings with efficient mycorrhizal associations. However, mycorrhizal deficiencies are frequent in forest nurseries, as current practices inhibit mycorrhizal development. Soil fumigation, which is more and more utilized to control pathogens and weeds in bare-root nurseries, kills natural mycorrhizal inoculum, while heavy fertilization and the use of fungicides reduce or eliminate mycorrhizas. Containerized seedlings grown in heavily fertilized, sterile artificial potting mixtures generally lack mycorrhizas.

3. Inoculation With Ectomycorrhizal Fungi

Several methods of inoculating soils have been or are currently in practice. They include:

Natural inoculation

Epigeous sporophores of epigeous ectomycorrhizal fungi produce large numbers of spores, which can be carried over long distances.

Spore dissemination occurs mainly in the autumn following sporophore production (Table 1). Outside of this period spore dispersal is restricted. Many forest nurseries are fumigated in the spring some weeks prior to sowing. The lack of air-borne spores at this time can cause erratic mycorrhizal infection. Fortunately, *Thelephora terrestris*, which is particularly well adapted to nursery conditions, generally produces spores at the end of spring. So this species is normally the first symbiont forming ectomycorrhizas. In Europe, *Hebeloma mesophaeum* and *Laccaria laccata* appear frequently after soil fumigation or in containers. They produce spores later than *T. terrestris* and form mycorrhizas only in August or September.

Table 1. Spores trapped in a fern heath in the Massif Central, France (September, 1980) (Le Tacon et al., 1984)

	Number of ectomycorrhizal spores per cubic metre air
Boletus sp.	235
Lactarius sp. and *Russula* sp.	225
Thelephora terrestris	196
Cortinarius sp.	172
Laccaria sp.	117
Inocybe sp.	37
Amanita sp.	17
Hebeloma sp.	14

Artificial inoculation with sporophores and spores

As natural inoculation with air-borne spores after soil fumigation is very erratic and several authors have proposed artificial inoculation using spores or sporophores.

In attempts to enhance truffle production during the 18th century in France, truffle sporophores were used to inoculate oak seedlings in nurseries or were placed directly in planting holes (Malençon, 1938). This method has been recently improved (Polenzona, 1969; Chevalier and Grente, 1973). Crushed sporophores are mixed with sterile substrate or fumigated soil used in seedling containers. *Rhizopogon luteolus* sporophores were used to inoculate nursery soils in Australia in the 1920's (Kessel and Sloate, 1938). In Europe, the first use of ectomycorrhizal sporophores in forestry was in a nursery at Wareham Heath, England, where *Rhizopogon luteolus* was introduced into a soil naturally infested with *Boletus bovinus.* Though the

soil was not previously fumigated, inoculation produced marked growth improvements in Sitka spruce seedlings. These results were reported in 1956 by Levisohn, but the experiment was carried out from 1943 to 1945 by M.C. Rayner. In Australia, Theodorou (1971), Theodorou and Bowen (1973), Lamb and Richards (1974) coated seeds of *Pinus radiata* from spores of either *Rhizopogon* sp. or *Suillus* sp. to increase ectomycorrhizal formation and seedling growth.

The largest effort to use spores as inoculum has been made in the United States. Marx and Bryan (1975) inoculated fumigated soils with freshly collected spores of *Pisolithus tinctorius* at a rate of $1.3 \times 10^{10}/m^2$, or $2.55 \times 10^9 m^2$. These spores were incorporated into the soil just prior or two months after sowing. Marx and his co-workers have also encapsulated seeds with spores of *Pisolithus tinctorius*. One mg of spores (1.1×10^6 spores) per seed was found to allow good ectomycorrhizal development. Seeds have to be watered daily for 1 week after sowing to release the spores from the coating materials. Encapsulation of seeds with spores could be a valuable technique for ectomycorrhizal fungi which produce large numbers of easily collectable sporophores. However, only some species such as *Thelephora terrestris*, *Pisolithus tinctorius* and *Rhizopogon* could be used, many difficulties having been encountered with other species.

Soil containing natural inoculum: spores, mycelium and mycorrhizas

The earliest attempts to introduce ectomycorrhizal fungi relied on the use of infested soils. A soil collected from natural forests, plantations or established nurseries, contains spores, mycorrhizas and mycelium. The first attempts to establish pines in Africa started in South Africa in the middle of the nineteenth century. No success was recorded until inoculation with ectomycorrhizal fungi taken from nursery soil of the Netherlands. It was a complete success, leading to the regular application of mycorrhizal soil as inoculum in South Africa. In 1919 soil inoculum was taken from South Africa to Kenya (Gibson, 1963), and from then the South African ectomycorrhizal inoculum became redistributed all over Africa. Soils of natural forests, plantations or nurseries are still the most commonly used form of mycorrhizal inoculum. Inoculation is usually done by spreading a 1–2 cm layer of infested soil on the nursery bed and then mixing with the rest of the soil. When containers are used, as is often the case in tropical countries, the infested soil is mixed with the rooting substrate in a proportion varying between 10 and 50%. The main objection to this method of inoculation is

the risk of inadvertently introducing pathogens or fungi poisonous to man. *Ammanita phalloides* was apparently introduced into both South America and South Africa as a mycorrhizal fungus imported from Europe (Mikola, 1970). Considerable risks are also taken when carrrying soil from one nursery to another. Most of the time, old nurseries are infested with *Pythium* and *Fusarium* which seriously affect seedling growth.

Mycorrhizas and mycorrhizal seedlings

Excised ectomycorrhizas or mycorrhizal seedlings are sometimes used to inoculate a new nursery. The method using mycorrhizal seedlings is called "indonesian" (Mikola, 1970). The infection spreads from the mycorrhizal plant to the surrounding transplants. This technique does not differ fundamentally from the soil inoculum technique and is not safer, the risks of introducing pathogenic fungi being similar.

Pure culture inoculation

The risk of introducing diseases is eliminated with pure culture inoculation. The ectomycorrhizal fungi can be chosen and more efficient fungal symbionts than those naturally present can be inoculated. The techniques of isolation and cultivation of ectomycorrhizal fungi were first developed by Melin (1936). Many ectomycorrhizal fungi grow well in pure culture, though some are very difficult to isolate and grow very slowly or not at all. This is true of some species of the genera: *Russula, Lactarius, Cortinarius, Inocybe, Tuber.* Among them, there are probably some very beneficial symbionts.

Two methods for producing large quantities of inoculum are available.

Culture on solid medium

According to Mikola (1970), Bokor (1954) in Hungary initiated this technique. Fungi were first grown in liquid medium (Hagem solution) and then transferred to flasks containing peat moistened with Hagem solution. This technique has also been used in Argentina by Takacs (1964). After two months incubation a mixture of three or four different fungi was used as inoculum (*Suillus luteus, S. granulatus, S. bovinus, Hebeloma crustuliniforme*). Four litres of inoculum were mixed with 100 kg of sterilized soil and kept moist for three weeks. One hundred kg of this mixture were enough to inoculate 500 m^2 of nursery bed.

Moser (1958a,b; 1959) has used a similar technique to inoculate *Pinus cembra. Boletus plorans* was gown in liquid medium, first in Erlenmeyer flasks and then in 10 litre tanks aerated for 2 to 3 hr daily. After 2 to 3

months, the mycelium was transferred into 5 litre jars containing a mixture of sterile peat and vermiculite. This substrate was completely colonized in 2 to 3 months. The inoculum was applied to nursery beds at a rate of 3 to 4 litres per m^2. One year old *Pinus cembra* seedlings or seedlings at cotyledon stage were transplanted immediately after soil inoculation. A modification of this technique is currently in use in Austria, for the production of *Suillus plorans* inoculum.

