Issues concerning the production and use of inocula of ectomycorrhizal fungi in increasing the economic productivity of plantations

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Abstract

Despite the clear evidence from small-scale experiments that ectomycorrhizal fungi can improve the growth rate of host plants, the use of inoculation to exploit this in plantation forestry is not widespread. The factors which tend to limit the use of inoculation as a management option are the lack of data from large-scale productivity trials to support the economic benefit achievable, and the lack of commercial producers of appropriate inocula. An economic analysis of the value of growth improvement in plantation forestry indicates that significant increases in revenue can be obtained. Such analyses can be used to persuade usage of the technology, argue the cost of inocula, and to encourage commercial interest in inoculum production. The linkage between field data and economic benefit dictates that only defined inocula containing known and field-tested fungi will be of significance in the commercial adoption of inoculation technology on a large-scale. Criteria for effective and practical inocula are presented. The relative merits of production of inocula via solid-substrate culture and submerged aerobic culture are considered and the superiority of the latter for higher quality inocula is indicated.

Introduction

There is now a wealth of information on the ecology and biology of ectomycorrhizal associations with plants (see Harley and Smith, 1983; Kropp and Langlois, 1990). Of particular interest to plantation forestry is the potential for improvement in biomass productivity achievable through exploiting the growth stimulating effects of ectomycorrhiza. These effects are real and measurable. It is now known that (i) some ecto-mycorrhizal fungi are better than others in improving the growth of host plants (*e.g.* Dixon *et al.*, 1984; Gagnon *et al.*, 1987; Grove *et al.*, 1991a) , (ii) to maximize benefits of the fungi, plants should be inoculated with particular fungi at the time of seedling production, (iii) there are few technical barriers to the exercise of inoculation as an option in current production practices for seedlings, (iv) unlike vesicular-arbuscular mycorrhizal fungi, ectomycorrhizal fungi are relatively easy to culture artificially.

Two factors determining whether ectomycorrhizal fungi will be used in plantations.

Despite the fact that under many conditions, the benefits of inoculation are clear and the option technically exercisable, use of ectomycorrhizal fungi in plantations is not as widespread as the situation might suggest. That inoculation technology is yet to be routinely applied in wood production shows that the evidence which is currently available is viewed conservatively. Two of the major factors determining whether ectomycorrhizal fungi will be used in, plantations are the economic efficacy of inocula and the availability of practical and efficacious inocula.

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Economic efficacy of inoculation in growth improvement

Management options in forestry are often simply different ways of minimizing costs and maximizing revenue. The efficacy of any new option has to be decided against practices already in place and which may be relatively well understood.

Field relevance. For inoculation to be economically efficacious, it must produce improvements in growth rates. The question which is always asked first of ectomycorrhizal technology is whether the growth improvements readily demonstrated under controlled conditions are reproducible in the field. The answer requires broad-scale productivity trials under various field conditions and over reasonable trial periods. Evidence of the beneficial effects of ectomycorrhiza is easily found in the literature (*e.g.* Ruehle, 1982; Valdes, 1986; Marx and Cordell, 1988). However, not all these results can be used to justify routine inoculation to increase the economic productivity of plantations because the conditions used may not be representative (Jeffries and Dodd, 1991) or of sufficient scale under realistic conditions (Kropp and Langlois, 1990). For example, the choice of nutrient levels may dictate that control plants perform poorly. In many cases, the obligatory requirement for mycobionts such as in pines, means that inoculations often lead to improvements over uninoculated controls. The results from inoculations in rehabilitation forestry can be accentuated by the rehabilitation site no longer possessing an appropriate population of ectomycorrhizal fungi. Lastly, ecological variability of field sites will tend to make extrapolations from trial results difficult.

Inoculation may not be advantageous on some sites because of existing populations of symbiotic fungi and the need to develop a capability to predict the advisability of inoculation on particular sites has been identified (Kropp and Langlois, 1990). Field evidence will be more difficult to obtain on sites where a naturally occurring population of relevant ectomycorrhizal fungi already exists because uninoculated plants may still form a relationship with fungi existing on the site. In these situations, the rationale for inoculation is based on the use of fungi which are demonstrably superior to the fungi found at the site. This has an important consequence for the type of inoculation that can be practiced, *i.e.* specific cultures rather than undefined cultures must be used to obtain efficacy data. Data obtained using undefined inocula such as soil or basidiospores can only support the general proposition that hosts are better off with mycorrhizal fungi than without. Such evidence does not satisfy Koch's postulates in that a relationship between specific microorganisms (by genus, species and strain) and hosts cannot be verified. As discussed later, this has implications for the selection of inoculum form and production method.

