

## Chapter (non-refereed)

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Wilson, J.; Coutts, M.P.. 1985 Exploiting tree crop-symbiont specificity. In: Cannell, M.G.R.; Jackson, J.E., (eds.) *Attributes of trees as crop plants*. Abbots Ripton, Institute of Terrestrial Ecology, 359-379.

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## EXPLOITING TREE CROP-SYMBIONT SPECIFICITY

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### I. INTRODUCTION

The principal symbiotic associations with tree crops are the ectomycorrhizal and vesicular-arbuscular (VA) mycorrhizal associations, and the nodule-forming associations with *Frankia* and *Rhizobium*.

Interest in the uses of mycorrhizal inoculation began with the introduction of exotic tree species. Many papers document the necessity of making parallel introductions of mycorrhizal fungi (Marx 1980; Mikola 1980) and attention has been brought to the requirements for introducing mycorrhizas when revegetating derelict and disturbed sites (Marx 1975; Marx 1977a). In this

paper, we outline the potential for matching symbiont species/strains to host tree species/provenances, particularly in relation to forestry.

Although Wilde (1944) was of the opinion 'that 99% of all *practising* foresters will not have to lose any sleep over the problem of mycorrhizal inoculation', research into the role of mycorrhizas and the nodule symbioses in the growth of tree crops has gathered impetus. Following the general appreciation that the formation of any symbiosis is better than none, it is now understood that the effectiveness of symbioses can depend upon the host and symbiont genotype, the climate and soil. There appears to be much potential for exploiting this variation, which at present is put to only limited use.

Forestry generally uses poor land unsuited for other crops and it is in those circumstances that trees are most dependent on their symbionts. Trees grown for other purposes tend to be planted on better sites, are more intensively managed, and receive larger applications of fertilizers.

Excessive use of fertilizers and pesticides produces its own economic and pollution problems (Alexander 1973). Nitrogen fertilizer production is costly in terms of fossil energy, and much phosphate fertilizer that is applied may be bound in unavailable forms in the soil (Dommergues 1978). The use of symbionts provides a *biological* means of improving plant nutrition – mycorrhizas by increasing the rate of nutrient absorption from the soil, with selective absorption of some ions, and nodules by fixation of atmospheric nitrogen. Their use may reduce or eliminate the need for fertilizers and also produce benefits such as increased survival, for which fertilizer application is no substitute.

Mycorrhizas, particularly ectomycorrhizas, can improve plant performance in a number of other ways, including improving tolerance to drought, heavy metals, soil toxins and extremes of pH and temperature. They can also provide protection against fine-root pathogens (Meyer 1974; Marx & Krupa 1978; Harley & Smith 1983).

## II. HOST AND MICROSymbiont DISTRIBUTION

Nearly all higher plants are mycorrhizal. According to Wilhelm (1966), plants 'do not, strictly speaking, have roots, they have mycorrhizae'. Worldwide, species forming VA mycorrhizas are far more abundant than those forming ectomycorrhizas. According to Meyer (1973), the latter comprise only 3% of phanerogams. Although ectomycorrhizal associations are much less frequent, the host plants are generally long-lived woody perennials, and many of them are important forest trees. Harley and Smith (1983) listed 133 woody genera containing at least one ectomycorrhizal species, distributed among 30 families. Many of those species are forest dominants of the north and south temperate and subarctic regions. An increasing number of ectomycorrhizal species are now being identified in the tropical and subtropical regions. Their distribution in these regions is mainly in areas where vegetative activity is seasonally restricted by rainfall, or at high altitudes (Singer & Morello 1960). However, this is not always so; some of them, including the economically important Dipterocarpaceae, are more widespread (de Alwis & Abeynayake 1980).

VA mycorrhizas occur on all coniferous families except the Pinaceae, and

on most other gymnosperms. They predominate on tropical hardwoods and occur on some temperate hardwoods including *Fraxinus*, *Liquidambar*, *Platanus* and *Liriodendron*. Other tree crops with VA mycorrhizas include apple, cacao, coffee, citrus, cherry, rubber and oil palm.

Nodule-forming associations occur with fewer hosts, and are found together on root systems with mycorrhizas. Until quite recently, *Rhizobium* was thought to be exclusively associated with legumes, but it has now been found in association with a non-leguminous tree belonging to the genus *Trema* (Trinick 1973) (now reclassified as *Parasponia*) that is widely distributed in the tropics (Akkermans 1978). *Frankia* forms associations mainly with trees of temperate regions (including *Alnus*) and with tropical genera at high altitudes, but also with the more widespread tropical genus *Casuarina* which is prevalent in coastal areas.

Research effort into these various symbioses with trees has been rather patchy. It has focused on ecto- rather than VA mycorrhizas, and the nodule symbioses have been comparatively neglected. This imbalance may be partly due to the difficulties of culturing VA fungi and, until recently, *Frankia*, *in vitro*.

### III. BALANCE OF THE SYMBIOSIS

In the symbiotic relationship, the host plant supplies the microsymbiont with all its carbohydrates, while the mycorrhizas supply the host with phosphate, other soil-derived nutrients, and water, and the nodule symbionts provide nitrogen.