The largest attempts to produce commercial inoculum are currently being made in U.S.A. with *Pisolithus tinctorius* by Marx and co-workers. They have gained much experience and the sources of practical difficulties of large-scale production of pure ectomycorrhizal inoculum are now become known. Before utilization, the inoculum has to be leached with running tap water to remove the sugars which have not been used by the fungus. After leaching, the inoculum can be dried at 20°C to 29°C and to 35 to 45 per cent relative humidity. In bare-root nurseries, the inoculum is mixed with the previously fumigated soil at a rate of 0.5 to 1.5 l/m^2. In containerized seedling production, the inoculum is mixed with the growth medium at the rate of 1/5 v/v to 1/10 v/v. In 1976, the USDA Forest Service and Abbott Laboratories started to develop commercial methods of ectomycorrhizal inoculum production. Abbott inoculum was produced in a solid-substrate fermentor containing a modified Melin-Norkran nutritive solution. The fermentor was inoculated with a liquid mycelium culture. All the results show (Marx et al., 1982; Marx et al., 1984) that vermiculite-peat moss inoculum of *Pisolithus tinctorius* can be produced industrially for large scale applications in forest nursery. Nevertheless some difficulties appeared in the fermentation process in large tanks, and in maintaining the sterility of the inoculum.

Two firms, Sylvan Spawn Laboratory, U.S.A., and Somycel, France, specializing in mushroom spawn production are also interested in ectomycorrhizal inoculum production. At the heart of their processes is a specially designed autoclavable plastic bag with a breather strip allowing oxygenation, while still maintaining sterile conditions. The peat-vermiculite mixture is either steam sterilized in the bag or is transferred to the bag after sterilization. The bags are then inoculated and incubated at 25°C for 1 or 2 months. Both firms have developed nutrient solutions that do not require to be leached from the peat-vermiculite mixture before the inoculum can be used. Somycel uses a diluted brewers malt, but that used by Sylvan Spawn Laboratories is not generally known. Several ectomycorrhizal fungi have now been tested by the two companies. They include strains of *Laccaria laccata*, *Hebeloma crus-*

tuliniforme, Paxillus involutus, and *Pisolithus tinctorius* coming from our laboratory and also from those of Trappe and Molina in Corvallis (U.S.A.) and Marx in Athens (U.S.A.)

Mycelium cultivated in fermentor and entrapped in polymeric gels

Liquid industrial fermentation procedures have been employed for ectomycorrhizal inoculum production, but the cultivated mycelium cannot be used directly for nursery inoculum. Cheetham et al. (1979), Dommergues et al. (1979) and Jung (1979) have proposed entrapping microorganisms in polymeric gels. This method can be used for ectomycorrhizal fungi. Mycelium produced in fermentor is first leached to remove the excess nutrients, then resuspended in a solution of sodium alginate (10 g/l). Small pellets of entrapped mycelium are then obtained by replacing the sodium ions by calcium ions. Clay or peat can be incorporated into the pellets, and have been shown to diminish water loss during storage (Maupérin et al., 1987). The inoculum can be stored at least 10 months at 4°C. The pellets are both easy to manage in the nursery and have been found to be more effective for mycorrhizal production than peat-vermiculite moss inoculum (Le Tacon et al., 1985). The effectiveness of the entrapped mycelium is probably related to its high metabolic activity. By this technique, the mycelium is produced in one or two weeks, while mycelium produced by the solid technique is often several months old when used. The entrapped mycelium can be used in the nursery after soil fumigation at a rate of 2 g/m^2 (dry weight).

4. Endomycorrhizal Inoculation

The VA endomycorrhizal fungi cannot yet be grown *in vitro* without the host plant, and they do not produce large fruiting bodies yielding many spores. Therefore, the only way at present of producing inoculum for nursery practice is to grow inoculated host plants in controlled glass-house conditions and to use infected roots and/or soil-borne spores as an inoculum.

Menge (1984) reviewed the different techniques presently in use. In all cases, the selected fungi originate from single spores sieved out of the soil. The spores are checked for the absence of parasites, surface sterilized, and a plant is aseptically inoculated with the spores in laboratory conditions. The infected roots of these plants are then used to multiply the inoculum in pot cultures or in beds in the glass-house. As VA endomycorrhizal fungi are not host-specific, many plants can be used. Among the most convenient ones are maize, sudan-grass, onion, leek, clover and other legumes. They can be

grown in sterilized soils or mixtures of soil, peat, vermiculite, etc. In this case, the inoculum is the whole medium containing infected roots, mycelium and spores. If the plants are grown in liquid culture, the inoculum will only be infected roots, and is lighter and easier to ship than soil inoculum. To date, the largest scale commercial application of VA mycorrhizal soil inoculum has been with Citrus in California (Menge et al., 1977 and 1978).

5. Mycorhizal Inoculation in Tropical Forestry

Mycorrhizal status of natural tropical forests

About 95% of the tree species occurring in tropical forests are purely endomycorrhizal. Ectomycorrhizas are only found in the families *Caesalpinaceae, Dipterocarpaceae, Myrtaceae* (e.g. *Eucalyptus* sp.), *Fagaceae* (*Quercus* sp. in southeast Asia), *Pinaceae* (*Pinus* sp. in southeast Asia, Caribean and Central America), and some *Euphorbiaceae* (Redhead, 1980).

As far as forestry practice is concerned, this predominantly endomycorrhizal status of natural tropical forests, as well as the specificity of the rare ectomycorrhizal associations, should be considered when introducing exotic ectomycorrhizal trees such as pines, Eucalypts and Casuarinas; which are the main species used in extensive afforestation programmes in the tropics. In such cases, compatible inoculum has to be introduced into the plantation areas at the same time as the trees, which otherwise will not attain a satisfactory development.

Another important characteristic of the tropical ecosystems is the generally very low nutrient level in the soil. Therefore, an efficient mycorrhizal association is necessary for successful plantations when the use of expensive fertilizers is impossible.

Ectomycorrhizal inoculation of pine plantations in the tropics

The tropical pines used for plantation forests come from three major regions: southeast Asia (*P. kesiya, P. merkusii*), the Caribean (*P. tropicalis, P. cubensis, P. occidentalis, P. caribaea, V. caribaea* and *V. bahamensis*), and central America (*P. caribaea, V. hondurensis, P. gocarpa, P. strobus, V. chiapensis*). Ivory (1980) gives a list of the fungal species associated with these pines in their natural habitat. Most of them do not exist in temperate regions, but some very common pine associated fungal species of the temperate zone, such as *Thelephora terrestris*, *Pisolithus tinctorius*, *Suillus granulatus, S. bovinus, Amanita muscaria* and *A. gemmata*, are also present.

When these pines were first introduced in tropical Africa, the seedlings were stunted and chlorotic in the nursery and never survived field transplantation until ectomycorrhizal inoculum (plants or humus from pine forests) was brought from overseas. These introductions were often accidental and came from Europe as well as from the tropical regions where pines are native. As a result, the distribution pattern of pine mycorrhizas in Africa is very irregular, depending more on chance than on ecological factors. It is interesting to note that typical temperate fungi such as *Rhizopogon luteolus* or *Hebeloma crustuliniforme* spread quickly in some areas. The productivity of new plantations is maintained by reinoculating the nurseries with fresh pine soil, or by the use of "mother seedlings" staying in the nursery beds during several rotations. Although these techniques made possible the successful introduction of pines into Africa and into other tropical regions lacking the adequate symbionts (Mikola, 1973; Marx, 1980), they are unsatisfactory in a long term for reasons previously discussed.

