COMMERCIAL MICROBIAL INOCULANTS AND INOCULATED TRANSPLANTS FOR RECLAMATION[1]

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Abstract.--Inoculation of seed and containerized plants with mycorrhizal fungi and nitrogen-fixing bacteria can enhance the establishment of grasses, forbs, shrubs, and trees on reclaimed lands. At present a variety of microbial inoculants and inoculated transplants are commercially available and should be considered for use by reclamation specialists. Guidelines for determining the need for inoculants, for specifying strains to be used, and for ordering and applying inoculants and inoculated transplants are discussed.

INTRODUCTION

Mycorrhizal fungi and symbiotic nitrogen-fixing bacteria should be considered by reclamation specialists as tools for improving plant establishment and speeding the recovery of disturbed lands. Four groups of these microorganisms are of particular importance. These are: (1) vesicular-arbuscular (VA) mycorrhizal fungi which associate with most grasses, forbs, and broadleaved trees and shrubs (Powell and Bagyaraj, 1984); (2) ectomycorrhizal fungi which associate with many woody plants including oaks, willows, poplars, pines, firs, and spruces (Marks and Kozlowski, 1973); (3) bacteria in the genus *Rhizobium* which fix nitrogen in association with most legumes (Burton, 1980); and (4) actinomycetes (filamentous bacteria) in the genus *Frankia* which fix nitrogen with a variety of woody dicots including species in the genera *Alnus*, *Cercocarpus*, *Purshia*, *Cowania*, and *Ceanothus* (Akkermans et al., 1984). Mycorrhizal fungi generally benefit plants through improved uptake of phosphorus, trace metals, and other sparingly soluble nutrients. Nitrogen-fixing bacteria convert atmospheric dinitrogen to ammonium-nitrogen, a form that plants can readily use. Both classes of organisms typically improve plant growth and survival, particularly on relatively sterile, infertile soils. As such, these microbes hold considerable potential for use as inoculants to improve the establishment of vegetation on reclaimed lands.

At present, a variety of microbial inoculants and inoculated plant materials are commercially available for use in reclamation. This paper discusses guidelines for determining the need for inoculation, for specifying the microbial strains to be used, and for purchasing and applying microbial inoculants and inoculated transplants.

DETERMINING THE NEED FOR INOCULATION

While many studies have shown that inoculations with mycorrhizal fungi and nitrogen-fixing bacteria can enhance the establishment of plants on disturbed lands (Daft and Hacskaylo, 1977; Berry and Marx, 1978; Lambert and Cole, 1980; Call and McKell, 1984), it is not safe to assume that inoculations will prove cost effective in all instances. On-site test plots, comparing the growth and establishment of inoculated and non-inoculated plants, offer a direct approach to analyzing the need for inoculation, and it is recommended that such tests be run whenever feasible. When trials are not feasible, the reclamation specialist can consider several rules of thumb in judging the potential need for inoculation.

(1) Severely disturbed soils (scalped subsoils, top soils stored for several years in deep piles, heavily eroded soils, badly burned soils) typically contain depauperate populations of nitrogen-fixing bacteria and mycorrhizal fungi (Reeves et al., 1979; Rives et al., 1980; Allen and Allen, 1980). Plants introduced into such soils frequently benefit from inoculation. (Note that careful management of topsoil resources can be used to maintain relatively high populations of indigenous soil bacteria and fungi and can offset the need for inoculation.)

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(2) If plants such as conifers or alders, with fairly specific inoculant requirements, are introduced onto sites where they have not grown before, they are likely to benefit from inoculation (Mikola, 1980). Again, natural populations of the required symbionts will probably be low.

(3) Inoculation typically proves most cost effective when conditions for plant growth are suboptimal-to-marginal, and when the principal limitations on plant growth are soil infertility and perhaps drought. If site conditions are so severe (e.g. extremes in soil fertility, moisture availability, salinity, and metal toxicities) that plants cannot survive regardless of their mycorrhizal or nodulation status, then inoculation isn't going to help. Similarly if conditions are extremely favorable for plant establishment, performance may be little enhanced by inoculation. (Note, however, that inoculation can generally improve the establishment of transplants even when conditions for plant growth are favorable. During the establishment period root systems of containerized plants are confined to fairly small soil volumes and are thus subject to moisture and nutrient stress. Inoculations with mycorrhizal fungi, in particular, can aid plants in more fully tapping that restricted soil volume for nutrients and water (c.f. Menge et al., 1978).)

(4) Plant species differ in their dependency on mycorrhizal fungi. Some plants including many grasses and annual forbs have finely divided root systems and have lesser requirements for inoculation than do plants with thicker roots and few root hairs (Baylis, 1974). Other plants are nonmycorrhizal and don't require or form the association (Gerdemann, 1968). Similarly, only a limited number of plants form symbiotic nitrogen-fixing associations. Specific information on the mycorrhizal dependency and nodulation status of given plants can be obtained from local specialists and inoculum producers.

