Beneficial Microorganisms

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The web of life depends on microorganisms, a vast network of small and unseen allies that permeate the soil, water, and air of our planet. For people who work with plants, the greatest interest in microorganisms is in the complex communities that are part of the soil. Beneficial microorganisms are naturally occurring bacteria, fungi, and other microbes that play a crucial role in plant productivity and health. Two types of beneficial microorganisms, mycorrhizal fungi (figure 14.1) and nitrogen-fixing bacteria (figure 14.2), are considered beneficial to plant health. Mycorrhizal fungi and nitrogen-fixing bacteria are called "microsymbionts" because they form a symbiotic (mutually beneficial) relationship with plants.

In natural ecosystems, the root systems of successful plants have microbial partnerships that allow plants to survive and grow even in harsh conditions. Without their microsymbiont partners, plants become stunted and often die. Frequently, these failures are attributed to poor nursery stock when the real problem was the lack of the proper microorganism. In the nursery, microsymbionts can be introduced by "inoculating" the root systems of the plants with the appropriate beneficial microorganism to form an effective partnership. Inoculation methods usually incorporate the microorganisms into the growing media.

WHY USE BENEFICIAL MICROORGANISMS IN THE NURSERY?

As discussed in Chapter 2, *The Target Plant Concept,* plants for land restoration may in some ways be considered a root crop. In natural ecosystems, the root

Ectomycorrhizae on pine by Thomas D. Landis.

Figure 14.1—*The three types of mycorrhizal fungi are (A) ectomycorrhizal, which are visible to the naked eye, (B) arbuscular, and (C) ericoid, which are seen only with the aid of a microscope.* Photos A and B by Michael A. Castellano, C by Efren Cazares.

systems of successful plants have microbial partnerships with mycorrhizal fungi and, if applicable, with nitrogen-fixing bacteria. In the nursery, where plants have easy access to water and fertilizer, the benefits of these partnerships may not be apparent and their absence may go unnoticed. But, in the field, plants need every advantage. Plants that leave the nursery with microbial partnerships are better able to survive independently. Uninoculated plants have to "fend for themselves" to find microbial partners in the field and, while doing so, may grow poorly or die due to the stresses of outplanting or the harsher conditions of the outplanting site. In addition, many plantings take place on deforested or degraded land. Because microsymbionts often do not survive in soil in the absence of their host plants, native populations of microsymbionts may be low or nonviable.

Inoculating plants in the nursery is an opportunity to introduce select microbial partners. Similar to using select seeds, the nursery manager can match plants with optimal microbial partners for site conditions. The use of select microsymbionts has been shown to greatly improve plant survival, productivity, and growth. For this reason, the presence of microsymbionts is often an important target plant characteristic.

Using microsymbionts in the nursery has the following benefits:

- Reduced fertilizer use in the nursery.
- Improved plant health and vigor.
- Improved resistance to disease during the germination and establishment phases.
- Provided the opportunity to introduce superior or selected partnerships to meet the needs of the site conditions.

Inoculated plants may establish more quickly with less external inputs such as fertilizers, thereby reducing costs. For situations in which revitalized ecosystem function is a project goal, using plants that are already fixing nitrogen or partnered with mycorrhizal fungi can help accelerate the process.

MYCORRHIZAL FUNGI

Mycorrhizal fungi form partnerships with most plant families and forest trees. "Myco" means "fungus" and "rhizae" means "root;" the word "mycorrhizae" means "fungus-roots." Many plants depend on their partnership with these fungi. The host plant roots provide a convenient substrate for the fungus and supply food in the form of simple carbohydrates. In exchange for this free "room-and-board," the mycorrhizal fungi offer benefits to the host plant:

1. Increased water and nutrient uptake—Beneficial fungi help plants absorb mineral nutrients, especially phosphorus and several micronutrients such as zinc and copper. Mycorrhizae increase the root surface area, and the fungal hyphae access water and nutrients beyond the roots. In the field, when plants lack

mycorrhizae, they become stunted and sometimes "chlorotic" (yellow) in appearance.

2. Stress and disease protection—Mycorrhizal fungi protect the plant host in several ways. With some fungi, the mantle completely covers fragile root tips and acts as a physical barrier from dryness, pests, and toxic soil contaminants. Other fungi produce antibiotics which provide chemical protection.

3. Increased vigor and growth—Plants with mycorrhizal roots survive and grow better after they are planted out on the project site. This effect is often difficult to demonstrate but can sometimes be seen in nurseries where soil fumigation has eliminated mycorrhizal fungi from the seedbed. After emergence, some plants become naturally inoculated by airborne spores and grow much larger and healthier than those that lack the fungal symbiont.

