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**MYCORRHIZAS**  
*An Evaluation based  
on Australian forestry*

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MYCORRHIZAS --

An evaluation based on Australian forestry

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SUMMARY:

The background of mycorrhiza in relation to silviculture is reviewed, particularly as this concerns Australian conifer plantations.

Current theories concerning mycorrhizal functions are discussed very briefly and the wider implications of mycotrophy, that is, whether introduction of suitable mycorrhizas can be controlled in nursery beds or if indeed any purpose would be served by controlling mycorrhizas, are also discussed.

The desirability of new techniques in nursery practice is stressed.

## Introduction

Ever since the observations of Kessel (12) in the mid-twenties of this century, Australian foresters have accepted that exotic pines have to be inoculated in the nursery with suitable mycorrhizas.

The usual and current practice is to carry leaf litter or 'duff' from established pine plantations and to plough this into the nursery beds before sowing or spread it around the young seedlings soon after they have emerged from the ground.

Success of the operation is judged from the appearance of the characteristic clusters of coralloid outgrowths on the roots of the young seedlings, failure by the lack of such outgrowths and/or the stunted or yellowish appearance of seedlings.

It is freely admitted that robust seedlings may sometimes not show the presence of typical mycorrhizas and, vice versa, that stunted seedlings may sometimes be strongly mycorrhizal. However these events are regarded as exceptional and not important enough to discard the general concept of the need for mycorrhizas in pines.

It is not always convenient to carry litter to nurseries and in any case it can be a costly operation, so means of improving the inoculation process are sought.

Before instigating such research however it might be desirable to reconsider the evidence available from work already done and, in particular, the currently held views of mycologists about the function and value (if any) of mycorrhizas in plants generally.

Literature on the subject is copious and it is easy to be lead astray by local enthusiasm.

## Present position

Perhaps the most surprising thing about the reported work on mycorrhiza is that the lines of controversy drawn by Frank and Hartig in 1885, with relatively minor modifications, still persist today.

Ainsworth and Bisby (1) define Mycorrhiza as "a Symbiotic, nonpathogenic or feebly pathogenic association of fungi and the roots of plants". They then go on to say that the "old and widespread interpretation of mycorrhiza is that the association is a symbiotic one, of use to the two organisms but today the view is taken (quoting Burges) that the association is generally an example of limited parasitic attack having no mutualistic value". This statement is then qualified by reference to Harley (6), Melin (16), Kelley (11) and Bjorkman, all of whom have argued for the mutualistic concept. These are the bare bones, but of course the problem goes much deeper.

When Hatch (1936) wrote about the failures of pine in new areas like Western Australia, Rhodesia, the Philippines and Hawaii and went on to indicate that these failures were overcome by introducing innocula one way or another from established forests, he rightly anticipated that this would excite considerable enthusiasm. In fact he understated

the position. Many foresters were convinced that the solution to growing pines in new areas was primarily a matter of the selection of suitable mycorrhizas.

Twenty years later Harley (6) observes that the question 'Is mycorrhizal infection beneficial to the hosts that possess it?' is often asked, but it is a meaningless question. This situation arises because the term 'mycorrhiza' is difficult to define and he (Harley) tries to define what he understands by mycorrhiza later in his book.

It is only fair, of course, to point out that a lot of water has flowed under the bridge since Hatch published his early papers and we now know a lot more about mycorrhizas than we did then. Radioactive tracer work has demonstrated that carbon assimilated by the higher plant appears later in the fungus associate as organic metabolite and similarly phosphorus is absorbed by mycorrhizas from the soil to appear later in the higher plant.

The assimilation of nutrients by mycorrhizal hyphae and their storage by the fungus until they are required by the higher symbiont points clearly to the value of mycorrhizas in certain circumstances and is of course justification for the term 'symbiont'. On the other hand there is evidence to suggest that even ectotrophic mycorrhizas are not steady structures, at least in the sense that obtains for root nodules of legumes and other plants. Furthermore mycorrhizas appear to be strongly influenced by environmental conditions.