In the production of mycorrhizal seedlings, the nutrient and watering regime in nurseries must be appropriately managed if mycorrhiza are to develop (Beckford *et al.,* 1980; Beckford *et al.,* 1984; Bougher *et al.,* 1990). After outplanting, the inoculated plant may lose its inoculant fungus due to succession of other ectomycorrhizal fungi (Fleming, 1985; Gardner and Malajczuk, 1988), and the nutrient status of the soil will influence the effect of the mycorrhiza (Grove *et al,* 1991a; Grove *et al.,* 1991b). Thus, it may not be surprising that quantitative field evidence of the use of inoculations in wood production has been slow to emerge. In the case of eucalypt plantations, there have been few instances of the use of ectomycorrhizal fungi (Grove *et al.,* 1991a) and accordingly, there is little field evidence. However, a small-scale field trial in the Congo showed a volume increase of 30% at 50 months after outplanting of eucalypts inoculated with *Pisolithus tinctorius* (Garbaye *et al.,* 1988). Similarly, in south-western Australia, growth increases of up to 80% were found 7 years after inoculation with *Amanita xanthocephala* (Malajczuk *et al.,* in press). Such positive albeit limited results show the promise of ectomycorrhizal technology. As has been noted by others (Jeffries, 1987; Malajczuk *et al.,* in press), the demonstration of the benefits of ectomycorrhiza under plantation conditions is vital to the adoption of the technology and the current use of inoculation is limited by a lack of positive demonstration. The corresponding low level of demand does not attract commercial production of inocula.

The cost of inoculation technology. If gains in biomass productivity can be shown to be attainable under field conditions, the second question that is asked by the potential end-users is about the cost of inoculation. Plantation economists or managers and indeed even mycorrhizal scientists (*e.g.* Cordell *et al.,* 1987; Jeffries, 1987; Jeffries, 1987; Le Tacon *et al.,* 1988) often pronounce that for inoculation technology to be adopted, it should be cheap. "Cheap" is often defined with reference to the cost

of seedling production. Indeed, an economic justification for inoculation has been made on the basis of improvement in seedling survival and thus reduction in overall costs associated with seedling establishment (Kropp and Langlois, 1990). This way of putting the cost of inoculation technology in perspective, while short-sighted, is understandable because to inoculate is to incur an immediate cost while the potential economic reward is years away. The method of costing which is likely to be most beneficial towards commercialization of inoculation technology is to relate cost to the economic value of increased biomass productivity. Thus, if a biological advantage can be conferred by ectomycorrhizal fungi, then the second question should be about its economic benefit, not the cost of using it.

The economic value of improved growth rate. Since inoculation as a management option will increase operational costs, the advisability of adopting ectomycorrhizal technology will be a measure of cost (inoculation) against benefit (increased productivity). The rationale for incurring the cost of inoculation is that the gain in improved biomass productivity will be greater than the cost of inoculation. The ratio of cost to benefit which will be acceptable, will depend on the overall economics of the plantation operation and the degree of certainty of growth improvement. The potential economic benefit is in the future because the monetary gains in improved biomass productivity are realized at the end of the rotation period of the plantation stand. The economic value of such a deferred return can be estimated using net present value analysis to correct for the time value of money. Representative costs (Australian dollars) for woodchip plantations in south-western Australia (Table 1) were used in an analysis completed in 1989 for Biosynthetica Pty. Ltd. (formerly Interbac Australasia Pty. Ltd.) by the Australian Agricultural Consulting and Management Company Pty. Ltd., both of Perth, Western Australia. The analysis was based on the planting of *Eucalyptus globulus* on 40 hectare, leased, ex-pasture sites. A discount rate of 5% was used to equate all future revenue to present day values. Estimation was made of the monetary effect of the increase in mean annual increment (MAI) of the biomass of plantation trees in the order of 10, 20 and 30% over a base MAI of 22 m^3 ha^{-1} $year^{-1}$. At the base MAI rate, it was assumed that the stands would be harvestable in ten years. In the analysis, the increase in MAI was translated to a shortening of rotation period and the effect on revenue was calculated on that basis. If the growth improvements are attributable to inoculated ectomycorrhizal fungi, then the analysis would in effect be one of the economic value of inoculation. It can also be used to compare the relative merits of the growth stimulating capabilities of specific ectomycorrhizal fungi.

Table 1: Major costings used in an analysis of the economic value of improvement in the growth rate of plantation *E. globulus.*

Item	\$ ha^{-1}
Annual lease payment for land (6% of land value)	72
Site preparation and establishment	668
Additional weeding (Year 2)	31
Additional fertiliser (Year 5)	96
Annual maintenance (Years 1- 5)	38
Annual maintenance (Years 5 - 10)	25