The symbioses impose a significant drain on the host photosynthate, particularly ectomycorrhizas, which produce large fruiting bodies and have considerable quantities of fungal matter in the mantle. In a spruce forest, fruit body production alone could use carbohydrate, equivalent to 10% of potential timber production (Romell 1939), or 160 kg ha<sup>-1</sup> yr<sup>-1</sup> of carbohydrate in a *Pseudotsuga menziesii* forest (Fogel & Hunt 1979) and 450 kg ha<sup>-1</sup> yr<sup>-1</sup> in an *Abies amabilis* forest (Vogt *et al.* 1982) (Harley & Smith 1983). Despite the imposition of this large energy drain, in situations where the nutrient supply is limited or other stresses are imposed, the symbiotic associations are essential for survival.

When selecting 'effective' symbionts, we must seek ones that (a) improve the host's carbon balance and enhance the production of marketable parts, and (b) are able to compete with naturally occurring types – bearing in mind that mycorrhizal symbionts are so widespread in nature.

### IV. SELECTION OF ECTOMYCORRHIZAL FUNGI

The genotype of the host and fungus, and both edaphic and climatic factors, should be considered when exploiting mycorrhizal symbioses (Bowen 1965; Mikola 1973; Marx 1977a; Molina 1977; Trappe 1977). Moreover, it is now becoming clear that there is the factor of the 'mycorrhizal succession' to be

considered, in that, when young trees are grown apart from more mature trees (as occurs in nurseries), only certain of the fungi that are capable of forming mycorrhizas with trees of that species may be able to form mycorrhizas with the juvenile plants (Fleming 1983; Last *et al.* 1983; Mason *et al.* 1983).

Some ectomycorrhizal fungi are rather host-specific, whereas others have a broad host range. *Suillus grevillei*, for instance, occurs only on a few species including *Larix*, while *Pisolithus tinctorius* forms ectomycorrhizas with over 73 species of trees (Marx 1977b). Conversely, some tree species, such as *Alnus*, have only a few ectomycorrhizal associates (Molina & Trappe 1982) while others have many. Although a fungal species may have a broad host range, this does not mean that all isolates are equally effective in forming mycorrhizas with all hosts or in producing beneficial effects.

Lamb and Richards (1970, 1971), working with *Pinus radiata* and *Pinus elliottii* var. *elliottii*, tested a range of inocula isolated from the roots of these two species. They found that, although some isolates would form mycorrhizas with both hosts, the largest growth increments of the hosts occurred with fungi that had originally been isolated from the same host species. Similarly, Reddy and Khan (1972) found that, although *Pinus roxburghii* would form mycorrhizas with soil inoculum taken from beneath that species or beneath *Eucalyptus* or when interplanted with *Pinus patula*, the only significant growth increase occurred with inoculum taken from *P. roxburghii*.

Maronek and Hendrix (1980) found that an isolate of *Pisolithus tinctorius* taken from beneath *Pinus taeda* formed mycorrhizas with most inoculated plants of *Picea abies* and *Tsuga canadensis*, but with only half the plants of *Pinus nigra*. However, the symbiosis increased height growth only in *Picea abies*, although the degree of infection was similar among all successfully inoculated plants. Molina and Chamard (1983), testing an isolate of *Laccaria laccata* with *Pseudotsuga menziesii* and *Pinus ponderosa* at various fertilizer levels, found little effect on plant size although mycorrhizal formation was excellent.

The importance of isolate selection was further demonstrated by the work of Theodorou and Bowen (1970). In glasshouse studies, they inoculated *Pinus radiata* with four different isolates of *Rhizopogon luteolus* and with *Suillus granulatus* and *S. luteus*. All inoculation treatments enhanced growth and, as a group, the *Rhizopogon* isolates were superior to the *Suillus* species. Considerable differences existed among the *Rhizopogon* isolates, the best promoting nearly double the height increment of the worst. Differences between treatments could also be obtained in the field (Bowen *et al.* 1971). Height increments between treatments diverged up to 32 months and then began to converge, apparently as inoculum spread from plot to plot. These data are important, in that they are one of the earliest pieces of evidence for the beneficial effects of mycorrhizal inoculation against an existing mycorrhizal background. However, a similar, repeat experiment failed to show any benefit of inoculation, apparently because there was a large background population of effective mycorrhizal fungi.

Interactions between host genotypes and inoculation treatments were demonstrated by Berry (1982) who tested the growth of *Pinus rigida* and *Pinus taeda* families, and hybrids between these species, on surface-mined coal spoil

in Tennessee. Among plants with naturally occurring mycorrhizas (mostly *Thelephora terrestris*), no single family produced a significantly greater stem volume than any other, whereas inoculation with *Pisolithus tinctorius* stimulated the growth of all families, with some responding significantly more than others to the inoculation treatment.

No account of mycorrhizal exploitation would be complete without reference to the extensive work by Marx and others on *P. tinctorius*. As indicated, this fungus can greatly enhance the growth of host plants. It occurs extensively on mining wastes (Schramm 1966) and appears to be ecologically adapted to poor conditions (Marx 1976) where few other ectomycorrhizal fungi can survive (Marx & Krupa 1978). The exceptional ability of *P. tinctorius* to form mycorrhizas at high temperatures may explain its importance on mine wastes (Marx *et al.* 1970). *Pinus taeda* seedlings inoculated with *P. tinctorius* have survived and grown as well at soil temperatures of 40°C as at 25°C (Marx & Bryan 1971).

