REVIEW/SYNTHÈSE

Can plant productivity be increased by inoculation of tree roots with soil microorganisms?

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Fostering symbiotic associations between appropriate soil microorganisms and their compatible hosts lies within the management capabilities of agriculturalists and foresters. Using knowledge of the fundamental scientific bases for these associations, one can facilitate the development of beneficial symbioses by inoculation of seeds, seedlings, or growing plants with selected microorganisms to establish and perpetuate effective symbioses leading to increased productivity. Of particular interest to the forester are four major groups of symbiotic associations: *Rhizobium* or *Bradyrhizobium* – leguminous trees; *Frankia* – actinorhizal plants; ectomycorrhizae – host trees; and endomycorrhizae – host trees, including vesicular–arbuscular mycorrhizae. Summarized here are the isolation, characterization, and culture of the microbial symbionts; the demonstrated specificity for infection and effectivity for facilitating nutrient uptake in each case; and the development of the technology for field inoculation to achieve effective symbioses in forest plantations. The factors involved in successful inoculation procedures are reviewed, and recommendations are made as to some of the necessary steps to further the development of this biotechnology.

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Les agronomes et les forestiers, en tant que gestionnaires, sont en mesure de favoriser les associations symbiotiques entre microorganismes du sol et leurs hôtes végétaux. Connaissant les principes fondamentaux régissant ces symbioses bénéfiques, il est possible de favoriser leur développement en inoculant les semences, les semis ou les plantules avec des microorganismes appropriés, avec l'objectif d'en accroître la productivité. Les forestiers s'intéresseront particulièrement à quatre principaux types d'associations symbiotiques : *Rhizobium* ou *Bradyrhizobium* – légumineuses arbustives; *Frankia* – plantes actinorhiziennes; ectomycorhizes – arbres hôtes; endomycorhizes – arbres hôtes, incluant les mycorhizes à vésicules et arbuscules. L'isolement, la caractérisation et la culture des symbiotes seront abordés, de même que leur capacité à coloniser spécifiquement certains hôtes, leur effet bénéfique sur la nutrition de la plante et la technologie disponible pour les appliquer sur le terrain. Les points importants pour le succès d'une inoculation seront recensés, avec des conseils pour promouvoir l'essor futur de cette biotechnologie.

[Traduit par la rédaction]

Introduction

The rhizosphere, that is, the soil that surrounds and is influenced by the roots of a plant, is a complex environment populated by myriads of microscopic and macroscopic organisms that interact in dynamic ways. Some soil organisms are detrimental and some are beneficial to the growth and development of plants whose roots invade, occupy, and draw upon the water and nutrients available in the edaphic environment. The association of two dissimilar organisms, e.g., microorganisms and higher plants with their extended root systems, living together in mutually beneficial relationships, is the topic of this review. What are these symbioses, how beneficial are they to the plant, especially trees, and can these associations be improved by man's intervention to maximize the productivity of the plants involved?

The dimensions of the interactions range from atomic to molecular to microscopic to morphological. Each organism in the rhizosphere has complex functions to achieve maximum benefit to itself in time and place. Symbiotic relationships have evolved that accomplish this result. The photosynthetic host plant provides the sources of chemical energy, usually in carbohydrate form. The microsymbiont brings some specialized function to the partnership. In the cases of rhizobia or *Frankia*, nitrogen availability is facilitated via the prokaryotic enzyme nitrogenase, which catalyzes the fixation of atmospheric dinitrogen. Since nitrogen is the major limiting nutrient to the growth of trees in most temperate and boreal forests, symbiotic nitrogen fixation from the dinitrogen of the atmosphere is an important process for plant productivity.

Mycorrhizae provide increased access to inorganic nutrients in the soil, especially phosphate, as well as to organic components, including organic nitrogen in the soil, through elaborated mycelial hyphae extended from infected host root tissues. Some species of mycorrhizal fungi benefit host plants during drought. Their presence may extend the life of feeder roots. Some species of mycorrhizal fungi may protect hosts against pathogens. Mycorrhizal fungi play other important roles in affecting soil aggregation and physical attributes.

The problem for the forester is to optimize these soil–root–microorganism interactions at the lowest cost with the creation of the maximum quality product. Achieving all of this requires soil and site preparation, propagation of select host trees, and inoculation at the seedling stage with appropriate microorganisms for immediate and continuing symbiotic success. The benefits are both short term and long term, with a payoff within a year in forest nurseries using fumigated soils or in as few years as a decade or as long as a century for average outplantings.