Three selected scenarios from the analysis representing single, coppice and multiple rotation operations are presented in Figures 1- 3. As expected, all scenarios showed an increase in revenue resulting from increment in MAI. The type of plantation operation affects the size of the potential return with single rotations poorest and multiple rotations the best. Coppiced rotations would best reflect the average scenario in plantation operations in Western Australia. With such rotations, an increase in MAI of 30% and a stumpage price of \$20 m^{-3}, would result in an extra \$952 ha^{-1} (net present value) return from the plantation at harvest. Therefore, in Western Australia, should ectomycorrhizal fungi be capable of improving MAI by 30% then significant gains in revenue can be realized. In terms of the commercialization of inoculation technology, the significance of such analyses goes beyond quantitative data to persuade usage of ectomycorrhizal fungi, to a revelation of the price which inocula may command by applying a cost to benefit ratio to the gain in revenue. For example, at an increased return of \$952 ha^{-1}, a reasonable cost to benefit ratio of 1:5 indicates an inoculum cost of \$190.40 ha^{-1}. At a tree density of 1000 ha^{-1}, this is a cost of 19 cents per seedling. The 1:5 ratio also means that with ectomycorrhiza boosting growth by 30%, the plantation can afford to spend 95.2 cents more per seedling for inoculation and be no worse off. Herein lies the potential for inoculum producers to argue the cost of inoculum. Experience with fermentation methods for the production of biomass indicate that the cost to produce a single inoculum dose would be well inside 19 cents. Thus, such analyses can also

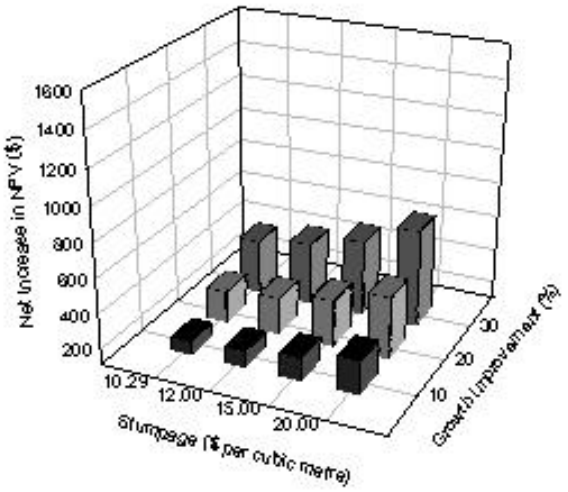

Fig. 1 The economic value of increased growth rates as indicated by the net increase in the net present value (NPV) of revenue at harvest in single-rotation eucalypt plantations in south-western Australia.

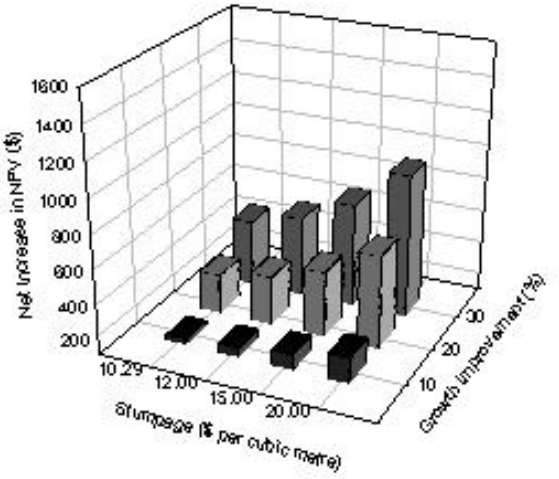

Fig. 2. The economic value of increased growth rates as indicated by the net increase in net present value (NPV) of revenue at harvest in coppiced eucalypt plantations in south-western Australia. The analysis was based on two coppice rotations with yields which were 100% and 90% of the initial rotation. Growth increment was assigned to only the initial rotation.

development funds in inoculation technology for provide an economic rationale for the investment of research and now these may be tallied against potential returns from sales of inocula as estimated from prices which may be commanded. The analyses can also be used by ectomycorrhizal scientists to gauge the potential value of different fungi or to set minimum targets in growth increment in their search for efficacious fungi. Using the same example above, if for various reasons, the price of inoculum had to be a minimum of 19 cents per dose and the end-user requires a cost to benefit ratio of greater than 1:5, then the growth increment which would have to be delivered has to be greater than 30%.

Clearly for such economic analyses to be of any practical significance, firstly, they must be reliable. Given the influence of the environment on the ecology of the mycorrhizal association, the data must either be available for the specific environmental regimes or be of a fundamental nature, capable of intra- and extrapolation. In general, business sense dictates that the higher the risk and cost of an option, the greater is the return on investment which is expected. If field evidence is poor, then the potential economic gain

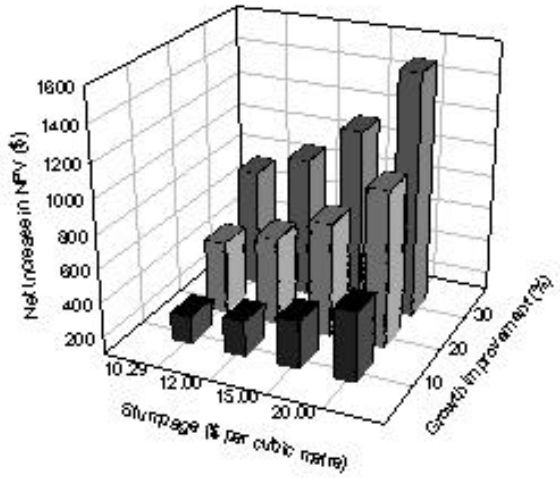

Fig. 3. The economic value of increased growth rates as indicated by the net increase in the net present value (NPV) of revenue at harvest in multiple-rotation eucalypt plantations in south-western Australia.