Mycorrhizal fungi and nitrogen-fixing bacteria are the two main microsymbionts of concern to nurseries. Mycorrhizal fungi form partnerships with most plant families and all forest trees. Most trees depend on their partnership with these fungi. The fungi, present on plant root systems, enhance plant uptake of nutrients (especially phosphorus) and water and protect the roots from soil pathogens. The following three types of mycorrhizal fungi are important to native plant nurseries:

- **Ectomycorrhizae (ECM).** These fungi form partnerships with many temperate forest plants, especially pines, oaks, beeches, spruces, and firs (figure 14.1A)
- **Arbuscular Mycorrhizae (AM).** These fungi are found on most wild and cultivated grasses and annual crops; most tropical plants; and some temperate tree species, including cedars, alders, and maples (figure 14.1B)
- **Ericoid Mycorrhizae**. These fungi form partnerships with plants in the families of heath (Epacridaceae); crowberry (Empetraceae); sedge (Cyperaceae); and most of the rhododendrons (Ericaceae), including the genera with blueberries, cranberries, crowberries, huckleberries, kinnikinnick, azaleas, and rhododendrons (figure 14.1C).

Figure 14.2—*Nitrogen-fixing bacteria include* Rhizobium *that form relationships with plants in the legume family. Rhizobium nodules on the roots of (A) leadplant and (B) acacia.* Photos by Tara Luna.

These fungi may be obtained from either commercial suppliers or from soil collected around a healthy host plant of the species being propagated. In all cases, inoculum must physically contact living roots of the plant in order to colonize effectively. Ways to acquire and successfully apply mycorrhizal fungi are explained in subsequent sections. While the fungi are similar in how they function and in their benefits to host plants, they appear differently on roots. Each mycorrhizal type has a unique application method that must be described independently. However, management practices in the nursery are similar and will be discussed together. The important thing to remember is that different plant species have specific fungal partners that must be matched appropriately to be effective (table 14.1).

Table 14.1—Plants and their mycorrhizal partners
(after Landis and 1989)

Ectomycorrhizae	
birch	larch
Douglas-fir	oak
fir	pine
hemlock	spruce
Ectomycorrhizal and arbuscular mycorrhizal fungi	
juniper	
poplar	
walnut	
Arbuscular mycorrhizal fungi	
ash	sweetgum
cherry	sycamore
maple	tuliptree
plum	western redcedar
redwood	
Ericoid mycorrhizal fungi	
azalea	kinnikinnick
blueberry	pipsissewa
cranberry	rhododendron
crowberry	rushes
huckleberry	sedges

ECTOMYCORRHIZAE (ECM) FUNGI

What are ECM fungi and what plants do they affect? Many familiar mushrooms, including puffballs and truffles are fruiting bodies of ECM fungi. These fruiting bodies are a small, aboveground portion of the total organism; underground, the fungus covering the short feeder roots of plants may be enormous. They form easily visible structures on roots and have great importance to many temperate forest species, especially evergreens and hardwoods in the beech and birch families. ECM fungi extend the volume of the feeding area of roots by many times and protectively coat the feeder roots.

SOURCES AND APPLICATION OF ECM FUNGI

Three common sources of ECM fungi inoculants are soil, spores, or pure-culture inoculant.

Soil

Historically, topsoil, humus, or duff from beneath ECM fungi host trees has been used to inoculate nursery plants. This practice is more common in bareroot nurseries than in container nurseries. Sometimes litter, humus, or screened pine straw from the forest floor has also been used. Because sterilization would kill these beneficial fungi, unsterilized soil and organic matter are incorporated into the growing media, up to 10 percent by volume. Three disadvantages of using soil as a source of inoculants are that (1) large quantities of soil are required, which can make the process labor intensive and have a detrimental effect on the natural ecosystem, (2) the quality and quantity of spores may be highly variable, and (3) pathogens may be introduced along with the inoculant. Therefore, using soil as a source of inoculant is discouraged unless spores or pure-culture inoculum is unavailable. If soil is used, inoculum should be collected from plant communities near the outplanting site. Small amounts should be collected from several different sites and care should be taken not to damage the host plants.