Although *P. tinctorius* is undoubtedly very beneficial to trees under certain circumstances, it is not a panacea for reforestation problems. Marx *et al.* (1977a) found that five southern pine species responded differently to it at different outplanting sites. In poor soils, *P. tinctorius* had a competitive advantage over other fungi, whereas at better sites it was out-competed by other fungi. Raffle and Tinus (1982) outplanted pre-inoculated *Pinus ponderosa* and *Pinus sylvestris* on a grassland site. After five years, the only treatment which produced a significant increase in biomass was inoculation with pine duff from a 38-year-old *P. ponderosa* stand. None of the other inoculation treatments, including *P. tinctorius*, enhanced growth. Grossnickle and Reid (1982, 1983) found that neither *P. tinctorius* nor *Cenococcum geophilum* was suited to conditions at a high elevation mine site with a harsh climate and short growing season.

Relatively little is known about other symbionts and their preferences for particular types of site. *Cenococcum geophilum* appears to be more tolerant of drought than other species, although it may not be very beneficial to growth (Mikola 1948; Mexal & Reid 1973; Maronek *et al.* 1981). In culture, it is tolerant of sodium chloride concentrations in excess of those found in most salty soils of dry regions, and so it may prove a suitable symbiont for trees in these regions (Saleh-Rastin 1976). Although *C. geophilum* may not enhance growth, Pigott (1982) has found that, in association with *Tilia cordata*, the fungus could withstand desiccation, and it enabled *T. cordata* to maintain an intact absorptive system through periods of drought which could take up water and ions when conditions became favourable.

There is considerable variation in temperature tolerance between strains of ectomycorrhizal fungi (Trappe 1977). *Paxillus involutus* and *Suillus variegatus* are able to grow at low temperatures, which could be an advantage in some nurseries, allowing mycorrhizal formation in early spring, soon after seed germination (Slankis 1974). An ability to adapt to a changed temperature regime at outplanting is also important.

Measurements of the pH optima for the growth of ectomycorrhizal fungi *in vitro* have shown that best growth is obtained in acid conditions (Hung & Trappe 1983). Differences in the optimum pH are as great within as between

species, and some isolates have narrow optimal pH bands while others have broad bands. An isolate with a broad optimal band would presumably have wider applications in forestry.

The most successful fungus will be one which is competitive and able to adapt to soil and climatic conditions. This is an important consideration in tree inoculation, because a fungus which is well suited to conditions in the nursery may not be well adapted to conditions at the planting site. Molina (1977) has commented that *Thelephora terrestris*, *Laccaria laccata* and *Inocybe lacera*, that are common in *Pseudotsuga menziesii* nurseries in the Pacific Northwest, are aggressive and well suited to the highly fertile, irrigated nursery conditions, but they may not be well suited to many planting sites. Bledsoe *et al.* (1982) inoculated *P. menziesii* with *Hebeloma crustuliniforme* and *L. laccata* (both common species in seedling nurseries); neither was able to compete with a native fungus on outplanting. Both isolates originated from west of the Cascade mountains and may not have been suited to planting conditions east of the Cascades.

Microsymbionts may be selected to protect host plants from pathogens, and some mechanisms have been postulated (Zak 1964). Marx (1969a) grew five mycorrhizal fungi in agar plate culture with a number of root pathogenic fungi and soil bacteria; he found that the mycorrhizal fungi produced antibiotics and that 44% of the root pathogens were inhibited. *Leucopaxillus cerealis* var. *piceina* was particularly effective against *Pythium* sp., *Phytophthora* sp. and *Rhizoctonia* sp. The antibiotic produced by *L. cerealis* was identified as diatretyne nitrile (Marx 1969b). *Laccaria laccata* stimulates *P. menziesii* seedlings to produce phenols, which may protect them from *Fusarium oxysporum* (Sylvia & Sinclair 1983). In pot culture, Hyppel (1968) found that *Boletus bovinus* could protect *Picea abies* seedlings against *Fomes annosus*, even when no mycorrhizas were formed. The mantle and Hartig net of mycorrhizas may present a physical barrier to infection; thus, Marx (1970) found that the roots of *Pinus echinata* seedlings did not become infected by *Phytophthora cinnamomi* in those parts that had formed mycorrhizas with *Thelephora terrestris* or *Pisolithus tinctorius*.

Recently, Brown (1983) demonstrated that ectomycorrhizal infection can ameliorate zinc toxicity in *Betula*, but little research has been done on the role of ectomycorrhizas in heavy metal tolerance, despite the interest in selecting plants which could be used to reclaim mine waste.

## V. SELECTION OF VA MYCORRHIZAL FUNGI

Despite their great importance, comparatively few investigations have been made of the relationships between VA mycorrhizas and tree growth. VA fungal species are much less host-specific than ectomycorrhizal fungi. *Glomus microcarpus*, for instance, can form mycorrhizas with both angiosperms – monocots (*Phleum pratense*) and dicots (*Rubus spectabilis*) – and gymnosperms (eg *Taxus brevifolius*) (Gerdemann & Trappe 1974). Many VA fungal species have a worldwide distribution (Mosse 1973). This wide distribution might suggest that there is less need to inoculate plants with VA fungi than with

ectomycorrhizal fungi, but circumstances can still occur where VA fungi are absent. For instance, if they are eliminated from a soil by fumigation, it can take a long time before they are reintroduced naturally, because, unlike the majority of ectomycorrhizal fungi, VA fungi produce their spores below ground so they cannot be dispersed by wind. The fact that certain types of VA fungi predominate in (and perhaps are adapted to) particular regions (Mosse 1973) and soils (Hayman 1975) may indicate that it would be advantageous to introduce particular types when planting new areas. Species of VA fungi are known to differ in tolerance to pH (Green *et al.* 1976), salt (Hirrel & Gerdemann 1980) and temperature (Schenck & Schroder 1974).