must be high. This means that the price which may be commanded by inocula will be correspondingly low. This has a negative effect on encouraging the development of inoculation technology. Secondly, the data must be defined specifically by fungus (pure cultures used in trials), host tree, and environment for it is only this way that the results can be duplicated. The value of such data as argument for the use of ectomycorrhizal technology is diminished when the combination cannot be duplicated or knowingly not duplicated. The routine duplication of results obtained in field tests under operational plantation conditions will require that the specific fungi used be available as inoculum before the economic advantage of the ectomycorrhizal fungi can be exploited.

Availability of practical and effective inocula.

Inocula. Ectomycorrhizal fungi sought as inocula will be those which field testing and economic

analysis reveal as desirable. These must then be capable of being supplied as inocula on demand. Inocula whose use are justified on the basis of a verified beneficial relationship between specific fungi and hosts must in the first instance be capable of producing ectomycorrhiza of specific fungi on inoculated plants. This has implications on the type of inocula which are relevant for plantation forestry. The previous arguments make it clear that in economic terms, those inocula which are not strain specific cannot be justified and are therefore not considered here. Unspecific or undefined inocula include soil, forest litter, basidiospores and sporocarps. What then are the types and forms of inocula which are best able to deliver the promise of field trial results and which are practical and effective?

Criteria for practical and effective inocula. The effectiveness of an inoculum is associated with its characteristics as a source of propagules. Practicality is related to the ability to produce it satisfactorily, and to its ease of usage. In general, effectiveness and practicality are inversely related. Therefore, the ideal inoculum is a compromise between these two attributes. Fungal propagules (biomass) may be used directly as inoculum. However, for ease of handling or because of the culture process, inocula often consist of biomass and a carrier or particulate bulking material such as peat-vermiculite or cereal grains. Thus, inocula are available in different forms depending on the production process or other considerations such as the method of inoculation. Effective and practical inocula should satisfy the following criteria (Tommerup *et al.*, 1987): (i) the inoculant microorganism must be efficacious; (ii) it should be produced axenically and have a physiological state which is consistent and appropriate for storage, inoculation and initiation of mycorrhiza; (iii) carrier or bulking material if used, should protect the biomass against physiological stress during production; (iv) the inoculum should be of a form which will allow close control of growth conditions in large-scale production; facilitate handling, and be capable of being provided in repeatable dosages associated with consistent amounts of biomass. Most experimental and commercial inocula currently available are sufficiently below ideal as to pose a potential limitation on the routine practice of inoculation in plantation forestry.

Large-scale production of inocula. Inoculation can only be routinely practiced in plantation forestry if production of inocula can be scaled up to the size required. Fore-shadowing large-scale usage of inocula, and having defined what constitutes effective and practical inocula, the production technology which is required will involve the use of pure isolates in axenic-mass culture under controlled conditions. As most ectomycorrhizal fungi do not form yeast phases or asexual spores in culture, the production of vegetative mycelium will provide the basis for manufacturing inocula of many ectomycorrhizal fungi on a large-scale. The two main techniques for large-scale production are solid-substrate and submerged aerobic culture.

Solid-substrate culture. This form of culture is where the substrate is largely undissolved in an aqueous phase. Such culture may be static such as those within bags and other containers, or stirred such as those within rotary drum reactors. Examples of inocula produced using solid-substrate culture is mycelium grown on a substrate of cereal grain or a mixture of peat and vermiculite (Marx and Kenney, 1982; Cordell *et al.*, 1987; Le Tacon, *et al.*, 1988). The solid-substrate gives the inoculum physical form and no further formulation is usually required.

As there is little water in the system, the chemical environment of a fungus growing in solid-substrate culture consists of the film of water that may be present around substrate particles, and the zone within each substrate particle where diffusion of chemicals can occur. Regulation of the moisture level in the culture determines the amount of free water available but this can be difficult to achieve (Laukevics *et al.*, 1984; Larroche and Gros, 1986). The immediate gaseous environment consists of the inter-particulate space not occupied by water. In static systems, the supply of oxygen and removal of carbon dioxide is determined entirely by gaseous diffusion, the degree of which is in turn determined by the size and continuity of the inter-particulate space. Thus, in solid-substrate culture, the diffusion of gases and nutrients is of overriding importance in determining the rate and amount of growth, and is a serious limitation. This contributes to long incubation periods *e.g.* 2 to 3 months in a 2 L container for fast growers like *Pisolithus tinctorius* and 8 to 10 months for slow growers like *Cenoccocum geophilum* (Marx and Kenney, 1982). The regulation of incubation temperature is another problem in static cultures because it is reliant upon the inefficient heat exchange within and without the culture. These considerations tend to limit the size of culture batches. The mixing of cultures that is achieved in stirred solid-state fermentors reduces the problems of diffusion and

temperature control to some extent. However, mixing is a self-limiting solution because excessive mixing disrupts fungal growth (Silman, 1980; Moo-Young *et al.,* 1983; Larroche and Gros, 1986).