It is now generally agreed that a pure inoculum is preferable. Some recent field experiments show the advantages of this technique.

Table 2 gives 4 examples of inoculation experiments on *Pinus caribaea* with pure mycelial cultures in comparison with traditional soil inoculum from the region. *Pisolithus tinctorius* proved to be the most consistently efficient fungus, particularly when the young plantations suffered long dry seasons with high soil temperatures. The growth increase due to *P. tinctorius* can be very large and of highly significant economic value, as in examples 3 and 4. However, this is not always the case, as shown by example 1, where the soil inoculum proves to be as efficient as *P. tinctorius*. A similar result was obtained in Congo by Delwaulle et al. (1982), with soil inoculum from a very precise plantation area far away from the experiment. Thus, local fungi carried by soil inoculum with all the limitations discussed previously, can be quite satisfactory in some cases.

In very deficient soils, the full effect of fertilizers can be attained if the mycorrhizal status of the trees is correct. In these conditions, it is possible to obtain an inoculation-fertilization interaction (example 3).

P. tinctorius is presently the most interesting fungus for practical applications for the following reasons:

- It is fast growing on laboratory media and inoculum production is easy.
- Its golden-brown mycorrhizas are easy to recognize, which is important for checking the results of inoculation in the nursery.

Table 2. Results of field experiments comparing the effect of inoculum source (pure mycelial cultures of ectomycorrhizal fungi versus traditional soil inoculum) on growth of *Pinus caribaea*

Reference and country	Site conditions	Age of trees when measured	Treatments in order of decreasing efficiency	Survival in the best treatment per cent of control with soil inoculum	Mean height in the best treatment (per cent of control) with soil inoculum)
Marx, Hedin and Toe (1985) LIBERIA	Savanna on poor acid soil with short dry season	36	*Pisolithus tinctorius* soil inoculum *Thelephora terrestris*	100 (non fertilized) 104 (fertilized)	92 (non fertilized) 103 (fertilized)
Delwaulle, Garbaye and Okombi (1982) CONGO	Savanna on poor sandy soil with short dry season	20	*Pisolithus tinctorius* *Suillus granulatus* *Suillus bovinus* *Suillus bellini* *Hebeloma cylindrosporum* Soil inoculum	100	145
Krügner (1982) BRAZIL	Savanna on poor acid soil with long dry season	24	*Pisolithus tinctorius* *Thelephora terrestris* Soil inoculum	179 (non fertilized) 118 (fertilized)	155 (non fertilized) 239 (fertilized)
Momoh and Gbadegesin (1980) NIGERIA	Savanna with long hot dry season	22	*Pisolithus tinctorius* soil inoculum (mostly *Rhizopogon luteolus*	317	286

- The world-wide distribution of this species and its mycorrhizal habits are well documented (Marx, 1977).
- It is the most studied ectomycorrhizal fungus, and the selection of high performance strains is underway.
- It is highly competitive against other ectomycorrhizal fungi in (but only in) hot, nutrient poor, acidic and sandy soils with low organic matter content. Such soils are characteristic of many tropical afforestation areas. The survival and spreading of *P. tinctorius* on new roots after outplanting results in a long term growth improvement.

Another interesting aspect of *P. tinctorius* is its ability to quickly produce large quantities of puff-ball like sporophores releasing air-borne basidiospores in all seasons, which dramatically increases its dissemination. This contagiousness has been particularly obvious in Congo, where, within 2 years from planting the first experiment (cf. Table 1, example 2), nearby pine plantations started to grow faster and to yield sporophores.

Collecting sporophores and inoculating nursery beds with the spores is now replacing the handling of traditional soil inoculum as a routine technique. The spores are easy to collect and to store, cheap to transport, and make possible very efficient inoculation far away from the nursery where *P. tinctorius* was first introduced (Diangana, unpublished data).

Mycorrhizal inoculation of eucalypt plantations in the tropics

Twelve main eucalypt species, belonging to the subgenera *Symphomyrtus* (S), *Corymbia* (C) and *Idiogenes* (I) according to the classification of Pryor and Johnson (1971), are commonly planted in the tropics. These species are *Eucalyptus* (S) *grandis*, *E.*(S)*alba*, *E.* (S) *deglupta*, *E.* (C) *citriodora*, *E.* (C) *maculata*, *E.* (C) *torelliana*, and *E.* (I) *cloeziana*. They originate from northeastern Australia, eastern Indonesian Islands and Papua-New Guinea.

In its native area, the genus *Eucalyptus* forms both ectomycorrhizas and VA endomycorrhizas. However, little is known of the respective importance of the two types of symbiosis. Pryor (1956) and Uhlig (1968) made observations which strongly suggest that the subgenus *Monocalyptus* is much more dependent on ectomycorrhizae than are the other subgenera. The ecological distribution of eucalypt subgenera supports this conclusion. *Monocalyptus* species display some of the characteristics of temperate ectomycorrhizal trees: they occur in pure stands in the more temperate regions of Australia, on generally poor soils. The other subgenera grow in mixed stands on richer colluvial or alluvial soils (Florence, 1981).

Investigations on eucalypt mycorrhizas in Australia are rare, and where they have been made they only deal with species of the temperate zone. Lapeyrie and Chilvers (1985) found an endo-ectomycorrhizal succession associated with enhanced growth on young seedlings of *E.* (S) *Dumose*, but it was not clear whether this enhanced growth was due to endomycorrhizas or ectomycorrhizas. Malajczuk et al. (1981) inoculated young seedlings of *E.* (M) *marginata* and *E.* (S) *diversicolor* with the VA endomycorrhizal fungus *Glomus fasciculatus*. Both species formed the same amount of typical VA endomycorrhizas. However, mature trees of the same species only formed atypical VA infections, with well developed arbuscules near the entry points but no longitudinal extension.

Specificity of the symbionts is better documented for ectomycorrhizas, as, Malajczuk et al. (1982), have shown that *Hydnangium carneum* and *Hymenogaster albellus*, two common Australian ectomycorrhizal hypogeous gasteromycetes, are specific to eucalypts. In general, eucalypts in Australia seem to be mostly associated with gasteromycetes (*Scleroderma* sp., *Pisolithus* sp. and many undescribed species).

When introduced, outside their native area, tropical eucalypts belonging to the subgenera *Symphomyrtus, Corymbia* and *Idiogenes*, have grown well and not shown any of the symptoms displayed by introduced pines under the same conditions. However, the transfer of species in the subgenus *Monocalyptus* has been unreliable. When grown overseas, they were affected by root diseases in the nursery and poor growth after transplanting (Turnbull and Pryor, 1978). As a large choice of fast growing tropical eucalypts exist, *Monocalyptus* have been discarded before trying to understand and correct the cause of the failure.

Because of the generally good performance of other eucalypts when grown overseas, their mycorrhizal status has not received the same attention as that of pines. Some data exist on ectomycorrhizae and associated fungi occurring on eucalypts introduced into the tropics (Bakshi, 1966; Uhlig, 1968; Singh and Kumar, 1966). But, possibly because of the problems first encountered with pines, researchers seem to have been mostly interested in ectomycorrhizas tending to neglect the endomycorrhizal aspect which is perhaps as relevant, at least for subgenera other than *Monocalyptus*.