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Symbioses in forest trees

I plan to focus on symbioses involving tree species of importance in forestry worldwide and to assess briefly each of the four major symbiotic associations, viz leguminous trees – rhizobia; actinorhizal plants – frankiae; ectomycorrhizal fungi – host trees; endomycorrhizal fungi – host trees, especially vesicular–arbuscular mycorrhizae (VAM). This is a large subject and already much reviewed as I shall note, but seldom discussed comparatively (for an earlier effort see Torrey 1988) and with a focus on inoculation as an effective approach to the improvement of forest productivity.

It is regrettable that awareness of the importance of these symbiotic associations for forest trees worldwide is so low. Serious efforts are needed to educate horticulturists, foresters, and plant scientists in general on the subject. A major opportunity was lost, for example, in the recent volume entitled *Managing Global Genetic Resources: Forest Trees* (National Research Council 1991), which failed totally to mention microbial symbioses with forest trees!

What is interesting is that inoculation has been applied in each of these associations, has met with fundamental success in most cases, but the practical application of inoculation technology has suffered from numerous "trials and tribulations" and at the present time is foundering or failing in field application for want of appropriate cost-effective technology. Equally disturbing is that microbial inoculations are not routinely included in experimental greenhouse and field studies designed to elicit an understanding of whole-plant or plant population behavior in physiological or physiological–ecological terms. This failure may be attributed in part to the lack of understanding of the importance of these associations in the "real world" but also to the lack of readily available standardized inocula of the appropriate organisms that can be included in experimentation.

I want first to describe these different associations and to present the major microbial participants and their woody dicotyledonous host plant partners. One must turn to the specialized literatures of each group, where one finds the associations described, the nature of the symbioses, and the evidence of the benefits in terms of improved plant growth. The technology of inoculation procedures and successes or failures turns out to be harder to track down in the literature. Here, I will refer to published accounts and to personal correspondence with some of the research participants and to their publications. It is here one hears about the trials and tribulations.

A recent symposium edited by Keister and Cregan (1991) presents many aspects relating rhizosphere dynamics to plant growth. The structural and physiological mechanisms of plant–soil relationships are interestingly discussed by Lamont (1982). General reviews of the symbioses bring the subjects up to date. On leguminous plants – rhizobia, broad-based discussions have been published by Sprent (1979), Vincent (1982), Da Silva *et al.* (1987), Elkan (1987), and Sprent and Sprent (1990). Recently *Frankia* biology was reviewed comprehensively in a book edited by Schwintzer and Tjepkema (1990). A general treatment of mycorrhizae was written by Harley and Smith (1983). The potentialities and problems of practical application of mycorrhizal management in forestry were summarized by Peterson *et al.* (1984). Regular publications of the *Proceedings of the North American Conference on Mycorrhizae* cover a wide range of more specialized topics. Recent volumes include those edited by Molina (1985) and Sylvia *et al.* (1987). A discussion of biotechnology applied to fungi – host plant beneficial relationships is edited by Whipps and Lumsden (1990).

Symbiotic associations in woody species

Rhizobium *and tree legumes*

The major symbiotic N_2-fixing associations involving rhizobia are with herbaceous leguminous agricultural crops, including soybeans, bush beans, alfalfa, clover, vetch, and the like. Among woody species, the most important genera are tropical trees, including widespread *Acacia*, *Leucaena*, and *Prosopis*. A few north temperate genera such as *Robinia*, *Gleditsia*, and *Cercis* serve as horticultural plants. Only the first of these last three genera is reported as nodulated. For a comprehensive listing of leguminous host species, both herbaceous and woody, consult Allen and Allen (1981).

The infective, effective symbiotic organism producing nodules in these genera, leading to N_2-fixation, is usually the slow-growing *Bradyrhizobium*, often grouped with the "cowpea miscellany." Although there are cross-inoculation groups, there exists considerable specificity between host genus and *Bradyrhizobium* strain, that is, the bacterium from nodules of one host plant will infect other plants of the same genus but not other related genera. Unlike many of the herbaceous legumes where infection is via root hair entry, these tree species are characteristically infected by direct entry of the motile rod-shaped bacterium between root radial epidermal cell walls and thence via intercellular spaces before finally penetrating cortical cells, which multiply to form the root nodule. In some cases, bacteroids are formed, that is, the modified bacterial cell is enclosed in a host membrane; in other cases, the rhizobia are retained in the infection thread, where nitrogenase forms and is active (de Faria *et al.* 1986).