The lack of water in solid-substrate culture makes the measurement of the physicochemical environment such as pH, gaseous and nutrient status difficult. This means that fine control of the environment to optimize growth is not possible. Even if mensuration of the physicochemical parameters is made easier, there remains the problem of control because of the inherent heterogeneity of the culture and the lack of a good medium (water) through which control may be exerted.

The characteristics of solid-substrate culture contribute to heterogeneity in the growth conditions among individual inoculum particles, and within and between production batches. There are difficulties in large-scale production especially in static culture. The process has a large requirement for space in terms of the volume of inocula produced. At a commercial scale, quality and axenicity have been found to be difficult to maintain (Kendrick and Berch, 1985; Le Tacon *et al.,* 1988). The inocula produced often contain undesirable levels of residual nutrients which can adversely affect the success of inoculation (Marx, 1980). On the positive side, any ectomycorrhizal fungus which can be isolated and cultured on agar plates are likely to be culturable via solid-substrate culture. The main advantage of solid-substrate culture is that it is a relatively simple technology requiring relatively low capital investment.

Submerged aerobic culture. In this form of culture, microorganisms are grown within a liquid medium which is stirred and/or sparged with air in a bioreactor. Bioreactors are specialized vessels for the conduct of axenic microbial growth. Mixing of the culture is generally achieved by impellers. Control of pH, oxygen tension, degree of agitation and temperature of the culture broth is readily implemented using sensor probes and correcting actuators *e.g.* automatic addition of acid or base to maintain a set pH. Thus, the main advantages of submerged aerobic culture are the high level of homogeneity which may be achieved within an entire culture, and the ability to precisely control the physicochemical conditions for growth. Such control enables the physiological state of the microorganism to be finely regulated. Incubation periods are shorter than solid-substrate culture and are typically measured in weeks (Boyle *et al.,* 1987; Gagnon *et al.,* 1988) or even days (Le Tacon *et al.,* 1985). Scale-up of production is not a technical problem because large-scale production of fungi has long been practised in the fermentation industry *e.g.* in the production of antibiotics. The superior features of submerged aerobic culture enable the production of inocula which are consistent as propagules, predictable, and reproducible in quality within a batch and among batches. Homogeneity in inoculum quality means that inoculation doses can be minimal as opposed to maximal in order to compensate for ineffective "propagules" when inoculum quality is heterogeneous.

However, submerged aerobic culture may not be suitable for all ectomycorrhizal fungi. Some fungi are presently difficult to grow in submerged aerobic culture (Harvey *et al.,* 1988)and the culture of slow-growers may not be economical. This difficulty is related in part to the requirement to fragment mycelia during culture. In submerged aerobic culture, batch volumes are increased sequentially where the contents of a batch is used as seed culture for the following batch which would typically be 8 to 9 times the volume of the seed culture. Since ectomycorrhizal fungi only form vegetative mycelium in submerged aerobic culture, and since a maximum number of growing points is optimal, the seed culture is fragmented, usually by homogenization prior to use in the subsequent batch. Fragmentation of mycelia is also performed post-culture (see below) to evenly distribute the fungus in a slurry and maximize the number of propagules. But, ectomycorrhizal fungi can differ in their ability to withstand fragmentation (Boyle and Robertson, 1988) and it is most likely that such treatment at least reduces viability. More studies on aseptic fragmentation techniques for large volumes of mycelial suspensions would advance the technology. The increased handling of the cultures and translation of small initial volumes to what may ultimately be very large volumes in fermentation under growth-optimised conditions also means that the axenicity of fungal cultures must be absolute. This is a potential problem area not always apparent when the culture of ectomycorrhizal fungi is restricted only to small scale agar or other solid-substrate culture. Biomass from submerged aerobic culture may be used directly as an inoculum slurry with or without prior fragmentation (Boyle *et al.,* 1987; Gagnon *et al.,* 1988;). It can also be formulated to achieve different forms. For example, fragmented biomass can be mixed with sodium alginate and then gelled onto bare roots (Deacon *et al*., 1988), or formed into beads (Le Tacon *et al,* 1985;

Mauperin *et al.*, 1987), or granulated (Kropacek *et al.*, 1989). The most advanced form of inoculum is that where the biomass is cultured within hydrogel beads (Kuek *et al.*, 1992; Kuek, *et al.*, 1990; Jeffries and Dodd, 1991). Mycelium cultured in beads should be physiologically superior to fragmented mycelia and therefore act more rapidly as a propagule. Unlike entrapped fragmented mycelium, growth within beads results in mycelium being established immediately below the surface of the beads where it can readily grow outwards (Kuek *et al.*, 1992). Mycelium grown within beads is protected at all stages of the production process and inoculation procedure. Bead size can be varied and be as small as 0.5 mm in diameter. Using this technology, several species and isolates of *Laccaria*, *Hebeloma*, *Descolea*, *Elaphomyces* and *Pisolithus* have already been successfully produced as inoculum and other genera are being investigated (Kuek *et al.*, 1992).