STRAIN SPECIFICITY

Considerable variation in effectiveness (plant growth promoting ability) exists between strains of mycorrhizal fungi and nitrogen-fixing bacteria. Estimates suggest that for a given plant host in a given soil, the most effective strains should be 2-3 times more active in promoting plant growth than the average strain (Abbott and Robson, 1977; Wood and Bollinger, in press). Given this variability, considerable attention should be paid to selection of elite bacteria and fungi, best adapted for specific hosts and site conditions.

Rhizobium bacteria are host specific and have been divided into a number of cross-inoculation groups, i.e. groups of plants that are nodulated by a common strain (Burton, 1980). Six Rhizobium species have been named in conjunction with these groups. Four of importance to reclamation are Rhizobium meliloti for Medicago (alfalfa) and Melilotus species, R. trifolii for Trifolium (clover) spp., R. leguminosarum for Vicia (vetch) and Lathyrus (pea) spp., and R. lupini for Lupinus (lupine) spp. Other cross inoculation groups exist, and complete lists of host-Rhizobium compatabilities can be obtained from inoculum suppliers (Burton and Martinez, 1980).

Rhizobium strains also differ in site preferences, i.e. in tolerances for extremes in soil pH, soil moisture, and soil temperature, and in susceptibilities to biotic stresses including competitors, predators, and parasites (Lowendorf, 1980). Again, information on the adaptations of specific strains can be obtained through inoculum suppliers and local specialists.

Frankia strains show a degree of host specificity. Two cross inoculation groups have been broadly defined but no species names have been assigned (Baker et al., 1981). Little is known about the ecological specificity of Frankia strains.

VA mycorrhizal fungi show little if any host specificity. Under proper conditions, most strains can colonize most plants. Some site specificity though has been shown. Some species (e.g. Glomus mosseae) tend to prefer soils with near neutral soil pH's, while others (e.g. Acaulospora laevis) prefer more acid conditions (Young et al., 1985). Some isolates appear more tolerant of high phosphorus soils than do others (Hayman et al., 1976). There is undoubtedly additional variation between strains for tolerances to extremes in soil moisture and soil temperature although these have not been well documented. Again, it would be prudent to contact inoculum producers and local specialists for advice on site-specific strain selection.

Ectomycorrhizal fungi and their hosts show varying degrees of microbe-host specificity. Many pines and firs, for example, can be colonized by a wide range of ectomycorrhizal fungi (Molina and Trappe, 1982) while plants such as alders and poplars tend to be more selective (Molina, 1981). Differences in tolerances for specific soil conditions (e.g. organic matter content) have been demonstrated between some fungi, but there are few such studies, and generalizations cannot be made at this time. Again, it would be worthwhile contacting inoculum producers and local specialists for advice on site-specific strain selection.

For situations in which little is known concerning strain specificity, on-site test plots comparing the performance of several inoculant strains should be considered as a means of obtaining specific information.

COMMERCIAL AVAILABILITY OF INOCULANTS AND INOCULATED TRANSPLANTS AND GUIDELINES FOR ORDERING MATERIALS

Table 1 summarizes information on the availability of microbial inoculants. Names of producers/distributors, and their product lines are given for the various groups of nitrogen-fixing bacteria and mycorrhizal fungi.

While many of the Rhizobium inoculants commonly used in agriculture can be purchased off of the shelf from local distributors of seed and agricultural products, the majority of inoculants suitable for reclamation purposes must be ordered through source manufacturers. The Nitragin Company, a leading manufacturer of Rhizobium inoculants, produces over 100 inoculum formulations for use with almost 500 species of legumes (Burton and Martinez, 1980). Only a few of these formulations are produced on a regular basis. The majority are custom produced and require a lead time of 3-4 weeks from order to delivery.

Most distributors of native and rangeland seeds will precoat legume seed with appropriate Rhizobium strains upon request. Precoating is not a standard practice in the Intermountain West, and reclamation specialists should specify preinoculation if it is desired. Lead times of 3-4 weeks may be required for precoating.

Frankia inoculants are available on a limited basis from two companies, Rhizotec in Canada and NPI in Salt Lake City, UT. All Frankia inoculants must be custom ordered several months in advance, as these organisms grow slowly. Strains suitable for use with Alnus spp. are the most available and easy to use. Production and use of inoculants for Purshia, Cercocarpus, and many other actinorhizal hosts are not yet routine.

VA mycorrhizal inoculum is available from NPI in Salt Lake City. The company currently produces five species, Glomus mosseae, G. intraradices, G. deserticolum, G. etunicatum and Gigaspora margarita. The first three are most suitable for reclamation purposes in the intermountain west. Small batches of inoculum (less than five liters) are generally available. Larger batches (up to several hundred liters) should be custom ordered 6-8 months in advance (again these organisms are slow growing). Because application of VA mycorrhizal inoculum is not a routine practice at this time, it may be preferable to purchase inoculum in the form of preinoculated transplants.