Spores

Nurseries can make their own inoculum from spores. Collected from the fruiting bodies of mushrooms, puffballs, and especially truffles (figure 14.3), these fruiting bodies, full of spores, are rinsed, sliced, and pulverized in a blender for several minutes. The resulting thick liquid is diluted with water and poured into the growing media of germinating seedlings or newly rooted cuttings. Plants are usually inoculated 6 to 12 weeks after sowing or striking (figure 14.4). Two applications 2 to 3 weeks apart are recommended to ensure even inoculation. Some spore suspensions are also available from commercial suppliers of mycorrhizal inoculants. The quality of commercial sources varies, however, so it is important to have this verified.

Pure-Culture Inoculum

Mycorrhizal fungi are also commercially available as pure cultures, usually in a peat-based carrier (figure 14.5). Most commercial sources contain several different species of ECM fungi. The quality of commercial sources can be variable; it is important to make sure a product with a verified high spore count is applied. Commercial inoculum can be purchased separately and mixed into the growing medium as per the instructions on the product and prior to filling containers, or purchased already mixed into bales of growing medium. Using commercial sources may be the easiest way to begin learning how to acquire and apply inoculant. Because the inoculum is from pure cultures, finding selected strains to match site needs may be difficult.

VERIFYING THE EFFECTIVENESS OF ECM FUNGI INOCULATION

With practice, nursery staff can learn to recognize ECM fungi on the root systems of plants—they are fairly easy to see. During the hardening phase, short feeder roots should be examined for a cottony white appearance on the roots (figure 14.6A) or a white or brightly colored mantle or sheath over the roots (figure 14.6B). Unlike pathogenic fungi, mycorrhizae will never show signs of root decay and the mycelia around the root will be visible. Sometimes, mushrooms or other fruiting bodies will occasionally appear in containers alongside their host plants (figure14.6C). While these structures are visible to the unaided eye, it is also recommended to send plant samples to a laboratory for verification. A local soil extension agent or university can likely assist with this process.

ARBUSCULAR MYCORRHIZAE (AM) FUNGI

What are AM fungi and what plants do they affect? More than 80 percent of the world's plant families are associated with AM fungi. AM fungi can be observed only under a microscope and are essential for many

Figure 14.3—*Fruiting bodies of ectomycorrhizal fungi, such as (A)* Boletus satanus *common under oaks, (B)* Cantharellus cibarius *(chantrelles) common under spruces, and (C)* Amanita muscaria *common under pines, can be collected and used as a source of inoculum for native plant nurseries.* Photo A by Dan Luoma, B by Thomas D. Landis, C by Michael A. Castellano.

Figure 14.4—*Inoculating tree seedlings with ectomycorrhizal fungi. Two applications 2 to 3 weeks apart are recommended to ensure even inoculation.* Photo by Michael A. Castellano.

Figure 14.5—*Forms of commercial inoculant available for nurseries include spores in a carrier to be used at the nursery or at the outplanting site.* Photo by Thomas D Landis.

temperate hardwood trees, most annual crops and grasses, and most tropical trees and plants. AM fungi mycelia penetrate plant roots and extend far into soil, taking up nutrients and water for the plant. Unlike ECM fungi, however, AM fungi on the roots of plants are not visible to the unaided eye, and their much larger spores are not easily disseminated by wind or water.

SOURCES AND APPLICATION OF AM FUNGI

Inoculant for AM fungi may be collected from soil underneath AM fungi host plants and incorporated into the growing media. As with ECM fungi, this method is discouraged because of damage to natural ecosystems, variability of effectiveness, and risk of introducing pests and pathogens. Two main sources of AM fungi inoculant for nurseries are "pot culture," also known as "crude" inoculant, and commercially available pure cultures. Because AM spores are relatively large, it is critical to ensure that spores come in to direct contact with the root systems or seeds. Spores will not easily pass through irrigation injectors or nozzles, and do not move downward or through the soil with water. Therefore, thorough incorporation into the growing media is necessary.

Pot Culture

In pot culture inoculant, a specific AM fungi species is acquired either commercially or from a field site as a starter culture and then added to a sterile growing medium. A host plant such as corn, sorghum, clover, or an herbaceous native plant, is then grown in this substrate; as the host grows, the AM fungi multiply in the medium (figure 14.7). Shoots of host plants are removed and the substrate, now rich in roots, spores, and mycelium, is chopped up and incorporated into fresh growing medium before containers are filled and seeds are sown or cuttings stuck. This technique is highly effective for propagating AM fungi in the nursery. For details on how to use this method, consult the publications in the Literature Cited section of this chapter, particularly the book *Arbuscular Mycorrhizas: Producing and Applying Arbuscular Mycorrhizal Inoculum* (Habte and Osorio 2001).