As with ectomycorrhizas, not all VA mycorrhizas are equally effective with all hosts. Marx *et al.* (1971) found that *Endogone mosseae* significantly increased the growth of rough lemon, but not sour orange. Infection occurred with both hosts, but the degree of infection was greater (83%) with lemon than with orange (52%). Some citrus cultivars need to form mycorrhizas more than others in order to grow satisfactorily, depending on the fertilizer regime (Menge *et al.* 1978). Schultz *et al.* (1981) experimented with eight hardwood species (*Acer negundo*, *A. saccharum*, *Fraxinus pennsylvanica*, *Juglans nigra*, *Liquidambar styraciflua*, *Platanus occidentalis* and *Prunus serotina*) using a mixed inoculum of *Glomus mosseae* and *G. etunicatus*. Inoculation increased the height growth of all species except *A. saccharum* and *J. nigra*. Species differences in the degree of infection did not correspond with species differences in growth, although the degree of infection has been found to be important in other instances, such as on *Khaya grandifoliola* in Nigeria (Redhead 1975).

Host species can respond differently to different inoculum species. Bryan and Kormanik (1977) inoculated *Liquidambar styraciflua* seedlings with *Glomus mosseae* or naturally occurring inocula from *Liquidambar* soil. *G. mosseae* increased height growth six-fold (compared with an uninoculated control), but the natural inoculum increased height growth eight-fold and was superior to *G. mosseae* in increasing root growth. This experiment was done at high fertilizer levels, comparable to those used in hardwood nurseries, and demonstrated that seedlings have an obligate physiological requirement for VA fungi. Kormanik *et al.* (1982) inoculated the eight hardwood species tested by Schultz *et al.* (1981), with either *Glomus fasciculatus* (GF), a mix of *G. mosseae* and *G. etunicatus* (GM), or a mixture of several *Glomus* and *Gigaspora* species (GG). All tree species were infected, but this did not increase the height growth of *Juglans nigra* in any of the inoculation treatments, and *Acer saccharum* did not respond to GM, which was generally the least effective treatment. The authors commented that the period during which a root system is infected with VA fungi is probably more important than the percentage infection at a particular time. Early infection, promoting early growth, is probably very important (Kormanik *et al.* 1981, 1982). Other instances of host species differing in their response to different types of VA fungal inoculum have been reported by Kabre *et al.* (1982) using *Acer pseudoplatanus* and Furlan *et al.* (1983) using *Fraxinus americana*.

Work by Kormanik *et al.* (1977) suggested that there can be ecotypic variation within host species in their response to the same VA fungal inoculum, in that families of *Liquidambar styraciflua* from poor upland sites responded more to inoculation with *G. mosseae* than families from fertile lowland sites.



Most work with trees has been done using sterilized soils, so that the effects of inoculation have been judged by comparison with controls that lacked a natural endomycorrhizal flora. However, Plenchette *et al.* (1981) recently demonstrated that the growth of apple seedlings growing in an unsterilized phosphate-deficient soil could be enhanced by inoculating the soil with a VA mycorrhizal fungus, and this effect could not be mimicked by applying P fertilizer.

As with ectomycorrhizal fungi, competition between VA fungi may be important when attempting to establish or maintain particular symbioses (Wilson & Trinick 1983; Daft 1983). Evidence for interactions between VA fungi and other soil organisms is somewhat equivocal (Hayman 1978). However, the indications of increased tolerance to root pathogens and resistance to nematodes suggest that this field might be worth exploring.

Evidence for zinc and cadmium tolerance in a strain of *G. mosseae* with clover (Gildon & Tinker 1983) suggests that VA fungal strains differ in heavy metal tolerance – so trees might be ‘tailored’ with suitable mycorrhizas for particular mine sites.

At present, there is a conspicuous lack of evidence on which to base the rational selection of VA fungi. With the possible exception of the citrus industry, there is little information on which inoculant types might enhance tree growth in different site conditions. More work is needed on the benefits of inoculation against an existing mycorrhizal background, and on the extent to which effects persist after outplanting.

## VI. SELECTION OF *FRANKIA* AND *RHIZOBIUM*

### A. *Frankia*

*Frankia* strains are markedly host-specific and, indeed, this is used as the basis for the subdivision of the genus (Akkermans 1978). Particular strains may infect species other than their ‘own’ even in different genera, but the nodules formed are not always effective in fixing  $N_2$ .

Poor nodulation may result (a) when exotic species are introduced – for instance, *Ceanothus*, *Coriaria* and *Casuarina* do not normally form nodules with *Frankia* in the UK (Bond 1974), or (b) when there is little inoculum in the soil – as may be the case on sites which have not previously supported the host. However, good nodulation of *Alnus glutinosa* has been recorded on a site which had not supported alders for ten years (Akkermans & Houwers 1979).

