Proc. 7th Nth. American Conference on Mycorrhizae, Gainesville, Florida, May 3-8, 1987.

ECTOMYCORRHIZAL INOCULUM PRODUCTION AND UTILIZATION IN AUSTRALIA.

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Keywords --Propagule, biomass, carrier-delivery system, differential strains, *Laccaria laccata*, *Pisolithus tinctorius*

Introduction

The relationship between utilization and production of inoculum can be discerned from a juxtapositioning of the definitions of these two areas of mycorrhizal research. Utilization of ectomycorrhizal inoculum is a management option which relies on understanding the particular circum-stances which result in improved economic yield and/or survival of plants due to the application of inoculum. Produc-tion is an activity involving the generation of a biomass capable of acting as propagules and packaging the biomass in a conve-nient form. Effective utilization depends on the availability of high quality inoculum, the technology for applying noculum and an understanding of the phenological stage of plant development most appropriate for inoculation. The propagules can be naturally produced or they can be developed in a number of other ways, for example by the mass culture of an ectomycorrhizal fungus in a fermentor. Inoculum production encompasses the body of knowledge and under-standing associated with the identification of structures which can act as propagules, their physiology, biochemistry and genetics, and with the development of an appropriate delivery system for each of the various forms of inocula. This paper proposes physical and physiological criteria for ideal inocula, discusses current and newer production processes, and evaluates current Australian practices.

Criteria for Practical and Effective Inocula

Physical form. Fungal inocula are normally delivered to the soil via a carrier or bulking material to which they are attached. Thus an inoculum has two components *viz*. biomass and particulate carrier material. In a practical and effective inoculum: a) the particles of the carrier material should be associated with a constant amount of biomass, protect the biomass against physical and chemical stress during production and handling procedures, and not present problems in delivery of inoculum to soil; b) the biomass should have a consistent and appropriate physiological state; c) the inoculum should be in a form which is practical for large scale production and use, allows close control of production parameters, is low in volume and cost effective.

Fungal strain. Alongside selecting/breeding trees for particular attributes, there is an equal need to select/breed their mycorrhizal fungi for both their capacity to make effective associations with hosts probably involving increased phosphorus uptake, and to inhabit particular soil and climatic regimes. Similar views have been expressed by others (*e.g.* Levisohn, 1959; Trappe 1977; Marx and Kenney 1982). Climatic factors to be taken into account when selecting fungi include the frequency and distribution of rainfall, and the daily and seasonal temperature fluctuations especially in relation to rainfall. Soil factors include pH, salinity, texture, organic matter and availability of inorganic minerals. The cultural characteristics of the fungus and the survival strategies of the propagules are also important criteria. Having selected an appropriate strain, its genetic character must be guaranteed in every batch of inoculum produced. This is best assured by L-drying, freeze drying or cryogenic preservation of each strain.

Production Processes

Current inocula for direct application of fungi to soil are granular and produced in two ways:a) Solid-substrate culture e.g. mycelial growth on agar, peat/vermiculite and cereal grain (Fig. 1). Such inocula do not adequately meet the criteria for practical and effective inocula because solid substrate fermen-tations are characterised by difficulties in close control of the physicochemical requirements for fungal growth e.g. pH and gaseous and nutrient transfer. These difficulties result in inocula with inconsistent quality making large-scale prod-uction more problematical than it should be. b) Submerged aerobic culture followed by the attachment or association of mycelia/spores to/with a carrier material (Fig. 1). While liquid culture is superior to solid-substrate culture in terms of bioengineering and quality control, the requirement for recovery of biomass and a formulation step makes the process less attractive and raises questions about changes in the viability and effectivity of the resulting inoculum. If the potential problems with recovery of biomass and formulation can be avoided, there is no doubt that submerged aerobic culture would be the preferred approach.