Commercial Culture

Commercial sources of AM fungi inoculant are also available, usually containing several species or strains. These products are thoroughly incorporated into the growing medium before filling containers. Because AM fungi spores are so small and fragile, they are usually mixed with a carrier such as vermiculite or calcined clay to aid in application. Products are meant for incorporation into soil or growing media. Inoculation effectiveness has been shown to vary considerably between different products so it is wise to test before purchasing large quantities of a specific product. Laboratories can provide a live spore count per volume, which is the best measure of inoculum quality.

Verifying the Effectiveness of AM Fungi Inoculation

Unlike ECM fungi, AM fungi are not visible to the unaided eye. To verify the effectiveness of AM fungi inoculation, roots must be stained and examined under a microscope. This verification can often be done easily and inexpensively through a soil scientist at a local agricultural extension office.

ERICOID MYCORRHIZAL FUNGI

Plants that form partnerships with ericoid mycorrhizal fungi are able to grow in nutrient-poor soils and harsh conditions, including bogs, alpine meadows, tundra, and even in soils with high concentrations of certain toxic metals. Ericoid mycorrhizal fungi form partnerships in the plant order Ericales in the heath (Epacridaceae), crowberry (Empetraceae), and most of the rhododendron (Ericaceae) families. Similar to ECM fungi and AM fungi, ericoid mycorrhizal fungi must come into contact with the roots of host plants to form partnerships. Ericoid mycorrhizal inoculant is available as commercial cultures or from soil near healthy host plants. The product or soil is mixed into nursery growing medium. The fungus forms a net over the narrow "root hairs" of the plants, infecting the outer cells. The plant's cell membrane grows to envelop the fungal hyphae. Nutrients are shared through the membrane that forms the boundary between the fungus and plant roots. Laboratory confirmation is recommended to verify that successful inoculation has taken place.

MANAGEMENT CONSIDERATIONS FOR MYCORRHIZAL FUNGI

As with the introduction of any new practice, it takes time to learn how to work with mycorrhizal fungi. In some cases, working with a pure-culture product may be the easiest way to begin; the nursery can then expand into collecting and processing its own inoculant sources, if desired. It should be noted that some select or pure-culture inoculants may support high productivity in certain site conditions but may be less productive than native strains on other sites. If possible, the nursery can work with a specialist to help with the following tasks:

- Determining the best sources of inoculant and evaluating their effectiveness in the nursery.