Only one genus outside the family Leguminosae has a symbiotic association with *Bradyrhizobium*. That is the tropical tree genus *Parasponia*, a member of the Ulmaceae, where *Bradyrhizobium* infects by direct entry, induces actinorhizal-like nodules involving modified lateral roots, and fixes nitrogen within the infection threads in which it is retained (cf. Lancelle and Torrey 1985). Becking (1992) has written a comprehensive review of this model symbiotic system involving a natural extension of the host range specificity for rhizobia.

Frankia *and actinorhizal plants (cf. Schwintzer and Tjepkema 1990)*

Frankia is a filamentous bacterium of the Actinomycetales. It resides saprophytically or as dormant spores in the soil and infects roots of many diverse genera of woody plants. Actinorhizal plants encompass 8 families of angiosperms, 24 genera, and more than 200 species. Root nodules resulting from *Frankia* infection arise from invasion of multiple modified lateral roots. Infection may be via root hair entry, as in *Alnus* or *Casuarina*, or by direct epidermal entry, as in *Elaeagnus*. Cortical cells of the multiple lobes that develop from an initial infection are occupied by *Frankia* filaments, approximately 1 μm in diameter, and are retained within encapsulating polysaccharides of host origin. Nitrogenase may be localized in swollen terminal hyphal endings called vesicles, as in *Alnus* and *Elaeagnus*, or in the hyphal filaments enclosed in specially modified host cell walls, as in *Casuarina*. These different structural modifications are related to alternate mechanisms that have evolved and protect the nitrogenase enzyme from denaturation by molecular O_2.

Genera of actinorhizal trees that are prominent in forestry include north and south temperate species of the genus *Alnus*, including *Alnus rubra*, the red alder of northwestern North America and *Alnus hirsuta* of mainland China. The genus *Elaeagnus* includes several large shrubs of importance in horticulture. Several *Myrica* species forming shrubs or small trees are important as components of moist ecosystems in the temperate and tropical regions because of their N_2-fixing capacities. In the tropics genera of the Casuarinaceae are prominent members, originating in Australia and islands of the South Pacific but carried by man to areas of the tropics worldwide. The genera *Casuarina*, *Allocasuarina*, and *Gymnostoma* all reach considerable size in favorable habitats.

The microsymbiont *Frankia* shows a range of host specificities, which have been divided into several main groups. Within each host specificity group, cross inoculation with a *Frankia* strain will be successful but not between groups. The present non-all-inclusive groups are as follows: *Alnus–Myrica*, the Casuarinaceae, and the Elaeagnaceae. Some hosts are remarkably promiscuous, that is, they are nodulated by strains from several different host groups. The above groupings tend to exclude members of the Rhamnaceae, the Coriariaceae, and Rosaceae, which require further study. Other hosts are very specific, being infected only by organisms isolated from that host. Because of the diversity of host families, much research remains to be done on the question of host specificity.

Mycorrhizal fungi and host plant associations (cf. Harley and Smith 1983)

Many fungi are found associated with roots in the rhizosphere. These eukaryotic microorganisms have fungal hyphal diameters 5–10 × those of prokaryotic actinomycetes such as *Frankia*. The fungi are heterotrophic and may be saprophytes (living on organic matter in the soil), symbiotic with various host plant roots, or parasitic and pathogenic.

There are several types of symbiotic mycorrhizal associations, of which I will focus on two: ectomycorrhizae and VAM. These fungi depend upon the root for essential nutrients, especially carbohydrates, and they may or may not produce morphological changes in the host root. Mycorrhizal fungi facilitate uptake of many nutrients essential to plant growth, especially immobile mineral elements such as phosphorus.

Ectotrophic mycorrhizae

In this symbiotic association the fungus completely encloses the fine lateral root tips of the host plant in a sheath or mantle of hyphal filaments that usually penetrates between the cell layers of the root cortex, forming a complex termed the Hartig net. The hyphae do not penetrate the individual host plant cells. Ectotrophic mycorrhizae are usually evident externally, with swollen root tips forming fans or thickened root branches of various colors that may persist several years. Presence of the Hartig net is considered diagnostic of this type of association.

Ectomycorrhizae are common in forest trees, both gymnosperms (especially the conifers, such as pines, spruce, and larches) and angiosperms (including such deciduous trees as oak, beech, birch, and the eucalypts). Thus members of the families Pinaceae, Betulaceae, and Fagaceae are commonly ectomycorrhizal. According to Meyer (1973), only about 3% of the species of vascular plants are ectomycorrhizal, but they are of special interest to foresters.

Many diverse fungi are capable of forming this type of relationship. Many of these fungi are readily isolated and grown in pure culture. They are mostly Basidiomycetes, characterized as toadstool- and truffle-forming fungi. Many genera and thousands of species are known, including the following common genera: *Boletus*, *Russula*, *Rhizopogon*, *Hebeloma*, *Cenococcum*, *Amanita*, and *Pisolithus*. Some of these are favorites of mushroom collectors; others are highly poisonous if eaten by man.