Unlike mycorrhizas, *Frankia* nodules are long-lived; on *Alnus* they can live for seven to eight years (Akkermans 1978). Their nitrogenase activity per unit dry weight decreases with increase in nodule size. Unlike ecto- and VA mycorrhizal fungi, *Frankia* cannot grow along the root surface to keep pace with an extending root system; each nodule is induced by a separate point inoculation (Quispel 1954), so that few nodules may be produced if there are low levels of inoculum in the soil.

The importance of good nodule formation prior to outplanting on sites with

poor endophyte populations was indicated by Akkermans and Houwers (1979). They grew seedlings of *Alnus cordata*, *A. incana* and *A. glutinosa* in a nursery which had low levels of inoculum, and planted them on a clay polder site which had few nodule-forming *Frankia*. Two years later, many plants were poorly nodulated or lacked nodules, and these plants were significantly shorter than those which were well nodulated.

Evidence for host-genotype  $\times$  *Frankia*-strain interactions is accumulating for some species. Dawson and Gordon (1979), working with ten clonal lines of *A. glutinosa* growing in nutrient solutions, found significant differences in dry weight and N content among clones when they were not nodulated, but no significant differences among them when they were nodulated. Also, a strong correlation existed between nodule dry weight and growth. Hall *et al.* (1979) tested seedlot  $\times$  inoculum effects. Three seedlots of *A. glutinosa* and one of *A. rubra* were inoculated with each of four inocula (three from *A. glutinosa* and one from *A. rubra*). Three weeks after inoculation each seedlot, including *A. rubra*, had formed most nodules with the same *A. glutinosa* inoculum, although when growth was measured 13 weeks later a different *A. glutinosa* inoculum gave the best results. Dawson and Sun (1981) used *Frankia* isolates from *Comptonia peregrina* and *A. crispa* to inoculate clones of *A. glutinosa*, *A. cordata* and *A. incana*. Most of the uninoculated plants died. Among the inoculated plants, nodule numbers and dry weights differed significantly with host clone (*A. glutinosa* clones formed most nodules) and there were no significant clone  $\times$  isolate interactions (in nodule numbers and weights). However, plants inoculated with the *Comptonia* isolate grew more rapidly than the others and measurements of acetylene reduction suggested that the *Comptonia* isolate was more efficient in  $N_2$  fixation. Further evidence for strain differences in *Frankia* was furnished by Hafeez *et al.* (1984), who showed that isolates of the endophyte of *A. nitida* differed 20-fold in their  $N_2$  fixing ability.

### B. *Rhizobium*

The potential for selecting *Rhizobium* strains for tree crops has been little studied, despite their importance on poor tropical sites where woody legumes can provide timber and dry season fodder (Döbereiner & Campelo 1977).

Like *Frankia*, *Rhizobium* shows a considerable amount of host-endophyte strain specificity. Effective nodules are formed only when the appropriate *Rhizobium* species invades a particular host species or a taxonomically related species. With other hosts, no nodules are formed or else the symbioses are ineffective (Akkermans 1978).

Considerable intraspecific variation exists; the yield of different cultivars of white clover depends upon the strain of *Rhizobium trifolii* used (Mytton & Livesey 1983). Investigations with tropical trees suggest that specificity is not confined to agricultural crops. Thirteen species of *Acacia* could be categorized according to their ability to nodulate with fast- or slow-growing strains of *Rhizobium*; only four species formed effective nodules with fast-growing strains, six with slow-growing strains, while three could nodulate with both (Dommergues 1981). Under nursery conditions, differences were found in

the ability of strains to promote growth; fast-growing strains were the most effective, and produced the greatest quantities of nodules. Although fast-growing strains appear to be the most desirable, work with agricultural crops has indicated that slow-growing strains are more resistant to severe desiccation – a potentially important trait (van Rensburg & Strijdom 1980). Early inoculation is advantageous; good nodulation in the nursery seedbed increased the survival of *Mimosa caesalpiniaefolia* after transplanting from 54% to 94%, and increased growth rates by 60% (Dobereiner & Campelo 1977). Agricultural rhizobia differ in their ability to tolerate temperature extremes (Pate 1961; Lie 1971) and some are effective over wider temperature ranges than others (Roughley & Dart 1969). Also, pH tolerance differs between and within *Rhizobium* species (Graham *et al.* 1982).

As with *Frankia*, evidence exists for host-genotype  $\times$  *Rhizobium* endophyte-strain interactions. It appears that considerable benefits in growth may accrue from using appropriate host-endophyte combinations, provided the endophyte is selected for the right temperature, drought and pH conditions. Unlike selections for agricultural crops, rhizobia selections for trees must succeed at two locations – the nursery and the planting site. As with mycorrhizas, inoculation may only occasionally be essential, but it may frequently be beneficial, provided that selected strains can compete with naturally occurring inocula (Parker *et al.* 1977).

## VII. INITIATING AND SUSTAINING THE SYMBIOSES

### A. Features of successful microsymbionts

The tree is a long-lived woody perennial, often with seasonal growth. To be worthwhile, the microsymbiont must remain with the tree for some time.