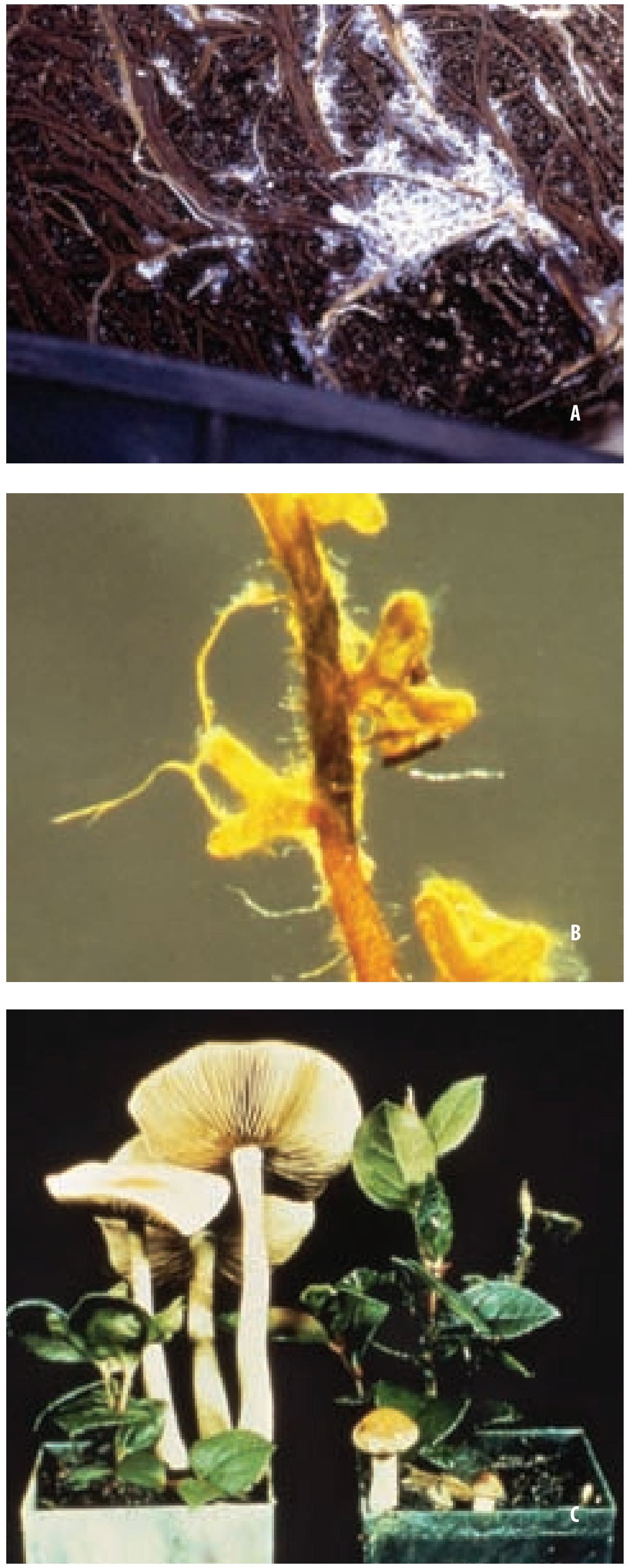

Figure 14.6—*Staff can learn to recognize the presence of ectomycorrhizal fungi (ECM) by examining plants during the hardening phase. (A) Spruce with and without cottony-white ectomycorrhizae on the roots, (B) brightly colored ectomycorrhizal fungi on the roots of lodgepole pine, and (C) fruiting bodies growing from containers.*

Photo A by William Sayward, B and C by Michael A. Castellano.

Figure 14.7—*In pot culture inoculant, a specific arbuscular mycorrhizal fungi species is acquired as a starter culture and added to a sterile potting medium with a fast-growing host plant. The shoots of host plants are removed, and the substrate, now rich in roots, spores, and mycelium, is chopped up and incorporated into the growing medium before containers are filled.* Photo by Tara Luna.

- Selecting optimal mycorrhizal partners for the species and outplanting sites.
- Designing outplanting trials to evaluate plant survival and mycorrhizal performance in the field and modifying the inoculant sources if improvements are needed.

In addition to learning how to effectively apply mycorrhizal fungi, some modifications of nursery management will be required to support the formation of mycorrhizal partnerships in the nursery. These modifications usually involve changes in fertilization and watering regimes and also likely will include changes in planting rates and scheduling.

Fertilization is probably the most significant adjustment. Mycorrhizal fungi extend the plant's root system and extract nutrients and water from the medium. High levels of soluble fertilizers in the growing medium inhibit formation of mycorrhizae. In some cases, the quantity of fertilizer used may be reduced by half or more due to the efficiency of nutrient uptake by mycorrhizal fungi. Fertilizer type and form is also important. An excessive amount of phosphorus in the fertilizer inhibits formation of the partnership; therefore phosphorus should be reduced. If nitrogen is applied, ammonium-nitrate is better used by the plant than is nitrate-nitrogen (Landis and others 1989). Generally, controlled-release fertilizers may be better than liquid fertilizers for inoculated plants because they release small doses of nutrients gradually rather than sudden higher doses. Water use is also affected; excessive or inadequate water will inhibit the presence of mycorrhizal fungi and the formation of the partnership, so watering schedules should be modified accordingly. Nursery staff who are willing to be observant and flexible as the nursery embarks on the use of mycorrhizal fungi will be the best decisionmakers in terms of modifying fertilization and watering regimes to support the microsymbionts.

Other adjustments may be necessary. Mycorrhizal inoculants may improve survival percentages of plants; these percentages will affect estimates and oversow rates. Scheduling may also be affected; inoculated plants may be ready for outplanting sooner than uninoculated plants. Applications of certain herbicides, insecticides, fungicides, and nematicides are detrimental to mycorrhizal fungi; susceptibility varies by species and pesticide applications should be assessed and adjusted.

When using mycorrhizal inoculants for the first time, it is recommended to start small and evaluate a few techniques and sources. Compare some trays or benches with and without mycorrhizae to determine how management and scheduling will be modified to support the presence of the fungi. Monitor the effectiveness of inoculation and keep records of crop development.

It should be noted that some plants form partnerships with both AM fungi and ECM fungi. These plants include juniper, poplar, and walnut (see table 14.1). In these cases, both kinds of mycorrhizal inoculant can be applied in the nursery for best results.

WHAT ARE NITROGEN-FIXING BACTERIA?