Inocula with lower volumes are less costly to produce, store, transport and apply. Those having low volume to biomass ratios are beads and slurries, whereas inocula produced via solid-substrate culture are bulky. Hydrogel bead inocula (Kuek *et al.*, 1992) for 10000 containerized seedlings (ten beads per seed) would occupy only 2 L. It has been estimated (Malajczuk *et al.*, in press) that mycelial-slurry or solid-state cultured inocula (Gagnon *et al.*, 1988; Boyle *et al.*, 1987; Cordell *et al.*, 1987; Le Tacon *et al.*, 1988) are between twenty to one hundred times the volume of hydrogel bead inocula at comparable dosage rates. Thus, in physical form, inocula can be prepared in a volume-efficient manner with submerged aerobic culture.

Submerged aerobic culture is a better approach to large-scale production than solid-substrate culture although the choice of process will often be made on the basis of the price that the inoculum can be sold for and the minimum acceptable level of inoculum quality. Inocula produced via submerged aerobic culture will be costlier than those via solid-substrate culture since the establishment of process equipment for the former is more capital intensive. However, if cost/benefit analysis such as those above are used, there is little doubt that in Western Australia at least if not elsewhere, submerged aerobic culture can be used profitably for the commercial production of inocula.

Storage life of inocula. Apart from maintaining quality, retention of viability during storage is advantageous because large requirements for inoculum can be met by storing the output from smaller batches. This means that production equipment can be smaller in scale, resulting in a lower capital cost for the production process.

The form of an inoculum influences its storage life. Bead inoculum in which mycelia of *Hebeloma westraliensis* and *Laccaria laccata* were grown, retained their ability to form colonies on agar plates after 7 and 5 months storage in water at 4^{o}C respectively (Kuek *et al.*, 1992). Manipulation of the composition of the medium used to suspend the beads is likely to further increase storage life. Fragmented mycelia of *Hebeloma crustiliniforme* entrapped in hydrogel had 90% of initial viability after five months in cold storage (Mauperin *et al.*, 1987). Solid-substrate cultured inocula of *Pisolithus tinctorius* or *Hebeloma crustiliniforme* can be stored for a month at 2 - 4^{o}C (Hung and Molina, 1986; Cordell *et al.*, 1987; Le Tacon *et al.*, 1988). Slurries of mycelium in water have been stored for as long as four months (Boyle *et al.*, 1987). Thus, if stored under refrigeration, inocula should have storage lives in excess of operational requirement (time for warehousing, shipment, and application).

Application of inocula. Row-drill applicators have been used for a solid-substrate cultured inoculum (Cordell *et al.*, 1987). For containerized seedlings, such inocula would be readily applied using the same machines which sow seed. The practicality of hydrogel bead inoculum (Kuek *et al.*, 1992) was demonstrated when 80000 containerised seedlings were inoculated via fluid-drilling in a commercial nursery in Western Australia in 1990. The resultant seedlings were nearly 100% mycorrhizal (Hardy *et al.*, 1992). Mycelial slurries can be delivered via fluid drilling or custom-built injectors (Fortin *et al.*, 1988). Thus, inocula are readily handled mechanically and therefore, the application of inocula is not likely to be a problem for adoption of inoculation technology.

Conservation of genotype. It is clear that an ectomycorrhizal fungus verified to be efficacious by several years of field trial results would be a valuable commercial resource. Yet, one of the weakest points in the thrust towards the adoption of ectomycorrhizal technology is the lack of effort put into research on the long-term storage of master cultures. Due to the unsuitability of other preservation techniques, conservation of ectomycorrhizal fungi is largely dependent on continued sub-culture on agar media. The potential for contamination through repeated sub-culture, and for spontaneous mutation and selection applied by

culture and storage conditions (Kidby, 1974) poses a real danger, the end result of which is loss of valuable genotypes. More stable methods of storage characterized by fewer sub-cultures and lower selection pressures are urgently required. Cryopreservation techniques are an obvious choice but these should be investigated to provide an understanding as to why not all ectomycorrhizal fungi can be preserved this way. A profitable avenue may be to adapt for ectomycorrhizal fungi, the cryopreservation techniques successfully developed for embryology.

In conclusion

It is reasonable to conclude that there are few technical barriers to the large-scale production and use of inocula of specific ectomycorrhizal fungi. Indeed, the main remaining items on the technological timetable for practicalization of inoculation technology are concerned with scale-up of processes. Such studies are not likely to proceed seriously until there is commercial justification for the investment in funds which would be necessary. This leads to the concluding remark that the technological timetable has been uncoupled from the evidence timetable *i.e.* production technology is better prepared for the advent of inoculation technology on a broadscale than is the overall scientific evidence to justify the use of the technology in increasing the economic productivity of plantations. On the positive side, this means that when the field evidence is sufficiently developed and accepted widely enough, ectomycorrhizal technology can be rapidly implemented.