Vegetative ectomycorrhizal inoculum is available in limited quantites from Sylvan Spawn Laboratory, Inc. in Kittanning, PA. Sylvan Spawn will produce commercial batches, in multiples of 90 liters, on an advanced order basis. Two to three months of lead time are required. At present the company produces inoculum of Pisolithus tinctorius, Laccaria laccata, and Hebeloma sp. A dozen other fungal strains are in various stages of research and development. The company expects to be in full scale commercial production of ectomycorrhizal inoculum by 1987.

Two companies, Mycor Tec in Greenville, CA and International Forest Seed Co. in Odenville, AL, sell spores of Pisolithus tinctorius, an ectomycorrhizal fungus that has benefited the establishment of pines on disturbed lands in the Southeast.

Starter cultures of many mycorrhizal fungi and nitrogen-fixing bacteria can be obtained from universities, the U.S. Forest Service, industrial laboratories, and the American Type Culture Collection (ATCC) based in Rockville, MD. Cultures are often provided free of charge although the ATCC does require a fee.

Preinoculated transplants, nursery grown stock that is pre-colonized by mycorrhizal fungi and/or nitrogen-fixing bacteria, can be purchased on an advanced-order basis from a variety of nurseries. Lead times of 12-18 months are typically required to satisfy production times for the inoculum and the plants. Care should be taken to specify the nature of the inoculum used. Pure inoculum is most desirable. Some growers will use untreated forest or field soils as inoculum sources, and this should be avoided because raw soils may carry pathogens. As mentioned earlier inoculation of many shrubs, trees and forbs is not yet a routine practice and most growers will perform inoculations on a best-effort, rather than a guaranteed, basis.

APPLICATION

Microbial inoculants are most efficiently applied to reclaimed lands in direct conjunction with plant materials. Rhizobium inoculants should be coated on seeds prior to sowing. Frankia, VA mycorrhizal, and ectomycorrhizal inoculants should be applied to seedlings in nurseries, and the preinoculated plants, with their microbial associations established, should then be transplanted into the field. Broadcast dispersal of inoculants directly onto reclaimed soils is an inefficient means of application and should be avoided. If inoculants have to be applied directly to field soils, they sould be placed directly beneath seed or transplants by hand or with a fertilizer drill. Reclamation specialists can consult with inoculum suppliers or other specialists concerning proposed application techniques. Again, because microbial inoculations are not routine in many instances, experimental test plots can be helpful in establishing effective application methods.

SUMMARY

Microbial inoculants, containing mycorrhizal fungi and nitrogen-fixing bacteria, are commercially available for use in reclamation. Inoculation should be considered, especially when reclamation plans involve severely disturbed soils and/or microbially dependent plant species. In purchasing inoculants, reclamation specialists should consider three points: (1) strains of mycorrhizal fungi and nitrogen-fixing bacteria show varying degrees of host and site specificity and efforts should be made to select the most effective strains for a particular project; (2) most microbial inoculants and inoculated transplants must be custom ordered with lead times varying from 3-4 weeks to 12-18 months; (3) application of many microbial inoculants is not a routine undertaking, and reclamation specialists should not only consult with inoculum suppliers and other experts in developing their reclamation strategy, but also consider the use of experimental plantings as a means for determining inoculation needs, identifying superior strains, and developing efficient inoculation techniques.

Table 1. Sources of microbial inoculants and inoculated transplants. Some materials are available on a regular commercial basis (+) while others must be custom ordered (custom).

Producer/Distributor	Bulk Inoculum	Inoculated Seed/Plants	Starter Cultures
RHIZOBIUM			
Nitragin Sales Corp., Milwaukee, WI	+/Custom		
Kalo Industries, Kansas City, MO	+		
Urbana Laboratories, Urbana, IL	+		
Local Seed Companies		Custom	
NPI, Salt Lake City, UT		Custom	+
Local Universities			+
FRANKIA			
NPI, Salt Lake City, UT	Custom	Custom	+
Rhizotec Laboratories, Canada		Custom	
Local Universities			+
VA MYCORRHIZAL FUNGI			
NPI, Salt Lake City, UT	+/Custom	Custom	+
Local Universities			+
ECTOMYCORRHIZAL FUNGI			
Sylvan Spawn Laboratory, Worthington, PA	Custom		
Mycor Tec, Greenville, CA	+		
International Forest Seed Co., Odenville, AL	+		
Select Nurseries		Custom	
NPI, Salt Lake City, UT			+
Local Universities			+
U.S. Forest Service			+

LITERATURE CITED

Abbott, L.K. and A.D. Robson. 1977. The distribution and abundance of vesicular arbuscular endophytes in some western Australian soils. Aust. J. Bot. 25: 515-522.