Nitrogen-fixing bacteria accumulate ("fix") nitrogen from the air. Many species live in root nodules and thereby share the nitrogen with their host plants. Unlike mycorrhizal fungi, which affect most trees and plants, only a fraction of native plants can form partnerships with nitrogen-fixing bacteria. The role of nitrogen-fixing bacteria and their partner plants in land restoration and ecosystem health is crucial. Nitrogen-fixing species are usually outplanted to help restore fertility and organic matter to the ecosystem. Soil at the outplanting site, however, may not contain a viable strain of bacteria to form a symbiotic partnership with the plant. Inoculating plants in the nursery ensures that nitrogen-fixing plants form an effective partnership to fix nitrogen. For nurseries that grow nitrogen-fixing plants, the introduction of nodule-forming bacteria to plant root systems is a key practice in meeting target plant requirements. Using these inoculants in the nursery can be an important part of accelerating rehabilitation of degraded land by enhancing plant survival and growth.

Without the bacterial partnership in place, plants are unable to make direct use of atmospheric nitrogen for their own fertility. Although plants that form this association are sometimes called "nitrogen-fixing plants" or "nitrogen-fixing trees," the plant itself is not able to accumulate nitrogen from the air. It is only through the partnership with nitrogen-fixing bacteria that these plants are able to obtain atmospheric nitrogen. It is a symbiotic partnership: the bacteria give nitrogen accumulated from the atmosphere to the plant, and in exchange the bacteria get energy in the form of carbohydrates from the plant.

Two types of nitrogen-fixing bacteria are used by plants: *Rhizobium* and *Frankia*. *Rhizobium* grow with some members of the legume family and plants of the elm family (Ulmaceae) (figure 14.8). *Frankia* are a kind of bacteria that form partnerships with about 200 different plant species distributed over eight families (figure 14.9). The species affected by *Frankia* are called "actinorhizal" plants (table 14.2).

Many, but not all, species of the legume family nodulate with *Rhizobium*. This family is made up of three subfamilies. Although most legume species in temperate regions of the world fix nitrogen, the subfamily Caesalpinioideae is mostly tropical in distribution and has fewer species that fix nitrogen.

HOW DOES BIOLOGICAL NITROGEN FIXATION WORK?

This symbiotic partnership between plants and their nitrogen-fixing microsymbionts consists of bacteria

Figure 14.8—*Legume species that form relationships with the nitrogen-fixing bacteria* Rhizobium *include (A) lupine, (B) American licorice, and (C) acacia. Because these species improve soil fertility on degraded lands, they are widely used for restoration projects* Photos by Tara Luna.

Figure14.9—*Species that form relationships with nitrogen-fixing* Frankia *bacteria include (A) buffaloberry, (B) deerbrush, and (C) mountain-avens. As with legumes, these species improve soil fertility on degraded lands.* Photos by Tara Luna.

living in nodules on the roots of the plant. Each nodule contains millions of the bacteria that accumulate atmospheric nitrogen. The bacteria share this nitrogen with the plant and in exchange receive energy (in the form of carbohydrates produced by photosynthesis).

When the nitrogen-fixing plant sheds leaves, dies back, or dies, the nitrogen stored in the plant's tissues is cycled throughout the ecosystem. This process, part of the nitrogen cycle, is the major source of nitrogen fertility in most natural ecosystems (figure 14.10).

BENEFITS OF INOCULATING WITH NITROGEN-FIXING BACTERIA

Application of inoculants of nitrogen-fixing bacteria can have some direct benefits in the nursery. If an effective partnership is formed, most of the plant's needs for nitrogen will be met. As a result, the need to apply nitrogen fertilizer is reduced or eliminated. This partnership also reduces management decisions associated with nitrogen fertilizer application, such as considerations about when to fertilize and what type of nitrogen to use. The plant is essentially self-rationing; if a productive partnership is formed, the plant can usually take up as much nitrogen as it needs through the partnership. The reduction of soluble nitrogen applications also decreases the nursery's contribution to pollution from fertilizer runoff. Nitrogen-fixing nursery stock with nodulated root systems have exhibited faster early growth than seedlings that were not inoculated.