Harley (6) deals with this aspect in some detail, pointing out that aeration, imbalance of nitrogen, calcium, phosphorus and potassium, and particularly the vigour of photosynthesis of the higher partner, are important factors affecting the development of mycorrhizas on roots.

The last factor has been further emphasised by Bjorkman who has stressed the importance of surplus carbohydrate in the root zone where the mycorrhiza works. Marks & Foster (1966) have produced supporting evidence in Pinus radiata mycorrhizas. This point has been checked by growing mycorrhizal fungi like Boletus in synthetic media.

A well authenticated example of the effect of soil conditions on mycorrhizal development, familiar to Australian foresters, is the Riverhead Pine plantation just outside Auckland, New Zealand. An extensive soil fertilisation programme was required before the physical condition recovered sufficiently to permit mycorrhizas to grow. It was not sufficient to inoculate the area with litter.

Although mycorrhizas spread from originally infected mother roots to other short roots either through the cortical cells of the plant or through the soil, the highest concentration of mycorrhizas remain in the upper soil horizons. This is because of the oxygen requirements of the root/fungus association and presumably is also an indication of accelerated chemical biosynthesis and energy release.

It is known that mycorrhizal short roots live longer than their uninfected counterparts so that fungus investment appears superficially at least to increase short root production. This observation is important in relation to Zaks (25) contention that mycorrhizas protect roots from attacks by root pathogens and will be referred to later.

Not only, then, are mycorrhizas dependent on environment but the actual root structure, and hence its liability to infection by mycorrhizal fungi, also varies in relation to mineral availability. This can be seen in pot culture work aimed at correlating distinctive foliage patterns with particular element deficiencies.

Nitrogen deficiency in water cultures of Pinus radiata results in longer than normal roots with sparse short roots (23); the same obtains for phosphorus deficiency.

Hatch (8) describes the roots of Pinus seedlings growing in sand culture under conditions of high nitrogen availability and those also are lacking in short roots, but the so-called long roots are less extensive than in the case where nitrogen is deficient. In effect this means that long roots (as opposed to short roots) are quite effective in mineral absorption providing the minerals are available and in a balanced relation or in silvicultural parlance the root/shoot ratio varies inversely with soil fertility.

Hatch explains the failure of non-mycorrhizal short roots to grow as being due in part to the invasion by 'casual soil organisms' (quoting Melin 1925). He calls such fungi 'Pseudo Mycorrhizas', as did Rayner (20). Marks (14) working with Pinus lambertiana seedlings concluded that pseudo mycorrhizas are favoured by poor soil drainage and the final result is destruction of the conducting tissue of roots.

Although Hatch does not say so, he implies that should soil conditions alter suddenly from luxury to poverty mineral supply a morphogenic stimulus is provided which results in short roots appearing.

Both Melin and Slankis (17) have hypothesised along these lines the one postulating an M. factor which stimulates the mycorrhizal fungus and the other the liberation of auxins by the fungus which causes dichotomous branching in rootlets.

Apart from the effect of nutrition on the morphology of the roots themselves Hatch also showed very convincingly how deficiency of a particular element affected mycorrhizal development, nitrogen having little obvious influence but calcium, potassium and particularly phosphorus deficiency being particularly encouraging to mycorrhiza formation.

Many workers in this field have since attested to the accuracy of these observations. It should be noted that these experiments are usually carried out with a luxury medium, less the particular element being tested. They do not therefore always correspond very closely with naturally poor soil conditions.

Finally in this context, Zak and Marx (24) have shown that the mycorrhizas on any given rootlet of pine may consist of several morphologically distinct types or even species if fungi. All these fungi are capable, presumably, of forming the mantle of hyphal threads and interstitial network characteristic of mycorrhizas, without mutual interference of function. Therefore, even if one regards the structure itself as a stable one (Marks 1966), the component fungi can vary from season to season.