Seedlings are generally produced in nursery conditions which favour the growth of the host, but may not necessarily favour the growth of the microsymbiont. The seedlings are then transferred to an outplanting site, where conditions are less favourable for growth, and where opportunities for cultivation are restricted. As they mature, the trees undergo a number of physiological changes, and bring about changes in their own environment – canopy development casts shade, alters soil temperatures and intercepts rain, water and nutrients are removed from the soil, leaf litter is deposited, old roots die and decompose and new ones are formed, and the root system extends to explore new ground.

Clearly, the successful microsymbiont must be adaptable if it is to remain with the host after transplanting and as it matures (or at least for a time), and it must survive during the host's dormancy periods when environmental conditions may be at their most stressful. Also, the microsymbiont must spread to colonize the enlarging root system and must be able to compete with naturally occurring populations of endophytes. Furthermore, for commercial use, the microsymbiont should be cheap to produce, and easily handled, transported and stored. Initiation of the symbiosis should not be a complex procedure.

## B. Inoculum production

Traditionally, natural mixed-genotype inocula, present in soil and leaf litter, have been used to initiate symbioses. But if we are to exploit particular strains, ways must be found to produce single-strain inocula of known infectivity.

Techniques have been developed for producing some ectomycorrhizal fungi in bulk liquid culture or vermiculite-peat (Marx & Kenney 1982). VA fungi cannot yet be cultured *in vitro*, but they can be grown on host plants (frequently fibrous-rooted monocotyledonous plants) in glasshouses under clean conditions to produce an inoculum of chopped roots and soil (Menge & Timmer 1982). *Glomus epigaeus* shows great potential for commercial application because it produces sporocarps on the soil surface which can be harvested repeatedly without destroying the pot culture (Daniels & Menge 1981). *Rhizobium* strains may be readily cultured *in vitro* (Date & Roughley 1977) and *in vitro* culture techniques for *Frankia* have been developed quite recently (Callaham *et al.* 1978).

## C. Nursery procedures

Special procedures may be necessary to ensure that good symbioses form in the nursery with known types of microsymbionts. At present, most of the information concerns the formation of ectomycorrhizas, but the principles involved probably apply to the other symbioses.

In nurseries where there is a large natural population of 'wild' types, soil fumigation may be necessary to reduce competition for infection sites on the root systems (Marx *et al.* 1976; Lamb & Richards 1978). If the soil is not fumigated, then placement of the inoculum close to the seedlings to allow rapid infection will be critical (Menge & Timmer 1982). The soil conditions and cultural regimes in nurseries seem to create conditions favouring certain species of microsymbiont, such as the ectomycorrhizal fungus *Thelephora terrestris* (Marx 1980). If the chosen microsymbiont is to dominate on the root systems, inoculation must be done soon after germination, allowing infection of new roots as they develop, and conditions must favour the desired symbiosis over one or more seasons (Ruehle 1980).

The levels of fertilizer applied, and the ways they are applied, often greatly affect ectomycorrhizal formation. If large amounts of fertilizer are applied to the soil, this can decrease the sugar content of short roots and limit mycorrhizal formation (Marx *et al.* 1977; Dixon *et al.* 1981). But if the same amounts of fertilizer are applied in a foliar mist, there may be good growth and mycorrhizal formation. Not all systems may be as sensitive; an isolate of *Laccaria laccata* can form good mycorrhizas at a wide range of fertility levels (Molina & Chamard 1983), and VA fungi appear to be less sensitive than ectomycorrhizal fungi to fertilizer application (Kormanik *et al.* 1977; Schultz *et al.* 1981).

For the grower of ectomycorrhizal plants there may be a conflict between the need to produce both large plants and mycorrhizal plants – high fertilizer rates may produce large but poorly mycorrhizal plants, whereas lower rates often produce smaller but mycorrhizal plants, so new methods of grading

plants for sale may be needed. This conflict does not appear to arise with VA hardwoods because fertilizer application does not prevent good VA mycorrhizal formation (Schultz *et al.* 1981). It may be possible to use cover crops to build up the inoculum of VA fungi on site (Kormanik *et al.* 1980).

For each proposed microsymbiont-host combination, it will be necessary to optimize nursery conditions – time of inoculation, fumigation treatment, fertilizer and watering regimes should all be investigated.

Having established the desired symbiosis, care with plant handling while lifting, storing and outplanting is essential if benefits are to be maintained, because mycorrhizas and nodules will be vulnerable to inadvertent root pruning and desiccation. The use of container-stock reduces this problem, but, provided bare-rooted stock is handled with reasonable care, sufficient mycorrhizas and nodules should remain to provide benefits after outplanting.

#### D. Alternative inoculation techniques

Inoculation at the time of outplanting is a possible alternative to nursery inoculation. Nursery procedures would not have to be changed and there would be no risk of losing the desired symbiosis when the trees are lifted. If the symbiont were applied by dipping the tree roots in an inoculum slurry before planting, the inoculum would be well placed to infect newly emerging roots. However, the symbiont would have to survive in the soil until the trees started to grow, and it would have to compete with wild types at the planting site, and with those that had infected in the nursery which would have their food base already established (from the photosynthesizing plant).

Clearly, inoculation at this time would not improve nursery growth and would be unlikely to improve outplanting survival. However, growth benefits might be obtained on sites where there was a low background of natural inoculum.