In the field, however, is where the benefits of the partnership are most apparent. Plants sent from the nursery with their root systems already nodulating have exhibited faster early growth than plants that were not inoculated. Nursery inoculation can reduce costs in establishment and maintenance; a few dollars worth of inoculant applied in the nursery can replace a lot of purchased nitrogen fertilizer over the life of the tree. Also, instead of providing spurts of soluble fertilizers (which may benefit surrounding weeds as well as the desired plant), the natural nitrogen fixation process provides a steady supply of nitrogen for the plant's growth. Faster growth early on can also lead to faster canopy closure, shading the soil and understory and reducing expenses of weed management. Early formation of the partnership with nitrogen-fixing bacteria also means a faster restoration of the natural nutrient cycling and fertility role of nitrogen-fixing species in

Table 14.2—What native plants form partnerships with nitrogen-fixing bacteria? (adapted from NFTA 1986)

Bacteria	Family	Subfamily (notes)	Common Names
Frankia	Birch (Beulaceae)		alder, birch
	Buckthorn (Rhamnaceae)		cascara, ceanothus, deerbrush
	Myrtle (Myricaceae)		myrtle
	Oleaster (Elaeaganaceae)		buffaloberry, silverberry
	Rose (Rosaceae)		bitterbrush, cliffrose, fernbush, mountain mahogany, mountain-avens
Rhizobium	Legume (Fabaceae)	Caesalpinioideae (about 1,900 species; about 23% fix nitrogen)	cassia, honeylocust, Kentucky coffeetree, nicker, redbud
		Mimosaideae (2,800 species; about 90% fix nitrogen)	acacia, albizia, mesquite, mimosa (2,800 species;
		Papilionoideae (about 12,300 species; about 97% fix nitrogen)	American licorice, black locust, clover, Indian breadroot, indigobush, leadplant, locoweed, lupine milkvetch, prairie clover, sesbania, sweetvetch, vetch

the ecosystem, which is usually the purpose of planting nitrogen-fixing species in the first place.

In many cases, uninoculated plants may eventually form a partnership with some kind of *Frankia* or *Rhizobium* strain after they are outplanted. These partnerships may not be with optimal or highly productive bacterial partners, and it may take months or even years for the partnerships to form if effective microsymbiont populations in the soil are low or inactive. Until the partnership forms, the plants are dependent on inputs of nitrogen fertilizers or the nitrogen available in the soil. Without fertilizer on poor sites, uninoculated plants will grow very slowly, sometimes outcompeted by weeds. Inoculating in the nursery ensures that plants form effective, productive partnerships in a timely fashion.

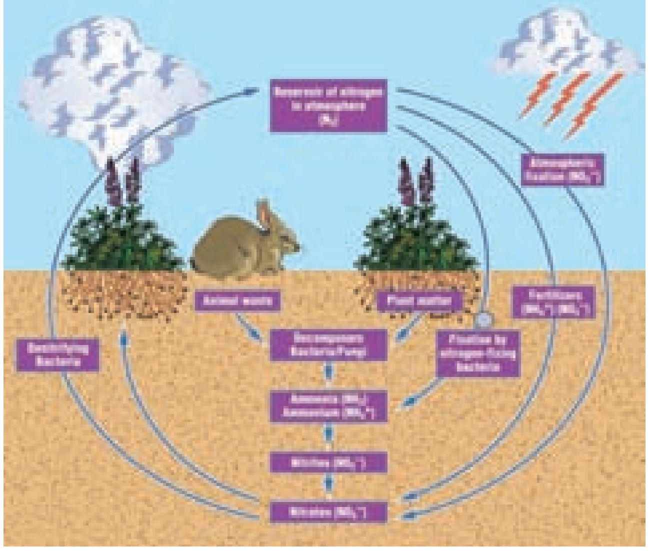

Figure 14.10—*The nitrogen cycle plays a central role in fertility in natural ecosystems; beneficial microorganisms are critical to this process.* Illustration by Jim Marin.

ACQUIRING INOCULANT FOR NITROGEN-FIXING BACTERIA

Inoculants for nitrogen-fixing bacteria tend to be very specialized. In other words, they are not "one size fits all." On the contrary, a different inoculant strain for each nitrogen-fixing species is usually necessary. Inoculants are live nitrogen-fixing bacteria cultures that are applied to seeds or young plants, imparting the beneficial bacteria to the plant's root system. Two forms of inoculant can be used in the nursery: pure-culture inoculant (figures 14.11A and B), and homemade (often called "crude") inoculant (figure 14.11C). Cultured inoculant is purchased from commercial suppliers, seed banks, or sometimes, universities. Crude inoculant is made from nodules collected from the roots of nitrogen-fixing plants of the same species to be inoculated. Whichever form is used, care should be taken when handling nitrogen-fixing bacteria inoculants because they are very perishable. These soil bacteria live underground in moist, dark conditions with relatively stable, cool temperatures. Similar conditions should be maintained to

Figure 14.11—*Nitrogen-fixing bacteria are commercially available as (A) pure-culture inoculants, (B) often in a carrier. (C) They can also be prepared by collecting nodules from plants in the wild.* Photos A and C by Tara Luna, B by Mike Evans.

ensure the viability of inoculant during storage, handling, and application.