Even on healthy and fast growing trees, infection rates are generally very low for both types of mycorrhizas. Ectomycorrhizas are mostly of the gasteromycete type, and the most commonly found fruiting bodies are *Pisolithus tinctorius* and *Scleroderma* species. VA infections are present but limited to

entry points with few lateral extensions. Thus, mycorrhizal status tends to be very similar to that of *Symphomyrtus* in their native areas.

It is not known, whether the fungal associates found outside Australia were introduced with the eucalypts, introduced with pine beforehand, or are native to the regions. Whatever the origin of these fungi, it is highly probable that the symbioses in question do not operate at optimal efficiency in their new environments. It is worth trying to improve this efficiency, due to the very high production potential of selected provenances and hybrids of eucalypts. For these reasons, there is a worldwide new interest in eucalypt mycorrhizas and their possible application in tropical forestry.

To date, field results are rare and only available for ectomycorrhizal inoculation. In a 27 month old plantation of a hybrid *E. europhyllax* (*E. tereticornis x E. robusta*) in Congo, Garbaye, Delwaulle and Diangana (unpublished data) recorded significant increases in basal area of 23 and 18% with *Pisolithus tinctorius* and *Scleroderma taxense*, respectively. The control trees were not inoculated, while the other treatments received mycelial inoculum (pure culture) in the nursery. These early results are far from being as spectacular as those with pines, but they demonstrate that improvement of eucalypt production in the tropics can be achieved by mycorrhizal inoculation.

Mycorrhizal status of Casuarina

The family Casuarinaceae includes 4 genera, all of them generally referred to as "casuarinas". Trees planted in the tropics belong mostly to the genus *Casuarina* and include *C. cunninghamiana, C. equisetifolia, C. glauca, C. junghuhniana* and *C. oligodon.* All species originated from Australia, Papua-New Guinea and the Indonesian islands.

Casuarinas form nitrogen-fixing root nodules with an actinomycete (*Frankia*), which makes them particularly interesting for afforestation in adverse conditions such as on sand dunes, eroded soils, mine spoils, etc.

The mycorrhizal status of Casuarinas has not been investigated until recently. Reddel et al. (1965) in an extensive survey of casuarinas' root symbioses in Australia, found that *Casuarina* species can form ectomycorrhizae as well as VA ecnomycorrhizas, with a predominance of the latter type. Nothing is known about the ectomycorrhizal fungi associated with *Casuarina.* Diem and Gauthier (1982) inoculated young seedlings of *C. equisetifolia* with the VA endomycorrhizal fungus *Glomus mosseae.* They found that VA infection occurred readily and greatly increased growth, nodulation and nitrogen

fixation. Thus, the nitrogen-fixing symbioses may not operate at its optimal efficiency if an adequate mycorrhizal association is not established.

6. Mycorrhizal Inoculation in Temperate Forestry

Mycorrhizal status of natural temperate forests

Ectomycorrhizal fungi can be found on about 90% of the trees in temperate forests, including those of the families Fagaceae, Tiliaceae, Betulaceae, Pinaceae and Abietaceae. Trees belonging to the Salicaceae and Rosaceae have both ectomycorrhizas and VA mycorrhizas, as do those of the genus *Alnus* which belongs to the family Betulaceae. Species of the Cupressaceae, Juniperaceae, Taxaceae, Fraxinaceae, Aceraceae and Juglandaceae are purely VA endomycorrhizal.

On very acid soils (mor-moder) there is a dominance of ectomycorrhizal trees. However, on mull soils above pH 5 pure VA mycorrhizal species such as *Acer, Fraxinus, Juglans* and *Sorbus* can compete with ectomycorrhizal species.

The main groups and genera of fungi that form ectomycorrhizas have been reviewed by different authors (Trappe, 1977; Mosse et al., 1981). Most are basidiomycetes and occur essentially in the order Agaricales, but some ascomycetes form ectomycorrhizas and often produce hypogeous sporophores. Each ectomycorrhizal tree species can be associated with several hundred or thousands of fungal species. Conversely, some fungi such as *Cenococcum graniforme* or *Pisolithus tinctorius* will infect many hosts (Marx, 1977), though others are restricted to single hosts, e.g. *Boletus elegans* with larch (*Larix europaeus*). The consequence of this is that a mixed stand is always richer in ectomycorrhizal species than a pure stand. In a mixed stand of oak, beech and birch one finds both the fungi common to all three hosts and those specific to each one. Table 3 gives for a natural deciduous forest in the centre of France, the species associated with oak (group 1), the species associated with beech (group 2), the species associated with birch (group 3), the species common to beech and oak (group 4), and the species associated with these three deciduous trees, which are able also to form mycorrhizas with introduced conifers (*Picea abies, Pseudotsuga menziesii*, and *Pinus silvestris* (group 5,6).

The fungal species of groups 5 and 6 are especially interesting, as they are able to form ectomycorrhizas with the two most common tree species used in Europe for reforestation, Norway spruce and Douglas fir. Seedlings of these trees planted immediately after the clearing of such a natural deciduous

Table 3. Ectomycorrhizal fungi of a natural beech, oak and beech forest of France (after, Le Tacon, Lamoure, Guimberteau, Fiket, 1984)

GROUP 1 fungi associated only with *Quercus robur*	*Lactarius vellereus* Fr. *Lactarius chrysorrheus* Fr. *Lactarius camphoratus* Bull. ex. Fr. *Lactarius quietus* Fr. *Cortinarius bolaris* (Pers. ex. Fr.) Fr. *Cortinarius alboviolaceus* (Pers. ex. Fr.) Fr. *Tricholoma columbetta* (Fr.) Kumar
GROUP 2 Fungi associated only with *Fagus silvatica*	*Lactarius blennius* Fr. *Lactarius subduculcis* Bull. ex. Fr. *Amanita umbrino-lutea* Secr. *Russula fellea* Fr. *Russula mairei* var. *fagiticola* (Melz) Küln et Romagn. *Tricholoma ustaloides* Romagn. *Boletus calopus* Fr.
GROUP 3 Fungi associated only with *Betula verrucosa*	*Lactarius theigolus* (Bull.) Fr. *Lactarius necator* (Pers. ex. Fr.) Karst. *Cortinarius pholideus* Fr. *Cortinarius violaceus* (L. ex. Fr.) Fr. *Cortinarius armillatus* (Fr.) Fr. *Cortinarius paleaceus* Fr. *Russula aeruginea* Lindl. *Leotia lubrica* Pers.
GROUP 4 Fungi associated with both *Quercus robur* and *Fagus silvatica.*	*Cortinarius anomalus* (Fr. ex. Fr.) Fr. *Cortinarius claricolor* Fr. *Russula cyanoxantha* Schff. ex. Fr. *Russula fellea* Fr. *Russula laurocerasi* Melzer *Zerocomus chrysenteron* (Bull. ex. St Amans). Quél
GROUP 5 Fungi associated with *Quercus robur* and *Fagus silvatica* and able to form associations with *Picea abies*, *Pseudotsuga menziesii*	*Russula nigricans* (Bull) Fr. *Laccaria amethistina* (Bolt) Muss *Hydnum repandum* L. ex. Fr.
GROUP 6 Fungi associated with *Quercus robur* and *Fagus silvatica* and able to form associations with *Picea abies*, *Pseudotsuga menziesii* and *Pinus silvestris*	*Laccaria laccata* (Scop. ex. Fr.) Bk. et Br. *Boletus edulis* Bull. ex. Fr. *Paxillus involutus* (Batsch.) Fr. *Russula ochroleuca* (Pers.) Fr. *Russula mairei* Sing. *Russula emetica* Fr. *Russula fragilis* (Pers. ex. Fr.) Fr. *Dermocybe cinnamomeus* (L. ex. Fr.) Wünsche *Cortinarius orellanus* (Fr.) Fr. *Cantharellus tubeaformis* Bull. ex. Fr. *Cantharellus cibarius* Fr. *Amanita gemmata* (Fr.) Gill. *Xerocomus badius* (Fr.) Kühn. ex. Gibb. *Xerocumus subtomentosus* (L. ex. Fr.) Quél.