## The wider implications of mycotrophy

Most of the practical applications of the studies on mycotrophy centre on forestry. In particular they concern the Pinaceae and Myrtaceae because species of *Pinus* and *Eucalyptus* are most involved in afforestation in various parts of the world.

These new areas may be locations which have never grown trees in living memory, for example grasslands, waste-lands or reclaimed swamps, or on the other hand they may be places which for reasons of economics or soil utilisation a low grade vegetation is to be replaced by pure stands of a suitable forest species. In either case it is reasonable to assume the introduced plant community will meet, initially at any rate, soil conditions which fall short of optimum for the species. In either case it is reasonable to assume the introduced plant community will meet, initially at any rate, soil conditions which fall short of optimum for the species.

This confrontation usually takes place in a nursery which is reasonably close to the area for reforestation. Investment of most short roots of the germinated seedlings with fungal hyphal usually place within the first twelve months and at this stage the forester is concerned to know the status of the root infection (if any) which has occurred because he considers that this could determine the success or otherwise of the field plantings. (10).

There are three major possibilities in the nursery:-

- (a) the roots are uninfected with mycorrhizas, which usually (but not always) means a lack of development of short roots.
- (b) short roots are plentiful and showing initial investment with mycorrhizal hyphae.
- (c) short roots are devoid of mantle but are infected with pseudomycorrhizas.

The identifying Coralloid mycorrhizas of the Pinaceae do not develop till somewhat later under normal conditions and they do not develop at all in eucalypts (3).

Attempts to discriminate between mycorrhizas using morphological features have been widely used (Melin, Dominik), but such distinctions lack utility unless they can be linked to particular fungal species and this is very difficult to do.

Careful excavation of sporocarps and tracing rhizomorphs through the soil profiles may indicate the general locus from which the initial fertile hyphae have developed but it does not prove that the mycorrhiza is causally related to the fungus in question.

This came down then to the question of definition of mycorrhiza which was mentioned earlier.

Obviously the term 'mycorrhiza' has been too widely used in the past, covering as it does a whole range of conditions from root parasitism through to a kind of tolerated symbiosis.

Analogies in the Ericaceae and Orchidaceae, Angiosperms and Gymnosperms, and so on have lead to wide discussions and comparisons of what should be referred to as the 'mycorrhizal condition' rather than 'mycorrhiza as such'. As Kelley (11) says, the emphasis that has been placed on mycorrhizal fungi would seem to be somewhat exaggerated because in so many cases the identity of the fungus seems a relatively inconsequential thing.

In Australian pine forests conspicuous of Boletus have lead to the assumption that Boletus spp. are the principal mycorrhiza-formers in Australian grown Pinus. Closer examination shows that Gasteromycetes and Hymenomycetes are well represented in these same forests and on the basis of studies made there is no reason to believe that representatives of these classes of fungi do not form ectotrophic mycorrhizas with the same facility and in the same proportion as do Boletus spp.

Attempts made in this laboratory to isolate mycorrhizal fungi from short roots (after Zak and Marx and Bryan) have not been very successful but these techniques are being persisted with because of their obvious attractiveness.

Whether a correlation based on the appearance of mycorrhizas on roots is more valid than one based on the appearance of fruiting structures on the forest floor is open to question.

Theoretically it should not make much difference which scheme is used, having in mind the object of such exercises which is to encourage 'good' mycorrhizas and discourage 'bad' mycorrhizas.

The use of the terms 'good' and 'bad' is not justified and it would be better to use the term mycorrhiza (meaning ectotrophic mycorrhiza) as representing a normal root/fungus condition and the term 'haustorial structure' for these other mycorrhizal conditions (endotrophic and pseudo mycorrhizas) which are different although not necessarily disadvantageous to either fungus or host plant. Lists of fungi (based on sporophore identification) which are deemed to be satisfactory associates with certain host species have been very laboriously put together but they must be regarded as being essentially of local significance and not necessarily applicable in other countries; they are also somewhat deficient in themselves because they do not include mycorrhiza-formers like Cenococcum graniforme (Mycelium radialis nigrostrigosum) which do not, as far as is known, form fruiting structures.