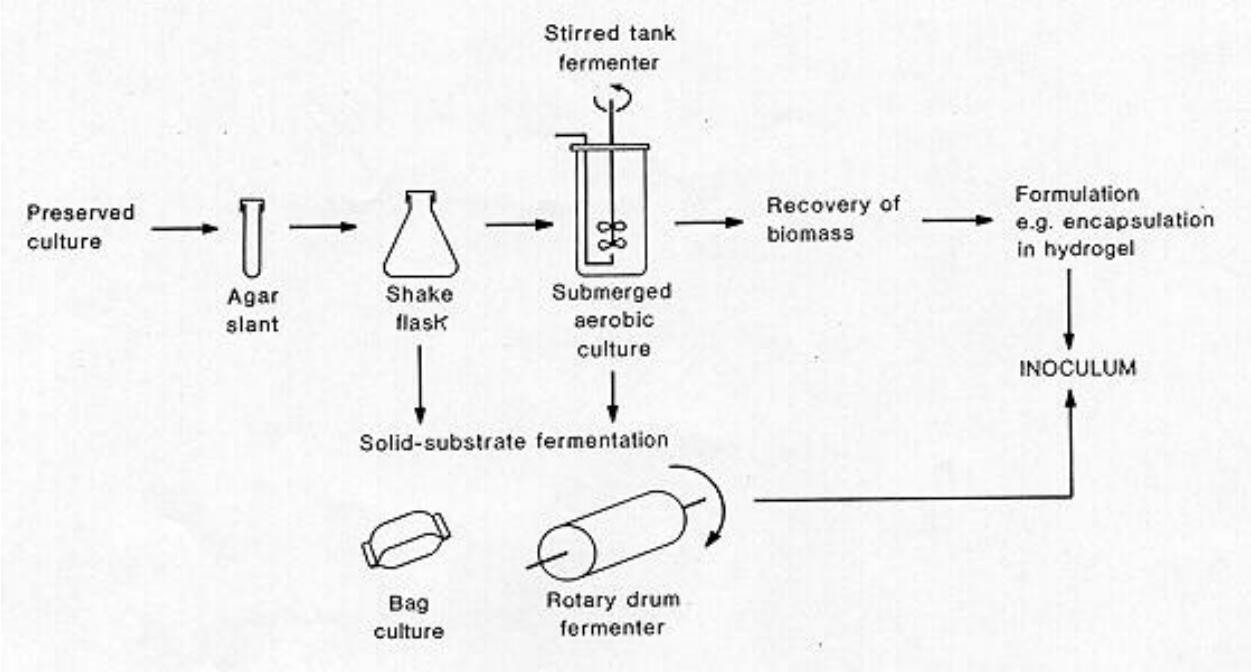
Background to Forestry Practice and Mycorrhizae

Large numbers of seedlings of indigenous species are produced in commercial forests by natural regeneration. In contrast there is no natural regeneration of *Pinus* as commercial forests in Australia, all the seedlings are planted out. As far as we are aware no naturally regenerating forests, even where there has been clear felling, are inoculated. For most indigenous species the mycorrhizal fungi which associate with seedlings have not been identified. Moreover there is no information about the stage of seedling development when mycorrhizae form, how effectively they function to increase seedling growth, compe-tiveness or survival, or whether certain species or strains would be more effective at promoting growth of seedlings and young trees. Yet this type of information could be economically valuable for forestry, particularly on clear-felled sites where regeneration is manipulated so that one species will be domi-nant. Recent and projected research aims to redress some of these imbalances (Malajczuk, these proceedings).

Current Production and Utilization of Inoculum in Australia

Large scale utilization of inoculum depends on the demonstration and acceptance of the advantages of inoculation, and the availability of sufficient quantities of inoculum having adequate and reliable quality, produced in a form convenient to store and for which application is compatible with other aspects of production. Indeed any experimentation to test the advantages of inoculation depend on the availability of inocula quantified for quality and reliability. In Australia, use of mycorrhizal fungi has been a management practice for more than sixty years. Inoculation with litter and/or soil from forests in Europe and North America resulted in improved survival and greatly increased tree growth of commercially valuable, exotic pine species in plantations. The method was first used in Western Australia and subsequently throughout the continent (Kessell, 1923). How has the production of inoculum and utilization changed in the intervening period?

In 1987 some of the techniques are little changed from those of 1923. Three types of inoculum are currently used in commercial operations and three others are being tested in pilot programs.



Natural inoculation. Wind borne spores from established pine forests adjacent to nursery beds is relied on for initiating mycorrhizae of seedlings raised for bare-root stock by some nurseries. This form of inoculation could be erratic in distribution and contribute to variable seedling and young tree establishment and growth. Theodorou and Bowen (1970) showed that additional inoculation could be increase the overall growth of pines which had been naturally inoculated. Pines in field-nurseries established on old plantation sites and those having more than one seedling crop without a break-crop are probably inoculated with a mixture of mycelium associated with old mycorrhizal roots as well as wind-blown spores. Naturally regenerating stands of indigenous species would also probably be inoculated with mixtures of spores and mycorrhizae. However the fungi may be late-colonizing types which can be less effective than early colonizing species (Malajczuk, these proceedings).

Soil/litter. A common form of inoculum for exotic pine species is soil litter containing one or more ectomycorrhizal fungi. Although it is a long standing method, this type of inoculum has several disadvantages. Firstly the types of mycorrhizal fungi in the soil and secondly, the number and distribution through the soil of propagules capable of establishing a mycorrhizal relationship are unknown. Inoculum failure is one reason for heterogenity in seedling establishment and growth. Because the inoculum is of unknown quality, quantification of its success or failure is difficult. Thirdly pathogens can be unwittingly disseminated with this form of inoculum. Soil/litter inoculum is used for both bare-root stock and containerized seedlings on a large scale. In a few operations the inoculum production is a carefully controlled process to produce high quality and disease free material. The type of system used to produce inoculum of VA fungi for *Araucaria* could be emulated for ectomycorrhizal fungi where fresh mycorrhizae are the best form of inoculum. Surface decontaminated seeds of an alternative host and spores of selected VA fungi are introduced into sterile soil. These pot cultures are watered with sterile water, physically isolated from other glasshouse operations and maintained with appropriate hygiene to avoid contamination with pathogens (B. Brown pers.comm.).