forest can develop symbiotic associations without any problem. It does not matter if the inoculum coming from the nursery with seedlings is poor, as the inoculum in the forest soil site is sufficiently abundant and diversified.

It is difficult to obtain good evidence about the survival of ectomycorrhizal fungi after the disappearance of the host. Most ectomycorrhizal fungi can probably survive for a few months. Dimbleby (1953) assumed that they persisted for several years in a saprophytic state after deforestation until stumps were completely mineralized. However, the deforestation that has occurred in temperate countries has resulted in the disappearance of many of the ectomycorrhizal fungi from the cultivated soil, and has favoured the spread of endomycorrhizal fungi. In Europe, large areas of cultivated land have been abandoned by agriculture for decades or even centuries. Such areas, particularly abundant on acid soils in France and Britain, are always difficult to re-afforest, being rarely colonized by ectomycorrhizal fungi. Often, the first trees to appear when grazing is discontinued are endomycorrhizal species, e.g. *Juniperus communis*. Except for birch and Scots pine, trees planted on *Calluna* moorland grow very slowly and have few or no mycorrhizal roots. This is probably due to toxic compounds coming from both *Calluna* roots and humus inhibiting the growth of many ectomycorrhizal fungi, except those specifically associated with birch or Scots pine (Handley, 1963).

Grassland areas that occur extensively in Russia and the midwestern U.S.A. are normally devoid of trees, except along stream banks. It is widely agreed that oaks and beeches in the steppe soils and pines in the prairies succeed only if the seedlings become mycorrhizal. Whereas the older literature (e.g. Vystskii, 1902) stressed the need for inoculation of oak and beech seedlings in steppe soils, Vlasov (1952) reported mycorrhizal development in oak seedlings without inoculation. Shemakhanova (1962), summing up Russian experience, concluded that young oak seedlings in Russia became mycorrhizal not only in soil long denuded of forests but also in soil far removed from forests. This may be explained by Chastukhin's (1955) observation that inoculum could be carried on the acorn seed coat. Mishustin (1955) observed that in the chernozem zone, oak seedlings formed mycorrhizas naturally with indigenous fungi, whereas they failed to do so in the more southern chestnut soils further removed from the inoculum of the temperate forest belt. One reason for the confusing and divergent opinions may be that the indigenous mycorrhizal fungi might be relatively inefficient, and thus foresters insist on the need for inoculation, while researchers simply note the presence of mycorrhizas in test seedlings. Dominik (1961) in Poland, and

Fassi and De Vecchi (1962) in Italy, showed that young seedlings became ectomycorrhizal in cultivated soils, but the number of fungal species involved is smaller than in normal forest soils and the fungi are often relatively inefficient. Most prairie soils of the midwest U.S.A. lack the mycorrhizal fungi necessary for the successful introduction of conifers (Hatch, 1936; Mikola, 1970).

Sites without previous vegetation (e.g. sand dunes, and reclaimed soils), or locations such as mine spoils, soil shale wasters, open-cast mining sites, gravel pits, and other rehabilitation sites where top soil has been brought to the surface or stored for prolonged periods are generally devoid of ectomycorrhizal and VA mycorrhizal fungi.

For the afforestation or reforestation of cultivated soil, calluna heathlands, grassland areas or adverse sites without previous vegetation, establishment of mycorrhizal associations is absolutely necessary. Most of the time, it can occur naturally by air-borne spores, but to assure the success of the plantations in these sites it is necessary to transplant seedlings with mycorrhizas formed with ecologically adapted fungi.

Ectomycorrhizal inoculation in temperate forest nurseries

Many experimental studies have been conducted on ectomycorrhizal inoculation techniques in temperate forest nurseries. These studies have consistently shown that with pure culture inoculation it is possible to favour the more efficient fungal species and to avoid the risk of introducing soil pathogens.

Pines

Pinus cembra is used for reforestation in subalpine areas often above the present timber line. The most efficient ectomycorrhizal fungi associated with *Pinus cembra* is *Suillus plorans.* This fungus is lacking in the Austrian nurseries which are located in the valleys. Moser (1958a,b) has shown that seedlings artificially inoculated with pure cultures of *Suillus plorans* have a better survival and initial growth after planting than naturally inoculated seedlings. In Austria, artificial inoculation of *Pinus cembra* has now reached the stage of practical application.

Few experiments have been conducted on the artificial inoculation of Scots pine (*Pinus silvestris*). Compared to natural inoculation by *Thelephora terrestris*, artificial inoculation with pure cultures of *Laccaria laccata*, *Hebeloma*

cylindrosporum and *Hebeloma crustuliniforme* improved seedling growth after soil fumigation in a bare-root nursery in the centre of France (Le Tacon and Bouchard, 1986).

In pot experiments with nursery soils from the east of France, *Hebeloma crustuliniforme* and *Paxillus involutus* improved the growth of Austrian pine (*Pinus nigra nigricans*) seedlings more effectively than did *Thelephora terrestris* (Garbaye and Lopez, unpublished data). In two U.S.A. bare-root nurseries, inoculation of Austrian pines with *Pisolithus tinctorius* after soil fumigation significantly increased seedling growth (Marx et al., 1984).

Large scale inoculation experiments are being done in the U.S.A. with *Pisolithus tinctorius* on different pine species, including *P. taeda, P. elliottii, P. echinata, P. clausa, P. virginiana, P. palustris, P. ponderosa, P. strobus, P. resinosa* (Marx et al., 1982, 1984). During several years, effectiveness of *Pisolithus* inoculation was tested on these pine species in 23 conventional bare-root nurseries located in 25 states. In 1978, positive results were obtained in 80% of the nurseries that fumigated soil in the spring. Similar successes were obtained by artificial inoculation of containerized pine seedlings on different growing substrates.

Since 1973, field experiments have been conducted by Marx and co-workers on adverse and routine reforestation sites. On adverse sites, e.g. coal spoils, kaolin spoils, eroded soils, and borrow pits, pine seedlings with mycorrhizas survived and grew better than seedlings lacking mycorrhizas. Seedlings with *Pisolithus tinctorius* mycorrhizas were, most of the time, better adapted to adverse soil conditions than seedlings inoculated with naturally occurring fungi such as *Thelephora terrestris*. In routine reforestation sites in Florida and North Carolina, *Pisolithus tinctorius* improved survival and early tree growth on almost all of the sites, but most notably on the poorest.