Ectomycorrhizal fungi depend for their growth on simple mono- or oligo-saccharides as carbon and energy sources. They exhibit limited ability to metabolize complex carbohydrates such as cellulose, starch, or pectins. Since most soils have a low supply of simple carbon compounds, these fungi tend to associate with root systems that provide available sugars or other carbon sources. Thus ectomycorrhizal fungi form symbiotic associations with appropriate hosts and are seldom free-living saprophytes in the soil. Most host plants in turn benefit from the association. The hyphal filaments of the fungus extend out into the soil around the root and provide access to inorganic nutrients for the root through the hyphal network, which is especially beneficial in phosphate-deficient soils.

Vesicular–arbuscular mycorrhizae

This type of symbiosis involves fungi that form a much more intimate intracellular association with the host plant roots than ectomycorrhizae. The hyphal filaments enter the host root cells of the epidermis or root hairs, penetrate into the root cortical cells, and there proliferate and differentiate without causing evident external morphological change to the root system. Diagnosis of the presence of these associations usually involves fixing, clearing, and staining the roots to demonstrate the presence of the fungus. Within the root cells the fungus typically produces enlarged storage structures called vesicles, and in other cells, highly and finely branched hyphal endings termed arbuscules. Such endomycorrhizae are of the VAM type. External hyphae extend several centimetres beyond the root and facilitate inorganic elemental uptake, especially phosphate. Also, external sporulation occurs with the formation of very large (~80 μm) spores.

These VAM associations are the most widespread in the plant kingdom, commonly occurring in the Bryophytes (mosses), the Pteridophytes (ferns), and the Gymnosperms and Angiosperms, and they occur from the tropics to the arctic regions of the world. VAM may involve several types of fungi. The most common genera of the VAM fungi are *Glomus*, *Gigaspora*, and *Acaulospora* of the Endogonaceae (Phycomycetes).

In a recent survey of the north temperate vegetation at the Harvard Forest (Berliner and Torrey 1989), 91% of 45 higher plant species studied were found to be mycorrhizal, 22% were ectomycorrhizal, and 71% were vesicular–arbuscular (VA) mycorrhizal. Most of the conifers were ectomycorrhizal, and most of the hardwood species were associated with VAM. Some were both. Ectomycorrhizae were more common in communities of low diversity, while VAM occurred more

TABLE 1. Sources in the United States of information and (or) cultures of microorganisms symbiotic with roots of woody plants

	Organization	Notes
Information database (catalogue of culture collectors)	The Microbial Germplasm Database and Network, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331	Organizers: L.W. Moore and F.J. Hanus. This database and network has recently (1991) received a significant grant from the National Science Foundation that extends the life of this operation
Culture collections	American Type Culture Collections, 12301 Parklawn Drive, Rockville, MD 20852	Produces catalogue of fungi–yeasts, also bacterial strains
	USDA, Beltsville *Rhizobium* Culture Collection, Beltsville Agricultural Research Center, Beltsville, MD 20705	Produces catalogue of *Rhizobium* culture collection
	University of Hawaii NIFTAL Project, College of Tropical Agriculture and Human Resources, University of Hawaii, P.O. Box O, Paia, Maui, Hawaii 96779	Produces catalogue of selected strains of *Rhizobium*
	USDA, Midwest Area Northern Regional Research Center, Microbial Properties Research, 1815 North University Street, Peoria, IL 61604	Microbiologist: D.P. Labeda. Storage site for cultures of *Frankia*, among others
	International Culture Collection of VA Mycorrhizal Fungi (INVAM), *First established at* the Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611.	Organizer: N.C. Schenck. Source of starter cultures of VAM
	Current location: Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506	Curator: Dr. J. Morton
Commercial sources	The Nitragin Company Milwaukee, Wis.	Commercial inoculum of *Rhizobium* for legume crops
	MycorrTech, Inc., University of Pittsburgh, Applied Research Center, Pittsburgh, Pa.	Produces commercial vegetative inoculum of ectomycorrhiza (*Pisolithus tinctorius*) for use on conifers and hardwood species; also *Hebeloma crustuliniforme* and *Laccaria laccata*

frequently in communities of high plant species diversity. Most cultivated crops, grasses, herbs, shrubs, and trees showed VA mycorrhizal associations.