### VIII. PROSPECTS FOR MAINTAINING THE DESIRED SYMBIOSIS

#### A. Natural succession, competition and dispersal of symbionts

A natural succession of ectomycorrhizal fungi has been observed on *Betula*; that is, different fungi occur as the trees become older (Last *et al.* 1983; Mason *et al.* 1983). Other observations on nursery and outplanted trees also suggest that the range of ectomycorrhizas associated with forest trees alters considerably as the trees age (Mikola 1961; Chu-Chou & Grace 1981; Riffle & Tinus 1982; Malajczuk *et al.* 1982; Danielson 1984). There is no evidence for succession in other symbioses with trees. Spore counts of VA fungi on crops planted annually for six years after clearing woodland do suggest that some systematic changes can occur with particular host plants, but this is a change with time, not with plant age (Schenck & Kinloch 1980).

Succession and competition among microsymbionts will inevitably diminish

the effect of any chosen inoculum. On sites which have a poor background inoculum (for which it is essential to establish a good symbiosis before outplanting), competition will probably be no problem, but competition will be a problem on sites where there is a high background of compatible microsymbionts. Evidence suggests that high backgrounds of ectomycorrhizal symbionts may occur on clearcut sites – which have been previously wooded – although this will be affected by the method of site clearance. Slash burning reduced mycorrhizal development on one- and two-year-old naturally regenerated *Pseudotsuga menziesii* seedlings, particularly in the surface layers of soil (Wright & Tarrant 1958). Also, the mycorrhizal inoculum for *P. menziesii* and *Thuja plicata* diminished with time after clear-cutting (Schoenberger & Perry 1982).

Malajczuck *et al.* (1982) raised an interesting point with reference to succession in plantations of ectomycorrhizal exotic tree species. They suggested that, in native stands of eucalypts and *Pinus radiata*, a natural succession of mycorrhizal fungi occurred as the stands matured, and that this succession began with fungi with a broad host range and ended with fungi that were host-specific. This observation has implications for plantation forestry, because host-specific fungi may be absent. Whether or not the lack of late-stage host-specific fungi causes problems is open to question, because the functions of the various fungi are not known.

As mentioned above, it is important for the microsymbiont to be able to spread into the new rooting zone after outplanting. Ectomycorrhizal fungi can produce numerous airborne spores and can grow along the root surface and out into the soil, and so should be well able to infect new roots as they emerge. This ability is one on which selection should be based – fungi which form mycelial strands are likely to be best. Marx *et al.* (1976) found that, in nursery conditions, *Pisolithus tinctorius* mycelium could spread 120 cm in one season. VA fungi can also grow along root surfaces and into the soil. Their spores are not airborne, but they can be moved in soil dust or in water; important vectors include rodents, earthworms, ants, wasps and birds (Janos 1983). *Frankia* and *Rhizobium* cannot spread by growing out into the soil; their spread will depend upon vectors carrying nodule fragments. Thus, of the microsymbionts, the ectomycorrhizal fungi are best able to spread and infect root systems as they develop, and *Frankia* and *Rhizobium* are least able. However, *Frankia* and *Rhizobium* can be very long-lived in the soil and the prospect of 'seeding' the site with resistant propagules should not be dismissed.

## B. Persistence of beneficial effects

Few substantial data have been published on how long the beneficial effects of inoculation with mycorrhizas persist after outplanting. Data published by Theodorou and Bowen (1970), Bowen *et al.* (1971) and Riffle and Tinus (1982) suggested that, in *Pinus radiata* and *Pinus ponderosa*, beneficial effects on height growth could persist for at least five years after outplanting and the benefits appeared to be increasing with time (Fig. 1). In *P. radiata*, the differences in heights between inoculated and uninoculated trees could be attributed to a greater height relative growth rate in the inoculated plants

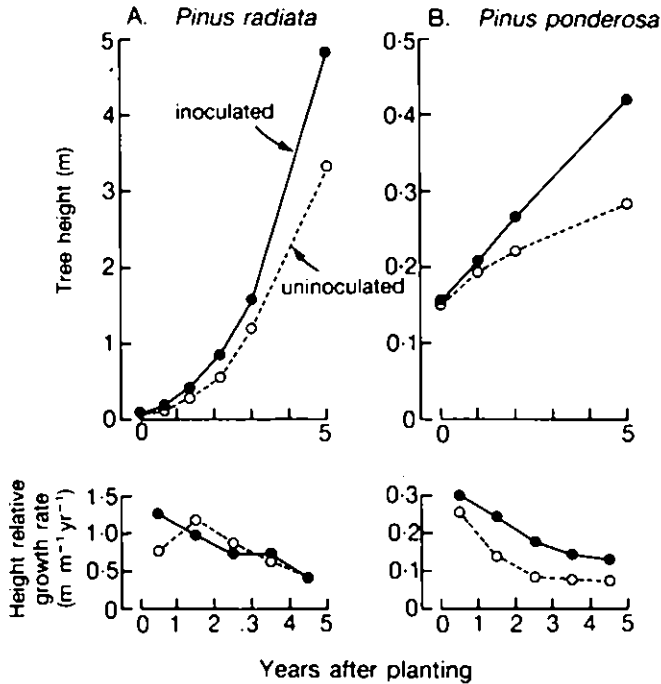


FIGURE 1. Heights, and height relative growth rates, of *Pinus radiata* and *Pinus ponderosa* trees for five years after outplanting, comparing trees that were inoculated with effective mycorrhizal fungi (closed circles, continuous lines) with trees that were not inoculated (open circles, broken lines).