Douglas fir

Molina (1982) succeeded in inoculating containerized Douglas fir (*Pseudotsuga douglasii*) seedlings with 4 isolates of *Laccaria laccata*. In several French bare root nurseries, growth of Douglas fir seedlings was also significantly improved after soil fumigation by inoculation of different *Laccaria laccata* and *Laccaria bicolor* isolates. Plantable seedlings can be produced in 2 years on a fumigated soil with the aid of *Laccaria laccata* inoculation. Without such fumigation and inoculation, 3 or 4 years are usually needed to produce plantable seedlings (Le Tacon and Bouchard, 1986). After outplanting on a reforestation site (*Calluna* heathland) Douglas fir seedlings with

mycorrhizas formed by *Laccaria laccata* grew better than seedlings with mycorrhizas formed by *Thelephora terrestris* (Le Tacon, unpublished data). In the same bare-root nurseries, *Hebeloma cylindrosporum* gave similar results. However, after field transplantation this fungus did not survive and seedlings mycorrhizal with *T. terrestris* had better survival and growth than seedlings mycorrhizal with *H. cylindrosporum* (Garbaye and Le Tacon, 1986).

Sitka spruce

Molina (1982) in the U.S.A. and Thomas and Jackson (1983) in the U.K. inoculated containerized Sitka spruce (*Picea sitchensis*) seedlings with *Laccaria laccata* and obtained well developed mycorrhizas. However, in both cases, the inoculated seedlings' growth was not improved on that of non-inoculated seedlings. This is generally observed with inoculated containerized seedlings where roots are confined to a small volume. In these conditions the carbohydrate demand placed on the plant by the fungus outweights the benefits it provides in terms of enhanced mineral uptake. In contrast, in two field experiments where rooting is not limiting a significant effect of *Laccaria laccata* inoculation was obtained in comparison with seedlings that became naturally infected with *Thelephora terrestris* or E strain (ectendomycorrhizal fungus) (Thomas and Jackson, 1983). Lastly, Mason and Wilson (1984) have also reported improved growth of out-planted Sitka spruce seedlings pre-inoculated with *Laccaria laccata* or *Paxillus involutus*, in the U.K.

Norway spruce

In several French bare root nurseries inoculation with *Laccaria laccata Laccaria bicolor, Hebeloma cylindrosporum, Hebeloma crustuliniforme* improved growth of Norway spruce (*Sicea excelsa*) seedlings (Le Tacon and Bouchard, 1986).

Western hemlock

Laccaria laccata formed well developed ectomycorrhizas on western hemlock (*Tsuga heterophylla*) in containers (Molina, 1982).

Beech and oak

In France, beech (*Fagus silvatica*) and oak (*Quercus robur*) are often produced on fertilized peat. *Hebeloma crustuliniforme*, *Paxillus involutus* and *Laccaria laccata* form abundant mycorrhizas with these two species if peat fertility is limited to 75 g m^{-3}N, 37.5 g m^{-3} P_2O_5 and 37.5 g m^{-3} K_2O (Le Tacon

and Garbaye, 1986). In bare-root nurseries or in containers *Pisolithus tinctorius* formed mycorrhizas with northern red oak (*Quercus robur*) and stimulated seedlings' growth (Marx et al., 1982, 1984).

VA mycorrhizal inoculation in temperate forest nurseries

Endomycorrhizal inoculum is often limiting in bare-root nurseries, due to soil fumigation practices and in artificial potting mixtures used to grow containerized seedlings. Thus, there is a need for inoculation of these soils with appropriate fungal strains. All the same, there have been relatively few experiments on the inoculation of commercially important temperate hardwood species, e.g. *Fraxinus, Liquidambar, Liriodendron, Acer*, which are all associated with VA mycorrhizal fungi.

Kormanik et al. (1977) showed on a fumigated nursery soil that artificial inoculation of *Liquidambar styraciflua* by *Glomus mosseae* enhances growth at any level of fertility. Furlan et al. (1983), have inoculated *Fraxinus americana* (white ash) seedlings with five different VA mycorrhizal fungi in a fumigated nursery soil, *Glomus epigaeum, Glomus monosporum* and a *Glomus* species obtained from roots of ash plants growing in another nursery all markedly improved seedlings' growth. Similar results have been obtained in France of *Acer pseudoplatanus* and *Fraxinus excelsior* inoculated with *Glomus mosseae*. A field experiment installed in east of France showed that *Glomus mosseae* continued to stimulate ash growth 4 years after outplanting (Garbaye and Le Tacon, 1986).

7. Conclusions

During the last decade, much progress has been realized in understanding the ecology, physiology and symbiotic efficiency of the mycorrhizal species associated with a range of forest trees. It is now clear that there is a need to produce tree seedlings with efficient mycorrhizae for planting in man-made forests. Mycorrhizal inoculation has two purposes: (a) introduction of mycorrhizal fungi into soils or substrates where they are lacking, (b) replacement of the natural occurring fungi by more efficient species. For artificial or natural inoculation, soil conditions must be in accord with the mycorrhizal fungi requirements: the most important factor is that the soil or substrate should not be over fertilized.

Even where its efficiency is clearly demonstrated, mycorrhizal inoculation cannot become a routine forestry practice if large amounts of cheap inoculum and practical inoculation techniques are not available. For mycorrhizal

inoculation the use of pure cultures is now considered the most biologically sound method of inoculation. New techniques of mass production of ectomycorrhizal inoculum are currently being explored in France and U.S.A. They are an adaptation of the techniques used for the highly mechanized industrial production of mushroom spawn in autoclavable plastic bags. Unfortunately nothing similar exists for the production of VA mycorrhizal inoculum.

Tropical plantation forests can benefit greatly from modern mycorrhizal inoculation techniques for the following reasons:

- most trees species planted in the tropics are exotic and cannot naturally form optimal symbiotic associations in their new environment
- tropical soils are generally very poor and are the first limiting factor for tree growth.
- mycorrhizal inoculation considered as a "biological fertilization" can save expensive chemical fertilizers or increase their efficiency at low fertility levels.
- tropical foresters are more aware of the importance of mycorrhizas than their colleagues in temperate regions, because of the early example of pines where empirical inoculation was compulsory.

Recent results of field experiments all around the world indicate that the technical conditions are now ready for a massive introduction of inoculation practice with pure ectomycorrhizal inoculum on pines, and probably on Eucalyptus too in the near future. VA endomycorrhizal inoculation in tropical forestry is less documented, but may prove to be decisive for successful establishment of eucalypts and casuarinas in adverse conditions or to increase the yield in high production plantations.

In temperate countries, it was thought for a long time that there was little need for mycorrhizal inoculation. However, cases in which mycorrhizal inoculation is beneficial are found more and more frequently. After soil fumigation, artificial inoculation assures a more rapid and uniform infection. The selection of efficient and competitive fungi improves seedlings' growth in nurseries and can improve survival and tree growth in adverse or routine reforestation sites.

REFERENCES

Bakshi, B.K. 1966. Mycorrhiza in Eucalyptus in India. *Indian Forester* **92:** 19–20.

Berry, C.R. and Marx, D.H. 1976. Sewage sludge and *Pisolithus tinctorius* ectomycorrhizae: their effect on growth of pine seedlings. *For. Sci.* **22:** 351–358.

Bokor, R. 1954. A mykorrhiza-gombákkal történő talajoltások új agrotechnikai eljárása. *Erdész. Kutatások, Budapest* **11:** 27–45.