Up to the present none of these commercially important VAM fungi have been grown in pure culture, so they are still commonly referred to as obligate symbionts. These fungal symbiotic associates are maintained in the greenhouse in pot cultures; that is, appropriate susceptible hosts are grown in pots in the greenhouse with infection from root or soil from around previously infected plants. Some inoculation success has been achieved with soil transfer when sufficient care has been taken concerning the soil source. Inoculum can be obtained as spores by wet sieving of the soil or by cutting up infected roots. This process is expensive, cumbersome, and filled with problems, including co-transfer of deleterious pathogens or soil components. Recently, the International Culture Collection of VA mycorrhizal fungi (INVAM), based on living collections in pot culture, was established at the University of Florida at Gainesville with National Science Foundation support (Schenck and Perez 1987). The collection has now been moved to West Virginia University (cf. Table 1), where it will serve as a repository and source of starter cultures for research. A new book by Sieverding (1991) (published in Germany) that was not available when this paper was written focusses on two subjects: the technique of inoculation of field crops with VAM and the management of VAM in agronomic practice.

Tripartite associations

Close examination of root systems of many species often shows that several different microbial associations may occur within one root system. Such complex relationships are referred to as tripartite or even tetrapartite associations and have been the subject of special studies, primarily among herbaceous plants (cf. Barea and Azcon-Aguilar 1983; Gardner 1986; Linderman 1988). Studies have shown that the presence of mycorrhizae enhance dinitrogen fixation by *Rhizobium*-nodulated plants (e.g., Bayne and Bethlenfalway 1987). In the forest tree *Casuarina equisetifolia*, Diem and Gauthier (1982) reported that inoculation with *Frankia* (as crushed nodules) and the VAM species *Glomus mosseae* significantly improved nodulation and plant growth.

To achieve optimum effects of microbial associations, one must examine the range of associations that occur in the root system of the host plants, the microorganisms in the soil, and the complex environment provided by the soil. As has been stated by Linderman (1991), "Managing the mycorrhizosphere is a target strategy whose time has come."

Biotechnical considerations

Researchers are interested in the development of practical procedures to facilitate and utilize the benefits to forest productivity resulting from effective symbiotic associations that lead to improved nitrogen and phosphorus utilization from the environment. The following steps in this process are involved:

(1) Recognition and development of an understanding of the symbiosis and the organisms involved. Studies of these associations are, for the most part, of long standing. We have still much to learn, especially about the genetic bases for these associations, to facilitate host–microorganism selection.

(2) The isolation, identification, and axenic cultivation *in vitro* of the microsymbionts. In the case of *Rhizobium* these steps were accomplished for the common herbaceous agricultural crops in the 1880s, although in the case of trees, many *Bradyrhizobium* still remain to be cultured for the first time. *Frankia* was first isolated, cultured, and used for pure-culture inoculum in 1978 (Callaham *et al.* 1978). The familiar ectomycorrhizal fungal species have been grown in culture for many decades. Organisms isolated, cultured, and made available for mycorrhizal inoculum were first listed as recently as the 1970s. The organisms in VA mycorrhizal associations have yet to be brought into continuous axenic culture.

(3) Experimental reestablishment of the symbiotic associations by bringing together in the greenhouse or the field appropriate symbionts by processes of inoculation.

(4) Demonstration by careful measurements of growth and development of the host plants of the benefits produced by the symbiotic association. Measurement of the increments of the essential elements that lead to increased productivity should also be made.

(5) The creation of permanent collections of the microorganisms that have been isolated, cultured, and tested, together with credentials documenting appropriate hosts and circumstances for effective inoculations. This step has been pursued vigorously for *Rhizobium* and *Bradyrhizobium* by governmental agencies, nonprofit organizations, and private companies, especially for important leguminous agricultural crops. For *Frankia* no permanent culture collections have yet been established, and foresters must rely on individual scientists conducting research on the organism or association with which they are concerned. Ectomycorrhizal fungi for designated associations (and hosts) are available from some national culture collections such as the American Type Culture Collection or from a few private companies. No existing sources for VAM inoculation are currently available.

(6) Development of effective inoculation procedures once pure cultures of the organisms are available from reliable and permanent culture collections. It is of no value to provide a forest nurseryman a test-tube culture of an infective, effective microorganism if he needs it for a million seedlings in his nursery. The forester asks for a ready-to-use inoculum to be applied in the seedling nursery or in the field, preferably with easy application and at low cost per plant. During the past century rhizobial inoculants have been composed of a peat base that can be processed with bulk microbial cultures, packaged cheaply, and stored for up to 1 year (effective inoculum life). The primary disadvantage is the cost of bulk shipment, especially if the inoculum is prepared in a developed country and is needed by a developing country halfway around the globe. For this reason, scientists supported by USAID (United States Agency for International Development) through the NifTAL (Nitrogen Fixation by Tropical Agricultural Legumes) program have encouraged developing countries to create their own inoculum production centers at the site of need to produce inocula on an appropriate scale to be applied in local crop production.