Nevertheless, for useful research it is essential that the identity of the fungal partners in a mycorrhiza be distinguishable in some way and this is the principal value of this taxonomical work and probably its only justification.

In the light of present knowledge a wide range of fungi such as Hygrophorus, Amanita, Russula, Boletus, Rhizopogon, Clitocybe, Tricholoma, Scleroderma, Cenococcum and so on (see Trappe 1962) are capable of forming ectotrophic mycorrhizas with coniferous and other host plants and there is nothing to indicate at this stage that any particular true mycorrhiza-former is superior to another. In fact a given root may support many different fungi, as mentioned above.

The position of pseudomycorrhizas and endotrophic relationships is different. Here there is obviously some degree of parasitism which varies in seriousness, from the point of view of the host plant, with the different fungi concerned. Fungi like Pythium, Phytophthora, Rhizoctonia, Thelleshora and Fusarium will penetrate unuberised roots with or without the aid of nematodes and on reaching the stele destroy the roots and cause damping-off in the above ground parts. This is straight-out root parasitism which varies in severity with environmental conditions and the age of the plant.

On the other hand Rhizoctonia along with Mycelium radialis atrovirens and probably other fungi which form pseudomycorrhizas (Levisohn, Rayner) may affect seedlings not at all or only slightly and this condition is very common in nursery beds particularly in the absence of suitable ectotrophic forms.

In practice it is not easy to be sure of the identity of pseudomycorrhizas. Kelley describes them as thinner and darker in colour than normal mycorrhizas; they are simple and monopodially branched but a microscopic section is needed for confirmation.

It seems to be axiomatic that if an ectotrophic mycorrhiza is not formed then a pseudomycorrhizal infection will occur, and in fact Levisohn asserts (13) that semi or partial sterilisation of a nursery soil will result in a definite balance in favour of pseudo-mycorrhizas. She was not able to show that pseudomycorrhizas following partial sterilisation actually harmed the seedlings so infected.

In the case of endotrophic mycorrhizas there is a progression towards parasitism in the sense that there are more haustorial structures penetrating cortical cells of the host plant. Host reaction is measured by the thickening of the radial and inner tangential walls of the endodermis and this reaction effectively limits the penetration of the endophyte. Mycophagy or digestion of the fungal hyphae and haustoria is frequent with endotrophic mycorrhizas but less common with ectotrophic forms and this phenomenon is looked upon generally as a defence reaction on the part of the host, apparently engendered in some subtle fashion by a balance of enzymes - the walls of the fungal elements being chitinous in contradistinction to the cellulose walls of the host plants.

It is becoming increasingly obvious to those who are working with Pinus spp. in the warmer parts of the world that Phycomycetes play an important role in early pine establishment and this leads to a closer consideration of that group of organism known as Rhizophagus (Harley) which seem to enjoy an enigmatic position somewhat intermediate between mycorrhizas and root pathogens.

Pythium and Endogone have been reported as endophytes in agricultural crop plants and Pythium and Phytophthora are very common in pine nurseries and plantations in Australia and New Zealand. Phytophthora cinnamomi Rands is often associated with late damping-off in Pinus radiata nurseries usually in association with nematodes.

Zak (25) has developed this theme and suggested that ectotrophic mycorrhizas may protect unuberised root tissue either by secreting antibiotics, using up residual food material, encouraging protective rhizosphere organisms

or simply providing a physical barrier to infection by parasitic fungi. He instances the case of a Boletus sp. which suppressed Phytophthora cinnamomi growing on agar.

This is a useful viewpoint and ties in with Australian experience where poor mycorrhizal development and Phytophthora damage often appear to go together.