Chastukhin, V.Ya. 1955. Biology and ecology of mycorrhizae forming fungi. In: *Mycotrophy in Plants.* Published for the U.S. Department of Agriculture by the Israel Program for Scientific Translations, Jerusalem, pp. 87–100.

Cheetham, P.S.D., Blunt, K.W., and Bucke, C. 1979. Physical studies on cell immobilization using calcium alginate gels. *Biotech. Bioeng.* **21:** 2155–2168.

Chevalier, G. and Grente, J. 1973. Propagation de la mycorhization par la truffle á partir de racines excisées et de plantules inséminatrices. *Ann. Phytopathol.* **4:** 317–318.

Delwaulle, J.C., Garbaye, J., and Okombi, G. 1982. Stimulation de la croissance initiale de *Pinus caribaea* Morelet dans une plantation du Congo par contrôle de la mycorhization. *Rev. Bois et Forêts des Tropiques* no. 196, 25–32.

Diem, H.G. and Gauthier, D. 1982. Effet de l'infection endomycorhizienne (*Glomus mosseae*) sur la nodulation et la croissance de *Casuarina equisetifolia*. *C.R. Acad. Sc. Paris* **294:** 215–218.

Dimbleby, G.W. 1953. Natural regeneration of pine and birch on the heather moors of northeast Yorkshire. *Forestry* **26:** 41–52.

Dominik, T. 1961. Trials on a natural isolation of mycorrhizal fungi from cultivated soils and wasteland in the surroundings of Szeczecin. *Pr. Inst. Badaw. Lesn.* **227:** 3–37.

Dommergues, Y., Diem, H.G., and Divies, C. 1979. Microbiological process for controlling the productivity of cultivated plants. U.S. patent 4, 155, 737. May 22, 1979.

Fassi, B. and De Vecchi, E. 1962. Richerche sulle micorrhize ectotrofiche del pino strobo in vivaio. 1. Descrizione di alcune forme piú diffuse in piemonte. *Allionia* **8:** 133–112.

Florence, R.G. 1981. The biology of the eucalypt forest. In: *The Biology of Australian Plants.* J.S. Pate and A.J. McComb, eds. University of Western Press, Nedlands, pp. 147–180.

Furlan, V., Fortin, J.A., and Plenchette, C. 1983. Effects of different vesicular-arbuscular mycorrhizal fungi on growth of *Fraxinus americana. Can. J. For. Res.* **13:** 589–593.

Garbaye, J. and Le Tacon, F. 1986. Effets de la mycorhization contrôlée après transplantation. *Rev. For. Fr.* **3:** 258–263.

Gibson, I.A.S. 1963. Eine Mitteilung über die Kiefernmykorrhiza in den Wäldern Kenias. In: *Mykorrhiza, Int. Mykorrhizasymp. Weimar.* W. Rawald and H. Lyr, eds. Gustav Fischer, Jena, 1960, pp. 49–51.

Handley, W.R.C. 1963. Mycorrhizal associations and Calluna heathland afforestation. *Forestry Comm. Bull.* **36**, HMSO, London.

Hatch, A.B. 1936. The role of mycorrhizae in afforestation. *J. For.* **34:** 22–29.

Ivory, M.H. 1980. Ectomycorrhizal fungi of lowland tropical pines in natural forests and exotic plantations. In: *Tropical Mycorrhizae Research.* P. Mikola, ed. Clarendon Press, Oxford, pp. 110–117.

Jung, G. 1979. Procédé d'inclusion de microorganismes dans une matrice de polymères et produits ainsi obtenus. *Demande Brevet Fr.* **79:** 908–597, Avril 05, 1979.

Kessel, S.L. and Stoate, T.N. 1938. Pine nutrition. An account of investigations and experiments in connection with the growth of exotic conifers in Western Australian plantations. *Forest Bull.* **50**, Perth, Western Australia.

Kormanik, P.P., Bryan, W.C., Schultz, R.C. 1977. Influence of endomycorrhizae on growth of sweetgum seedlings from eight mother trees. *Forest Science* **23:** 500–505.

Krügner, T.L. Associações micorrizicas em árvores florestais. VII. Seminário sobre atualidades e perspectivas florestais, 1–2 Abril 1982. Curitaba, Paraná, Emprapa, Brazil, Documentos no. 2.

Lamb, R.J. and Richards, B.N. 1974. Inoculation of pines with mycorrhizal fungi in natural soils. Effects of density and time of application of inoculum and phosphorus amendment on seedlings yield. *Soil Biol. Biochem.* **6:** 173–177.

Lapeyrie, F. and Chilvers, G.A. 1985. An endomycorrhiza-ectomycorrhiza succession associated with enhanced growth of *Eucalyptus dumosa* seedlings planted in calcareous soil. *New Phytol.* **100:** 93–104.

Last, F.T, Mason, P.A, and Wilson, J. 1984. Controlled inoculation of Sitka spruce with sheathing (ecto) mycorrhizal fungi. A commercial experience in 1982. *Scottish Forestry* **38:** 75–77.

Le Tacon, F. and Bouchard, D. 1986. Effects of different ectomycorrhizal fungi on growth of larch, Douglas fir, Scots pine and Norway spruce seedlings in fumigated nursery soil. *Acta Oecologia Applicata* **7:** 389–402.

Le Tacon, F. and Garbaye, J. 1986. La maîtrise des associations mycorhiziennes en pépinière forestière. *Rev. Forest. Fr.* **38:** 249–257.

Le Tacon, F., Lamoure, D., Guimberteau, J., and Fiket, C. 1984. Les symbiotes mycorhiziens de l'èpicea commun et du Douglas dans le Limousin. *Rev. Forest. Fr.* **4:** 325–338.

Le Tacon, F., Jung, G., Mugnier, J., Michelot, P., and Mauperin, C. 1985. Efficiency in a forest nursery of an ectomycorrhizal fungus inoculum produced in a fermentor and entrapped in polymeric gels. *Can. J. Bot.* **63:** 1664–1668.

Levisohn, I. 1956. Growth stimulation of forest tree seedlings by the activity of free living mycorrhizal mycelia. *Forestry* **29:** 53–59.

Levy, Y. and Krikun, J. 1980. Effect of vesicular-arbuscular mycorrhiza on *Citrus jambhiri* water relations. *New Phytol.* **85:** 25–31.

Malajczuk, N., Linderman, R.G., Kough, J., and Trappe, J.M. 1981. Presence of vesicular-arbuscular mycorrhizae in *Eucalyptus* ssp. and *Acacia* sp. and their absence in *Banksia* sp. after inoculation with *Glomus fasciculatus*. *New Phytol.* **87:** 567–572.

Malajczuk, N., Molina, R., and Trappe, J.M. 1982. Ectomycorrhiza formation in *Eucalyptus*. I. Pure culture synthesis, host specificity and mycorrhizal compatibility with *Pinus radiata*. *New Phytol.* **91:** 467–482.

Malençon, G. 1938. Les truffes européennes. *Rev. Mycol.* **3:** 1–92.

Marx, D.H. 1972. Ectomycorrhizae as biological deterrants to pathogenic root infection. *Ann. Rev. Phytopathol.* **10:** 426–434.

Marx, D.H. 1977. Tree host range and world distribution of the ectomycorrhizal fungus *Pisolithus tinctorius*. *Can. J. Microbiol.* **23:** 217–223.

Marx, D.H. 1980. Ectomycorrhizal fungus inoculations: a tool for improving forestation practices. In: *Tropical Mycorrhiza Research.* P. Mikola, ed. Clarendon Press, Oxford, pp. 13–71.