Experimental inoculations using *Frankia* on a large scale are not very common. M. Lalonde and his associates at Université Laval in Québec formed a commercial firm for producing *Frankia* inoculum for *Alnus* species and *Elaeagnus* species and reported (Périnet *et al.* 1985) using bulk liquid cultures of *Frankia* (hyphal filaments and spores combined) to inoculate more than one million seedlings at a time. Some reports of inoculum based on calcium alginate gels have been made (Diem *et al.* 1989; Burleigh *et al.* 1988), but no large-scale reforestation projects were involved. Cost still plays a major restrictive role in the development of *Frankia* inoculum.

Ectotrophic mycorrhizal inoculations involve the use of a peat–vermiculite carrier, and this type of commercial inoculum for a limited number of host species is now available (Marx *et al.* 1982, 1984). Experimentation with alginate gels or carriers has also been reported by Le Tacon and his associates in France (Le Tacon *et al.* 1985; Mauperin *et al.* 1987).

Despite considerable research and effort, no commercial inoculum for VAM fungi is available. Recent experiments (Mugnier and Mosse 1987) involved the *in vitro* propagation of transformed roots grown in bulk culture and inoculated with fungal spores to produce massive amounts of infected root tissue that could serve as inoculum homogenate. In another approach Native Plants Incorporated developed a large-scale pot-culture procedure that allowed regular harvesting of VAM spores free of contaminating material or organisms.

A new journal entitled *Microbial Releases*, to begin publication in 1992 by Springer-Verlag, is to be used to report viral, bacterial, or fungal releases; that is, "the introduction of manipulated or non-manipulated microorganisms into the environment in either large or small quantities for a particular, well-defined purpose" (editorial statement of policy).

(7) The development of an effective marketing system for the inoculation process. As suggested by the discussions of many of the points above, the final marketing is fraught with difficulties. For inoculation procedures to be developed for marketing, a reasonable profit must accrue to the manufacturer and retailer. These stipulations usually lead to development of patented organisms or at least processes that may be expensive and time-consuming.

Defining the market and the assurance of sales sufficiently to attract venture capital in developed countries is no small task, especially when the major markets are in developing countries desperately needing reforestation programs but having no capital to expend.

So, at present, with a few exceptions, inocula of microorganisms that demonstrably benefit productivity of many forest tree species in a range of environments and circumstances are usually not available when or where they are needed. Even information about the availability of cultured microorganisms to be used as starter cultures or inoculum available commercially is not easily accessible. In Table 1 are listed some of the current information sources in the United States for organisms of potential value as inoculum in symbiotic forest tree associations. Other individuals and agencies in the international scientific community can be reached through published literature.

Our present most urgent need in developed countries is to find the will and the funds to protect the biodiversity of germplasm already accumulated in individual researchers' laboratories in the form of pure cultured organisms and to preserve these resources in permanent culture collections until such time as necessity and economics make their use possible for commercial application.

The status and examples of specific biotechnological developments in inoculation for symbiotic associations

It seems useful to outline briefly for each of the associations that I have described the current status of a few exemplary cases to illustrate what is possible and what might be developed for other systems.

Rhizobium *and* Bradyrhizobium *for forest trees*

The American Type Culture Collection (ATCC) received many numbered strains of *Rhizobium* as early as 1916 from L.W. Erdman of the USDA, Beltsville, Maryland, which had begun testing commercial legume inoculants. Most of these bacteria were listed for herbaceous legumes. A few strains were designated as useful on tree species, including strains infective on *Acacia*, *Albizia*, *Erythrina*, and *Robinia*. These strains belonged to the "cowpea miscellany" and under modern nomenclature would probably be designated *Bradyrhizobium*.

Over the past decade or two *Rhizobium* or *Bradyrhizobium* inoculation of forest trees, especially for use in developing countries, has been pursued vigorously by scientists at the University of Hawaii NifTAL project supported by the USAID since 1974. In collaboration with the Nitrogen-Fixing Trees Association (NFTA) microbiologists have focussed on isolating, culturing, and testing *Rhizobium* and *Bradyrhizobium* strains for use in inoculating 11 important leguminous tree genera, viz *Acacia*, *Albizia*, *Cassia*, *Dalbergia*, *Erythrina*, *Gliricidia*, *Inga*, *Leucaena*, *Parkia*, *Prosopis*, and *Sesbania*. Pure cultures of microorganisms recommended for inoculants are available for most of these genera, some of which are reported to be infective and effective for the different species of importance in tropical or subtropical forestry and agroforestry. These inoculant cultures are provided by NifTAL and have been supplied to NFTA for distribution with tree seeds sent around the world in experimental plantings.