Attempts to establish this reciprocity on a quantitative basis with laboratory and field experiments have not been particularly successful mainly because it is hard to devise a technique which will give consistent results and is at the same time beyond the criticism that it is too artificial.

The possibility that biotypes of Phytophthora cinnamomi exist with different degrees of pathogenicity has been put forward but there is also the concept that Phytophthora may exist like the Rhizophagus complex, taking on a pathogenic character in response to external stimuli like waterlogging but otherwise existing as a tolerated endophyte within the host root.

Indeed Newhook (19) has already claimed something very similar to this in respect to P. radiata in a liquid culture experiment. Here the Phytophthora was confined to the cortical cells causing retardation but not death of the roots concerned.

#### Prospective work in silviculture

In terms of practical silviculture the presence of ectotrophic mycorrhiza is no longer disputed as constituting a healthy sign or good prognosis irrespective of the actual appearance of the seedling itself. What is in dispute really is the mechanics of the beneficial influence or in other words how the mycorrhizas do in fact further the survival of seedlings.

Marks (unpublished) has devised a technique for counting mycorrhizal fragments in the soil and hopes to relate counts with the site quality for growing pine. This may seem a little tendentious since ectotrophic mycorrhizas usually develop in response to a soil deficiency, particularly calcium or phosphorus (Hatch), and therefore what is being measured is the original mineral status of the soil which could be measured more simply in other ways. On the other hand if it can also be shown that a particular mycorrhizal pattern can be associated with some feature other than mineral deficiency, for example a adverse soil physical condition, then a new light will have been thrown on our thinking. It is certainly true, as Marks points out, that Australian foresters have an unusual opportunity to study the ecological patterns of mycorrhizas in pines which is denied workers in the countries of the northern hemisphere. By the same token, biological break-down of organic forest litter and the feedback to the living trees through soil organisms, including mycorrhizas, is likely to be significantly different in certain particulars under Australian conditions and these phenomena have to be explored here in this country. The early successes of people like Kessel and Hatch with soil inoculation in pine nurseries have tended to oversimplify the problem of mycorrhizal establishment. Jorgensen and Shoulders (10) have shown that despite the fact adequate mycorrhizal fungi can be present

in a nursery soil, nevertheless not all seedlings become infected (at least visibly) before leaving the nursery and they have correlated this condition with subsequent failure of plants to become established successfully.

How much the more could this position occur in Australian pine nurseries where some sort of conditioning has probably got to be made with a species new to the environment. Even if Basidiomycetes capable of forming mycorrhizas on Eucalyptus roots are also capable of infecting pines it is still very likely that auxin balances or predisposing root exudates (Melin) will be at least different if not actively hostile to the establishment of normal ectotrophic mycorrhizas in pines.

Attempts to effect synthesis of pure cultures of Boletus spp. with P. radiata roots in this laboratory using in vitro methods have not been very successful but it is presumed that this is because exposure time is limited with this sort of test. Seeds have been steeped in carboxymethyl cellulose thickened slurries of macerated colonies of Boletus and germinating radicals have been exposed to the fungus in petri-dishes but without noticeable success.

More successful has been the technique of germinating pine seed in polyurethane blocks and packing with pine litter (7). In other words a mixed culture seems to be more likely to succeed than a pure culture.

Success has been claimed in Europe (Moser) for inocula prepared by growing mycorrhizal cultures in peat moss and similar materials, but again our experience has been that although such cultures seem to survive they do not look promising enough, at this stage of development, to take the place of the litter treatment.

Some further work along these lines seems clearly desirable and foresters should take heart from mushroom growers who eventually, after much trial, found a convenient way of preparing concentrated spawn, which has been eminently successful in practice.

The pine nursery problem is to establish what the metabiotic progression is within the variable components of the fungal flora of the soil in a particular area. Certain relations and orders of progressive re-establishment have emerged following such treatments as steam-sterilising and so on but much more needs to be known. In the first instance at least it seems to be a problem of devising suitable techniques for studying the organisms concerned in situ.

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