Spores. These are obtained from mature sporocarps collected from pine plantations, stored frozen and applied as slurries to inoculate pines in some nurseries. In field trials fresh and dried spores of *Rhizopogon luteolus* from mature sporocarps increased the growth of *Pinus radiata* (Theodorou and Bowen, 1973). However both air drying and freeze drying considerably decreased the capacity of the spores to act as inoculum because compared with fresh spores 10 to 100 times more were required respectively to establish the same number of mycorrhizae. Spores were also effective as inoculum after drying in soil for two months (Theodorou and Bowen, 1973). While spores are theoretically a convenient form of inoculum, at present they are of limited or indeed no value for many isolates or species. For some species, spores have not been germinated under controlled conditions and their requirements for germination remain unknown, while for others germination is low and unpredictable (Birraux and Fries, 1981; Bowen and Theodorou, 1985; Fries, 1978; 1983a,b). Although spores of certain species have some resistance to drying nothing is known about this characteristic for the majority of Australian mycorrhizal species. Dessication resistance has practical advantages for inoculum storage, application and survival after application.

Mycelia. *Laccaria laccata* and *Pisolithus tinctorius* grown in peat/vermiculite with an organic and inorganic nutrient solution, have been used in pilot programs to inoculate containerized seedlings of *Pinus radiata* and *Eucalyptus* spp. respectively (Malajczuk, unpublished). Inoculated seedlings out planted at a site with several indigenous fungi had greater growth than those which became non- specifically inoculated during growth in the nursery or field. Results indicate that some of the variability in performance of experemintally inoculated plants is attributable to inconsistency in the inoculum, a difficulty with this type of inoculum recognised previously by others. There appears to be no test or even series of tests which is able to uniquivocably demonstrate the quality of this form of inoculum or that of the related form produced by growing mycelium with cereal grains or other plant material (Marx and Kenny, 1982).

Programs to produce progeny from selected trees of temperate and tropical species of both pines and eucalypts using cuttings, micropropagation and tissue cultures are being developed in several states of Australia. For the eucalypt program fungal isolates appropriate for the seedling stage of each tree species are being selected. Rooted cuttings are inoculated at transplanting with mycelium in peat/ vermiculite. Few experiments to discern the stage of root development most appropriate for inoculation of cuttings have been undertaken at this early stage in the program. Detailed experimentation of this and other aspects are envisaged as the range of host and fungal species/isolates increases.

While micropropagated plantlets of eucalypts and pines can be inoculated on a large scale with plugs of agar inoculum it is a time consuming operation (Malajczuk and Hartney, 1986). Production of the inoculum biomass in a fermentor could be technologically a more efficient process where the application is aseptically produced

plantlets. However some strains which grow rapidly on agar media generate biomass slowly in liquid systems. Clearly research aimed at resolving these problems is pertinent. The growth rate of some strains can be increased if the mycelium is provided with particulate support material such as vermiculite or shredded agar (Litchfield and Arthur, 1983). Other fermentation studies indicate strain variability in the tolerance of mycelium to physical damage and differential requirements for organic and inorganic nutrients (Unpub. obvs. of Kuek, Rowe, Tommerup & Malajczuk).

Mycelial forms of inoculum have a number of advantages and disadvantages as does spore inoculum. Many mycorrhizal fungi are difficult to culture and some have not been grown at all (Marx and Kenny, 1982). For practical reasons mycelia is often combined with a carrier or grown in association with a carrier. Most carriers currently widely used do not protect the mycelium from physical damage during preparation and handling. Loss of biomass viabilty due to operational procedures could account for the erratic performance in some large and small scale trials using inocula produced by solid state fermentation. Mycelia frequently fail to establish or establish few mycorrhizae under field conditions when the soil dries out or where there are biological antagonists (Theodorou, 1971). For field applications, biomass generated by fermentation would need to be attatched to some form of carrier for ease of handling and storage. The system used for many animal,plant and microbial cells, including some mycorrhizae, involving capsulating cells in a matrix gel such as agarose or calcium alginate (Le Tacon *et al*., 1985) should be adaptable for large scale production systems once the quality of the biomass can be controlled (Fig. 1).

In conclusion

For some commercially useful plant species the range of candidate mycorrhizal fungi is rapidly expanding while for other economically or environmentally desirable plants nothing is known of their mycorrhizae and for still others their fungi have proven difficult to culture. These challenges are being addressed by some of Australia's current research programs. Once candidate fungi have been selected for incorporation into an inoculum production program, procedures will depend on a basic knowledge of factors promoting the generation of biomass in an appropriate state and formulation of high quality, well characterised inocula for particular types of application. An examination of the characteristics of the current forms of inocula used in Australia would show that none adequately satisfy all the criteria which we have suggested would make an ideal inoculum. Some of the inocula have an adequate level of performance for a few criteria but an ideal inoculum must necessarily satisfy all the criteria. To be otherwise is to jeopardize inoculum efficacy. The next decade should result in a number of alternative production technologies which maximise production and application efficiencies and approach the goal for ideal inocula. Now that criteria for ideal inocula have been have been evaluated, the required specific technologies have to be developed and these are of particular interest to us.

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