Major culture collections of *Rhizobium* and *Bradyrhizobium*, especially for agricultural use, have been maintained at centers throughout the world. My own experiences have involved seeking strains from the Department of Soil Microbiology, Rothamsted Experiment Station, Harpenden, Hertfordshire, England (P.S. Nutman); the USDA Research Station, Beltsville, Maryland (D. Weber); and the Nitragin Company, Inc., Milwaukee, Wisconsin (J.C. Burton). Individual laboratories have also been generous in providing cultures for experimental studies conducted in my laboratory. Many of these sources have now disappeared with the closing of the laboratories or retirement of individuals.

Efforts have been made to decentralize inoculant production by designing and implementing the installation locally of fermenters and inoculant processing and packaging stations in proximity to the sites for inoculant use. NifTAL has pioneered these methods for agricultural and agroforestry uses in many developing countries throughout the tropical and subtropical world. Courses of instruction and advisory assistance have accompanied these efforts.

Frankia *for forest trees*

The ATCC catalogue lists only one strain of Frankia, strain HFPCpI1 (*Frankia* catalog number HFP 070101), sent to ATCC in 1979 following its isolation from *Comptonia peregrina*, an actinorhizal shrub used in roadside plantings (Callaham *et al.* 1978). Many isolates of *Frankia* have been sent by several different investigators to the Agricultural Research Culture Collection maintained by the Northern Regional Research Center, USDA in Peoria, Illinois, for maintenance and safekeeping. But a centralized systematic culture collection of *Frankia* strains does not exist at the present time. Individual researchers have stocked strains they have isolated themselves for use in their experimental studies. Several culture collections are being discontinued because of retirements (e.g., J.G. Torrey effective 1990; M.P. Lechevalier effective 1991). Various industrial firms involved during the 1980s in *Frankia* research directed toward inoculation of forest trees or inoculum production (e.g., Weyerhaeuser Company, Tacoma, Washington, and Native Plants Incorporated, Salt Lake City, Utah) have discontinued these research programs and closed their culture collections.

Several laboratories have reported large-scale tree seedling inoculation with *Frankia* under various circumstances, and their procedures have been described (Stowers and Smith 1985; Berry and Torrey 1985; Périnet *et al.* 1985). These studies included inoculation of several *Alnus* species, *Elaeagnus angustifolia*, *Hippophaë rhamnoides*, and *Shepherdia argentea*. A recent review of methods of production for forestry has been written by Benoit and Berry (1990), and a summary of management practices of actinorhizal plants for the tropics and subtropics (primarily *Casuarina*, *Allocasuarina*, and *Gymnostoma*) was published by Diem and Dommergues (1990). None of these publications deal with the problems centering on availability of cultured strains of *Frankia* or publicly or privately maintained culture collections.

Diem *et al.* (1988) presented some of their views on biotechnological developments in actinorhizal symbioses, including some evidence on successful inoculation of *Casuarina* using alginate-bead technology. Similarly, Burleigh and Torrey (1990) have reported the use of spore preparations from *Frankia* isolated from *Casuarina* as source of inoculum. Scattered small-scale efforts continue in the improvement of inoculum preparation and use for actinorhizal forest trees.

Ectomycorrhizal fungi for forest plantations

Isolations and culture of basidiomycete fungi associated with specific tree hosts have been performed for many years. Interest in fungal cultures for use as inoculum of tree species for mycorrhizal establishment was initiated in Europe and in the United States (Hatch 1937). As early as 1942 experimental inoculations of pine seedlings with appropriate mycorrhizal fungi for forestry application were reported (Finn 1942).

Cultured basidiomycetes described as mycorrhizal for tree species were accessed by the ATCC as early as the 1970s and thereafter. Genera included *Pisolithus* (loblolly and slash pine), *Lactarius* (oak and pine), *Laccaria* (pine and spruce), *Thelephora* (pine and spruce), *Rhizopogon* (Douglas-fir, pine, spruce), *Suillus* (pine, spruce, fir, hemlock), and in the Fungi Imperfecti, *Cenococcum* (pine, willow, fir).