Akkermans, A.D.L., D. Baker, K. Huss-Danell, and J.D. Tjepkema (eds.). 1984. Frankia Symbioses. Martinus Nijhoff/Dr. W. Junk Publ., Boston. 258 pp.

Allen, E.B. and M.F. Allen. 1980. Natural re-establishment of vesicular-arbuscular mycorrhizae following strip mine reclamation in Wyoming. J. Appl. Ecol. 17: 180-147.

Baker, D., W.L. Pengelly and J.G. Torrey. 1981. Immunochemical analysis of relationships among isolated frankiae (Actinomycetales). Int. J. Syst. Bacteriol. 31: 148-151.

Baylis, G.T.S. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. pp. 373-389. In F.E. Sander, B. Mosse, and P.B. Tinker (eds.). Endomycorrhizas. Academic Press, New York.

Berry. C.R. and D.H. Marx. 1978. Effects of Pisolithus tinctorius ectomycorrhizae on growth of loblolly and Virginia pines in the Tennessee Copper Basin. USDA For. Serv. Res. Note SE-264. 6 pp.

Burton, J.C. 1980. New developments in inoculating legumes. pp. 380-405. In N.S. Subba Rao (ed.). Recent Advances in Biological Nitrogen Fixation. Edward Arnold Ltd., London.

Burton, J.D. and C.J. Martinez. 1980. Rhizobia inoculants for various leguminous species. Technical Bulletin No. 101. The Nitragin Co., Milwaukee.

Call, C.A. and C.M. McKell, 1984. Field establishment of fourwing saltbush in processed oil shale and disturbed native soil as influenced by vesicular-arbuscular mycorrhizae. Great Basin Naturalist 44: 363-371.

Daft, M.J. and E. Hacskaylo. 1977. Growth of endomycorrhizal and non-mycorrhizal red maple seedlings in sand and anthracite spoil. For. Sci. 23: 207-216.

Gerdemann, J.W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Ann. Rev. Phytopath. 6: 397-418.

Hayman, D.S., J.M. Barea, and R. Azcon. 1976. Vesicular-arbuscular mycorrhiza in southern Spain: its distribution in crops growing in soil of different fertility. Phytopath. Medit. 15: 1-6.

Lambert, D.H. and H. Cole. 1980. Effects of mycorrhizae on establishment and performance of forage species in mine spoil. Agron. J. 72: 257-260. http://dx.doi.org/10.2134/agronj1980.00021962007200020003x

Lowendorf, H.S. 1980. Factors affecting survival of Rhizobium in soil. Adv. Microb. Ecol. 4: 87-124. http://dx.doi.org/10.1007/978-1-4615-8291-5_3

Marks, G.C. and T.T. Kozlowski. 1973. Ectomycorrhizae. Academic Press, New York. 444 pp.

Menge, J.A., R.M. Davis, E.L.V. Johnson, and G.A. Zentmyer. 1978. Mycorrhizal fungi increase growth and reduce transplant injury in avocado. Calif. Agric. April, 1978: 6-7.

Mikola, P. 1980. Mycorrhizae across the frontiers. pp. 3-10. In P. Mikola (ed.). Tropical Mycorrhiza Research. Clarandon Press, Oxford.

Molina, R. 1981. Ectomycorrhizal specificity in the genus Alnus. Can. J. Bot. 59: 325-334. http://dx.doi.org/10.1139/b81-045

Molina, R. and J.M. Trappe. 1982. Patterns of ectomycorrhizal host specificity and potential among Pacific Northwest conifers and fungi. For. Sci. 28: 423-458.

Powell, C.L. and D.J. Bagyaraj. (eds.). 1984. VA Mycorrhiza. CRC Press, Boca Raton, FL. 234 pp.

Reeves, F.B., D. Wagner, T. Moorman, and J. Kiel. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. Am. J. Bot. 66: 6-13. http://dx.doi.org/10.2307/2442618

Rives, C.S., M.I. Bajwa, A.E. Liberta, and R.M. Miller. 1980. Effects of topsoil storage during surface mining on the viability of VA mycorrhiza. Soil Sci. 129: 253-257. http://dx.doi.org/10.1097/00010694-198004000-00009

Wood, T. and W.H. Bollinger. In press. Tree improvement from the ground up: the potential for select microbial inocula in forestry. In S. Gliessman (ed.). Research Approaches in Agroecology.

Young, J.L., E.A. Davis, and S.L. Rose. 1985. Endomycorrhizal fungi in breeder wheats and triticale cultivars field-grown on fertile soil. Agron. J. 77: 219-224. http://dx.doi.org/10.2134/agronj1985.00021962007700020011x