References

Beckford P R, Adams R E and Smith D W 1980 Effects of nitrogen fertilization on growth and ectomycorrhizal formation of Red Oak. Forest Sci. 26, 529-536.

Beckford P R, Melhuish J H and McIntosh M S 1984 Effects of nitrogen and phosphorus fertilization on growth and formation of ectomycorrhizae of *Quercus alba* and *Q. rubra* seedlings by *Pisolithus tinctorius* and *Scleroderma auranteum.* Can. J. Bot. 63, 1677-1680.

Bougher N L, Grove T S and Malajczuk N 1990 Growth and phosphorus acquisition of karri (*Eucalyptus diversicolor* F. Muell.) seedlings inoculated with ectomycorrhizal fungi in relation to phosphorus supply. New Phytologist 114, 77-85.

Boyle C D, Robertson W J and Salonius P O 1987 Use of mycelial slurries of ectomycorrhizal fungi as inoculum for commercial tree seedling nurseries. Can. J. For. 17,1480-1486.

Cordell C E, Marx D H, Maul S B and Owen J H 1987 Production and utilization of ectomycorrhizal fungal inoculum in the eastern United States. *In* Proceedings of the 7th North American Conference on Mycorrhizae, May 1987. Eds. D M Sylvia, L L Hung and G H Graham. pp. 287-289. Institute of Food and Agricultural Science, Gainesville, Florida.

Deacon J W and Fox F M 1988 Delivery of microbial inoculants into the root zone of transplant crops. *In* Proceedings of the Brighton Crop Protection Conference, November, 1988, Volume 2 pp. 645-653, British Crop Protection Council, the United Kingdom.

Dixon R K, Garrett H E, Cox G S, Marx D H and Sander I L 1984 Inoculation of three *Quercus* species with eleven isolates of ectomycorrhizal fungi. I. Inoculation success and seedling growth relationships. For. Sci. 2, 364-371.

Fleming L V 1985 Experimental study of sequences of ectomycorrhizal fungi on birch (*Betula* spp.) seedling root systems. Soil Biol. Biochem. 17, 591-600.

Fortin C, Fortin J -A, Gaulin G, Jomhe N and Lemay S 1988 Large-scale ectomycorrhizal inoculation of containerized-grown seedlings. *In* Canadian Workshop on Mycorrhizae in Forestry. Eds. M Lalonde and Y Piche. pp. 115-118. Universite Laval, Ste-Foy.

Gagnon J, Langlois C G and Fortin J A 1987 Growth of containerized jack pine seedlings inoculated with different ectomycorrhizal fungi under a controlled fertilization regime. Can. J. For. Res. 17, 840-845.

Gagnon J, Langlois C G and Fortin J A 1988 Growth and ectomycorrhiza formation of containerized black spruce seedlings as affected by nitrogen fertilization, inoculum type, and symbiont. Can. J. Forest Res. 18, 922-929.

Garbaye J, Delwaulle J C and Diangana D 1988 Growth response of eucalypts in the Congo to ectomycorrhizal inoculation. For. Ecol. Management 24, 151-157.

Gardner J H and Malajczuk, N 1988 Recolonisation of rehabilitated bauxite mine sites in Western Australia by mycorrhizal fungi. Forest Ecol. Management 24, 27-42.

Grove T S, Malajczuk N, Burgess T, Thomson B D and Hardy G 1991a Growth responses of plantation eucalypts to inoculation with selected ectomycorrhizal fungi. Proc. IUFRO Symp. "Intensive Forestry: the Role of Eucalypts", Durban, South Africa, Sept. 1991, pp. 86-93.

Grove T S, Malajczuk N and Dell B 1991b Effects of applied nitrogen and phosphorus on ectomycorrhizal formation, plant growth and nutrient uptake in *Eucalyptus diversicolor*

seedlings. *In* Productivity in Perspective. Ed. P J Ryan. p. 209. Forest Commission, Sydney.

Hardy G E ST J, Thomson B D, Malajczuk N and Grove T S (1992) Commercial inoculation of eucalypts with ectomycorrhizal fungi. *In* Abstracts of the International Symposium on Management of Mycorrhizas in Agriculture, Horticulture and Forestry. Eds. D Jasper and B Thomson. p. 110. Aust. Inst. of Agric. Sci., Perth.

Harley J L and Smith S E 1983 Mycorrhizal symbiosis. Academic Press, London.

Harvey L M, Smith J E, Kristiansen B, Neill J and Senior E (1988) The cultivation of ectomycorrhizal fungi. *In* Biotechnology of Fungi for Improving Plant Growth. Eds. J M Whipps and R D Lumsden. pp. 27-39. Cambridge University Press, Cambridge.