One of the most serious efforts to develop the technology of fungal inoculation for mycorrhizal establishment was pursued by D. Marx (~1970 and thereafter), using *Pisolithus tinctorius* for pine and other forest tree species. A comprehensive review of that work is published as a monographic supplement to *Forest Science* (Marx *et al.* 1984). Commercial inocula of ectomycorrhizal fungi are available in the United States and other countries. For a review of recent developments in this field of practical application, see Marx *et al.* (1992). Marx (1985) summarized some of the trials and tribulations of trying to develop practical methods of inoculation for large-scale operations. For a broad overview and detailed account of technological aspects of ectomycorrhizal inoculation of forest trees, especially conifers, including forestry practices, one also should consult the discussion by Perry *et al.* (1987).

Endomycorrhizal fungi for forestry

Serious efforts have been made in many laboratories to isolate, culture, and maintain the fungal organisms that develop endotrophic mycorrhizae, including VA mycorrhizal relationships, with a very high proportion of the vascular plants of the world. Success in culturing and propagating a fungus from an ericoid mycorrhizal association has been reported on a number of occasions since the first isolation of a slow-growing dark-colored mycelium from roots of *Vaccinium* by Doak (1928). These fungi have been designated as belonging to the genus *Pezizella* of the Ascomycetes. Back inoculation into ericaceous hosts with the successful reestablishment of the mycorrhizal association showed low host specificity (cf. Harley and Smith 1983).

Little success has been achieved, despite considerable effort, in propagating any of the VA mycorrhizal fungi in pure culture. Propagation and production of VA mycorrhizal fungi for inoculum have always involved the presence of a host plant, usually in pot culture, using solid substrates such as soil or soil substitutes, including vermiculite, montmorillonite clays, or other expanded clay aggregates. In recent years efforts to produce VA mycorrhizal inoculum have included the use of root systems grown with the nutrient film technique or isolated roots grown in nutrient culture and infected under axenic conditions with fungal spores. These methods and the degrees of success have been summarized by Mosse (1990).

Intense efforts to use a sophisticated pot-culture technique to produce spores of *Gigaspora intraradices* in large numbers that could be marketed as inoculum for forest trees that benefitted from VA mycorrhizal infections proved to be too expensive to be feasible (T. Wood, personal communication).

It remains to be seen whether such VA mycorrhizal fungi as *Glomus* or *Gigaspora* can be grown in pure culture at sufficiently rapid growth rates and on relatively simple media to allow them to be produced as inocula, in either hyphal or spore form. A thoughtful review of the problems and prospects for the use of VAM inoculation in agriculture and forestry has been written by Wood (1991). In future years, when we better understand the nature of the interdependence of these symbiotic associates, cultivation of VA mycorrhizal fungi may be possible and perhaps their management in forest production can be exploited.

Conclusions and recommendations

Much is known about the symbioses involving many important forest trees and beneficial soil microorganisms, both microbial and fungal. Many such symbioses occur naturally and play an important part in the success, survival, and spread of tree populations in natural ecosystems. The effectiveness of symbioses is especially apparent in situations of low soil fertility.

In production forestry there exist great opportunities for the management of these symbiotic relationships. Using knowledge of the fundamental scientific bases for these associations, one can facilitate the development of beneficial symbioses by inoculation of seeds, seedlings, or growing plants with selected microorganisms to establish and perpetuate effective symbioses leading to increased productivity. Among the four major symbiotic systems discussed, there exist many common technical problems in the development of inoculation technology, although the microorganisms are quite diverse.

The following recommendations are made, with the full understanding that we have much still to learn about each of the symbiotic associations and that even if successful inoculation procedures are developed, these steps do not offer a general panacea to increased productivity but present only one of the many important management choices available.

(1) Foresters and microbiologists interested and concerned in symbiosis should arrange to meet together, cutting across the specialist approach by which we are all so bound in our research. Common biotechnological problems may be solved by community efforts.

(2) Now is the time for preserving microbial diversity among symbiotic systems by establishing and maintaining effective centralized stock culture collections in the United States and elsewhere in the world.

(3) Inoculant development and production should be predicated on results of careful and extensive commercially relevant field testing of managed symbiotic associations. Only under circumstances where benefits to host productivity can be reproducibly demonstrated will inoculation prove successful in practice.

(4) Special efforts should be made by microbiologists to assure that appropriate inocula are readily available to physiological ecologists and population biologists who deal with

introduced populations of higher plants, either in greenhouse or in field plantations, to assure that more nearly natural soil microbiological situations occur during experimentation.

(5) More cooperative efforts should be applied to the development of recommendations and materials for effective inoculation procedures in forest management directed toward cost-effective increased forest productivity, and a greater awareness must be developed among the forestry community of the potential profitability of microbial inoculants for forest production.

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