A. The *P. radiata* height data are taken from Theodorou and Bowen (1970) and Bowen *et al.* (1971) who tested four types of inoculum at South Mount Bold, South Australia. Data shown here are for the most effective isolate (*Suillus granulatus*). Height relative growth rates are after Mexal (1980) (with some modification).

B. The *P. ponderosa* height data are from Riffle and Tinus (1982) who tested seven types of inoculum on trees outplanted at a grassland site in North Dakota, USA. Data shown here are for the most effective type (mixed natural inocula from pine duff).

(Because height data were not always collected annually, relative growth rates were estimated from the height graphs.)

only in the year following outplanting, which gave them a long-term height advantage. By contrast, in *P. ponderosa* there was a continuing difference in height relative growth rate between the inoculated and control plants to age five (Fig. 1). However, the *P. ponderosa* trees were growing slowly and they may have been just emerging from 'check' at age five, and so have been equivalent to the *P. radiata* trees at age one to two after planting.

Figure 2 speculates how such differences may continue over the lifetime of a tree crop, comparing the heights of inoculated and uninoculated plants (a) when natural background mycorrhizas are ineffective and not competitive with the introduced inocula, and (b) when uninoculated plants can acquire effective natural mycorrhizas. In both of these illustrations, 'uninoculated'

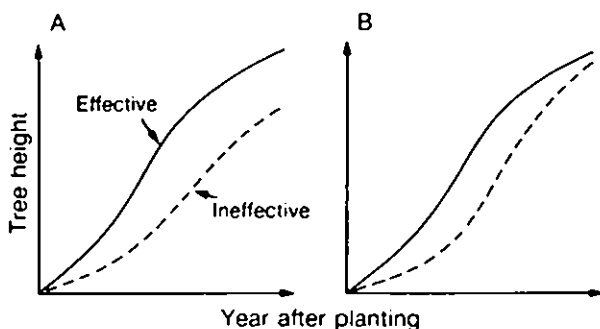


FIGURE 2. Postulated shapes of height/age curves for trees inoculated with effective (continuous line) or ineffective (broken line) mycorrhizas, and outplanted, A. on a site with poor natural, background inocula, and B. on a site with effective natural, background inocula.

is synonymous with 'has mycorrhizas of ineffective, unselected species'. Succession (replacement) with ineffective species would not substantially alter these speculations, because the differences which developed in the first few years after outplanting would persist, although they would represent a small relative difference in height at the end of the rotation. However, in both of these examples, the period during which the sapling is vulnerable to weed competition, frost and browsing damage is decreased, which could be most important.

### IX. PERSPECTIVE: BENEFITS OF SELECTIVE INOCULATION VERSUS GENETIC GAIN

Clearly, inoculation with selected mycorrhizal fungi can give substantial benefits in the nursery and soon after outplanting, but no data are available on which to judge the magnitude of the long-term benefits. We might note that an alternative technique for improving tree growth - genetic improvement of the host - is expected to provide a gain of 10-20% in tree height and stem volume. Except on sites where the benefits of inoculation are 'all or nothing', the long-term benefits of introducing symbioses are unlikely to be greater than this. Also, if genetic improvement is achieved by improving the ability to access nutrients and water, then the benefits of introducing selected mycorrhizas may not be additive with genetic gain. We need to know more about the physiological basis of genetic improvement, and to integrate tree breeding with studies on host responses to mycorrhizal inoculation.

### X. SUMMARY AND DISCUSSION

Mycorrhizal and nodule-forming associations are essential for the growth of tree crops. At present, most research has been done on the ectomycorrhizal



and VA mycorrhizal associations. The data obtained demonstrate considerable short-term benefits in the nursery and after outplanting; long-term benefits are a matter for speculation but may be of the same order as those obtained by genetic improvement of the host. There are strong interactions between host and inoculum genotype, and between environment and genotype, which should be taken into account when initiating trials. Although a particular microsymbiont cannot be expected to be equally beneficial in all circumstances, it is necessary to identify 'broad spectrum' microsymbionts, able to perform well in a range of environments. Just as tree provenances/species are grown on particular types of site, so might microsymbiont species/strains also be used on particular tree provenances/species. The ultimate refinements could be in clonal forestry, and in the use of clonal rootstocks in the fruit tree industry, where precise matching of microsymbiont to host could be achieved.

The discovery that a succession of different ectomycorrhizal fungi infect trees with increasing tree age has helped us to select inocula that are suited to the seedling stage, but the existence of succession implies that their beneficial effects may be short-lived. The longevity of a selected symbiotic association will depend upon a number of factors, including the presence or absence of background infective 'wild' inocula, changes in host physiology and the environment, and the ability of the microsymbiont to spread as the root system enlarges after outplanting.

One future development could be the use of mixtures of selected mycorrhizal fungi, which could maintain a physiologically active root system through a range of environmental conditions over time. Powerful combinations of selected  $N_2$ -fixing and mycorrhizal associations (on one root system) may be possible for trees that are host to both types of microsymbiont. Furthermore, there are some indications that it may be possible to incorporate  $N_2$ -fixing ability into strains of mycorrhizal fungi (Giles & Whitehead 1977). Even without these refinements, symbioses could be exploited much more by identifying and utilizing effective strains. However, we need to assess the costs and benefits of these techniques of tree improvement compared with other methods.

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