Hung L -L and Molina R 1986 Temperature and time in storage influence the efficacy of selected isolates of fungi in commercially produced ectomycorrhizal inoculum. For. Sci. 32, 534-545.

Jeffries P 1987 Use of mycorrhizae in agriculture. Crit. Rev. Biotechnol. 5, 319-357.

Jeffries P and Dodd J C 1991 The use of mycorrhizal inoculants in forestry and agriculture. *In* Handbook of Applied Mycology, Volume 1: Soil and Plants. Eds. D K Arora, B Rai , K G Mukerji G and G R Knudsen. pp 155-185. Marcel Dekker, New York.

Kendrick B and Berch S 1985 Mycorrhizae: Applications in agriculture and forestry. *In* "Comprehensive Biotechnology, Volume 4". Eds. C W Robinson. and J A Howell. Chapter 8. Pergamon Press, Oxford.

Kidby D K 1977 Culture maintenance and productivity. *In* Extracellular Microbial Polysaccharides. Eds. P A Sandford and A Laskin. pp. 1- 13. American Chemical Society, Washington, D C.

Kropacek K, Cudlin P and Mejstrik V 1989 The use of granulated ectomycorrhizal inoculum for reforestation of deteriorated regions. Agric. Ecosystems Environ. 28, 263-269.

Kropp B R and Langlois C-G 1990 Ectomycorrhizae in reforestation. Can. J. For. Res. 20, 438-451.

Kuek C, Tommerup I C and Malajczuk N 1990 Hydrogel bead inocula for the production of ectomycorrhizal eucalypts for plantations. Abstracts of the 8th Nth. Am. Conf. on Mycorrhizae, Jackson, Wyoming, Sept. 5-8, 1990, p. 175.

Kuek C, Tommerup I C and Malajczuk N 1992 Hydrogel bead inocula for the production of ectomycorrhizal eucalypts for plantations. Mycol. Res. 96, 273-277.

Larroche C and Gros J -B 1986 Spore production of *Penicillium roqueforti* in fermentors filled with buckwheat seeds: Batch and semi-continuous cultivation. Appl. Microbiol. Biotechnol. 24, 134-139.

Laukevics J J, Apsite A F, Viesturs U E and Tengerdy R P 1984 Solid substrate fermentation of wheat straw to fungal protein. Biotechnol. Bioeng. 26, 1465-1474.

Le Tacon F, Gabaye J, Bouchard D, Chevalier G, Olivier J M, Giumberteau J, Poitou N and Frochot H 1988 Field results from ectomycorrhizal inoculation in France. *In* Canadian Workshop on Mycorrhizae in Forestry. Eds. M Lalonde and Y Piche. pp. 51-74. Universite Laval, Ste-Foy.

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.

Malajczuk N, Grove T, Tommerup I C, Bougher N L, Thomson B D, Dell B and Kuek C *In press* Ectomycorrhizas. *In* Microorganisms that Promote Plant Productivity. Eds. P Daft and J Dawson. Martinus Nihoff, Amsterdam.

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

Marx D H and Cordell C E 1988 Specific ectomycorrhizae improve reforestation and reclamation in the eastern United States. *In* Canadian Workshop on Mycorrhizae in Forestry. Eds. M Lalonde and Y Piche. pp. 75-86. Universite Laval, Ste-Foy.

Marx D H and Kenney D S 1982 Production of ectomycorrhizal fungus inoculum. *In* Methods and Principles of Mycorrhizal Research. Ed. N C Schenck. pp. 131-146. American Society of Pathology, St. Paul.

Mauperin C, Mortier F, Garbaye J, Le Tacon F and Carr G 1987 Viability of an ectomycorrhizal inoculum produced in a liquid medium and entrapped in a calcium alginate gel. Can. J. Bot. 65, 2326-2329.

Moo-Young M, Moreira A R and Tengerdy R P 1983 Principles of solid-substrate fermentation. *In* "The Filamentous Fungi Volume Four". Eds. J E Smith, D R Berry, and B Kristiansen. pp. 117-144. Edward Arnold, London.

Ruehle J L 1982 Field performance of container-grown loblolly pine seedlings with specific ectomycorrhizae on a reforestation site in South Carolina. Southern J. Appl. For. 6, 30-33.

Silman R W 1980 Enzyme formation during solid-substrate fermentation in rotating vessels. Biotechnol. Bioeng. 22, 411-420.

Tommerup I C, Kuek C and Malajczuk N 1987 Ectomycorrhizal inoculum production and utilization in Australia. *In* Proceedings of the 7th North American Conference on Mycorrhizae, May 1987. Eds. D M Sylvia, L L Hung and J H Graham. pp. 293-295. Institute of Food and Agricultural Science, Gainesville, Florida.

Valdes M 1988 Survival and growth of pines with specific ectomycorrhizae after 3 years on a highly eroded site. Can. J. Bot. 64, 885-888