Marx, D.H. and Bryan, W.C. 1975. Growth and ectomycorrhizal development of Loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius. Forest Sci.* **22:** 245–254.

Marx, D.H., Cordell, C.E., Kenney, D.S., Mexal, J.G, Artman, J.D., Riffle, J.W., and Molina, R.J. 1984. Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorrhizae on bare root tree seedlings. *Forest Sci. Monogr.* **25**, 101 pp.

Marx, D.H., Hedin, A., and Toe IV S.F.P. 1985. Field performance of *Pinus caribaea* var. *hondurensis* seedlings with specific ectomycorrhizae and fertilizer after three years on a savanna site in Liberia. *Forest Ecology and Management* **13:** 1–25.

Marx, D.H., Ruehle, J.L., Kenney, D.S., Cordell, C.E., Riffle, J.W., Molina, R.J., Pawuk, W.H., Navratil, S., Tinus, R.W., Goodwin, O.C. 1982. Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorrhizae on container grown tree-seedlings. *Forest Sci.* **28:** 373–400.

Maupérin, Ch., Mortier, F., Garbaye, J., Le Tacon, F., and Carr, G. 1987. Viability of an ectomycorrhizal inoculum produced in a liquid medium and entrapped in calcium alginate gels. *Can. J. Bot.* (in press).

Melin, E. 1936. Methoden der experimentellen Untersuchung mykotropher Pflanzen. In: *Handbuch der biologischen Arbeitsmethoden.* Vol. XI, Part 4. E. Abderhalden, ed. Urban and Schwarzenberg, Berlin, pp. 1105–1108.

Menge, J.A., Lembright, H., Johnson, E.L.V. 1977. Utilization of mycorrhizal fungi in *Citrus* nurseries. *Proc. Int. Soc. Citriculture* **1:** 129–132.

Menge, J.A. and Johnson, E.L.V. 1978. Commercial production of mycorrhizal inoculum may benefit citrus growers. *Citrograph* **63:** 139–143.

Menge, J.A. 1984. V.A. inoculum production. In: *V.A. Mycorrhiza.* C.L. Powell and D.J. Bagyaraj, eds. CRC Press, Boca Raton, FL., pp. 187-200

Mikola, P. 1970. Mycorrhizal inoculation in afforestation. *Int. Rev. For. Res.* **3:** 123–196.

Mikola, P. 1973. Application of mycorrhizal symbiosis in forestry practise. In: *Ectomycorrhizae.* G.C. Marks and T.T. Kozlowski, eds. Academic Press, New York, pp. 383–412.

Mishustin, E.N. 1955. Mycotrophy in trees and its value in silviculture. In: *Micotrophy in Plants.* A.A. Imshenetskii, ed. Academie of Sciences of the USSR, Inst. Microbiol. Moscow, pp. 17–34 (English edition 1967, Israel Program Scientific Translations, Jerusalem for USDC, Springfield, VA).

Molina, R. 1982. Use of the ectomycorrhizal fungus *Laccaria laccata* in forestry. I. Consistency between isolates in effective colonization of containerized conifer seedlings. *Can. J. For. Res.* **12:** 469–473.

Momoh, Z.O. and Gbadegesin, R.A. 1980. Field performance of *Pisolithus tinctorius* as a mycorrhizal fungus of pines in Nigeria. In: *Tropical Mycorrhiza Research.* P. Mikola, ed. Clarendon Press, Oxford, pp. 72–79.

Moser, M. 1958a. Die Künstliche Mykorrhizaimpfung von Forstpflanzen. I. Erfahrungen bei der Reinkultur von Mykorrhizapilzen. *Forstw. Cbl.* **77:** 32–40.

Moser, M. 1958b. Die Künstliche Mykorrhizaimpfung an Forstpflanzen. II. Die Torfstreukultur von Mykorrhizapilzen. *Forstw. Cbl.* **77:** 273–278.

Moser, M. 1959. Die Künstliche Mykorrhizaimpfung an Forstpflanzen. III. Die Impfmethodik im Forstgarten. *Forstw. Cbl.* **78:** 139–202.

Mosse, B., Stribely, D.P., Le Tacon, F. Ecology of mycorrhizae and mycorrhizal fungi. *Advances in Microbial Ecology.* Vol. 5. M. Alexander, ed. Plenum Press, New York, pp. 137–210.

Palenzona, M. 1969. Sintesi micorrizica tra *Tuber aestivum* Vitt, *Tuber brumale* Vitt, *Tuber melanosporum* Vitt; e semenzali di *Corylus avellana* L. *Allionia* **15:** 121–132.

Pryor, L.D. 1956. Chlorosis and lack of vigour in seedlings of Renantherous species of *Eucalyptus* caused by lack of mycorrhiza. *Proc. Linn. Soc. N.S.W.* **81:** 91–96.

Pryor, L.D. and Johnson, L.A.S. 1971. *A Classification of the Eucalypts.* Aust. Nat. Univ. Press, Canberra.

Reddell, P., Bowen, G.D., and Robson, A.D. 1986. Nodulation of Casuarinaceae in relation to host species and soil properties. *Austr. J. Bot.* **34:** 435–444.

Redhead, J.F. 1980. Mycorrhiza in natural tropical forests. In: *Tropical Mycorrhiza Research.* P. Mikola, ed. Clarendon Press, Oxford, pp. 127–142.

Shemakhanova, N.M. 1962. Mycotrophy of woody plants. In: *Academy of Sciences of the USSR Institute of Microbiology.* Translated by Israel Program for Scientific Translations, Jerusalem, 1967.

Singh, S. and Kumar, A. 1966. Field survey on mycorrhiza in eucalypts and pines. *Indian Forester* **92:** 517–520.

Takacs, E.A. 1961. Inoculación artificial de pinos de regiones subtropicales con hongros formadores de micorriza. I. Formación de micorrizas ectotroficas en *Pinus taeda* L. con *Scleroderma vulgare* Horn. *Idia, supplemento Forestal* **12:** 41–44.

Theodorou, C. 1971. Introduction of mycorrhizal fungi into soil by spore inoculation of seed. *Aust. For.* **35:** 23–26.

Theodorou, C. and Bowen, G.D. 1973. Inoculation of seeds and soil with basidiospores of mycorrhizal fungi. *Soil Biol. Biochem.* **5:** 765–771.

Thomas, G.W. and Jackson, R.M. 1983. Growth responses of Sitka spruce seedlings to mycorrhizal inoculation. *New Phytol.* **95:** 223–229.

Trappe, J.M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. *Ann. Rev. Phytopathol.* **15:** 203–222.

Turnbull, J.W. and Pryor, L.D. 1978. Choice of species and seed sources. In: *Eucalypts for Wood Production.* W.E. Hillis and A.G. Brown, eds. CSIRO, Camberra.

Uhlig, S. 1968. A contribution to the mycorrhiza problem of eucalypts. *Zlb. Bakteriol. Parasitenk. Infektionkr. Hyg.*, (Abt 2), **122:** 271–274.

Vlasov, A.A. 1952. Methods of estimating the amount of mycorrhizae in seedlings. Tr. Kompleksn Nauch Exsped Po Voprosam Polezahchilnyhk. *Cesorajvedenii* **11:** 231–239.

Vysotskii, G.N. Mycorrhiza of oak and pine seedlings. *Lesoprom vestn.* **29:** 504.