PREPARING PURE-CULTURE INOCULANT

Pure-culture inoculants of nitrogen-fixing bacteria usually come in small packets of finely ground peat moss. Some manufactured inoculants contain select strains that have been tested for forming optimally productive partnerships with their host species. Select-strain inoculants should be used if they can be obtained; these substances contain optimal partners for the species they were matched for, providing a good supply of nitrogen at a low cost to the plant. Superior strains can yield significant differences in the productivity and growth rate of the host plant; in some cases, they yield over 40 percent better growth (Schmidt 2000). Manufactured products usually come with application instructions; these directions should be followed. In general, about 100 g (3.5 oz) of cultured inoculant is sufficient to inoculate up to 3,000 plants. Because they contain living cultures of bacteria, these inoculants are perishable and should be kept in cool, dark conditions, such as inside a refrigerator.

Peat-based inoculants are added to chlorine-free water to create a slurry. (Allowing a bucket of water to stand uncovered for 24 hours is a good way to let chlorine evaporate.) If a blender is available, using it to blend some inoculant in water is a good practice to ensure that the bacteria will be evenly mixed in the solution. If a blender is not available, a mortar and pestle or a whisk can be used. Five to 10 g (about 0.2 to 0.4 oz) of manufactured inoculant can inoculate about 500 plants, usually exceeding the recommended 100,000 bacteria per plant. After plants begin to nodulate, nodules from their roots can serve as the basis for making crude inoculant, as described in the following paragraphs. This way, if desired, inoculant need be purchased only once for each plant species grown and thereafter perpetuated using nodules.

PREPARING CRUDE INOCULANT

Crude inoculant is made by using nodules, the small root structures that house the bacteria. Nodules can be seen on plant roots when nitrogen is being fixed. Each of the nodules can house millions of bacteria. For *Rhizobium,* a brown, pink, or red color inside is usually a good indicator that the millions of bacteria in the nodule are actively fixing nitrogen. For *Frankia*, desirable nodules will be white or yellow inside. Gray or green nodules should be avoided, because they are likely inactive.

To make crude inoculant, select healthy, vigorous plants of the same species as the plants to be inoculated. Expose some of the root system of a nodulating plant in the nursery or field. If available, choose plants that were inoculated with select bacteria, as described previously. Young roots often contain the most active nodules. Search for nodules with the proper color and pick them off cleanly. If possible, collect nodules from several plants. Put nodules in a plastic bag or container and place them in a cooler for protection from direct sunlight and heat. As soon as possible after collection (within a few hours), put the nodules in a blender with clean, chlorine-free water. About 50 to 100 nodules blended in about 1 qt (1 L) of water is enough to inoculate about 500 plants. This solution is a homemade liquid inoculant, ready to apply in the same method as cultured inoculant, as described in the following sections.

APPLYING INOCULANT

Inoculation should take place as early in the plant's life as possible, when the plant will most readily form the association. In nursery conditions, inoculant for nitrogen-fixing bacteria is commonly applied when seedlings are just emerging, usually within 2 weeks of sowing, or just after cuttings have formed roots (figure 14.12A). This helps ensure successful nodulation and maximizes the benefits of using inoculants. The quart (1 L) of liquefied inoculant made from either nodules or cultured inoculant as per the instructions in the previous paragraphs is diluted in more chlorine-free water. For 500 plants, about 1.3 gal (5 L) of water is used. This solution is then watered into the root system of each plant using a watering can.

VERIFYING THE NITROGEN-FIXING PARTNERSHIP

Allow 2 to 6 weeks for noticeable signs, listed below, that the plant has formed a symbiotic partnership with nitrogen-fixing bacteria.

- Plants begin to grow well and are deep green despite the absence of added nitrogen fertilizer (figure 14.12B).
- Root systems give off a faint but distinctive ammonia-like scent.
- Nodules are usually visible on the root system after about 4 to 6 weeks (figures 14.12C).
- Nodules are pink, red, or brown (for *Rhizobium*), or yellow or white (for *Frankia*).

MANAGEMENT CONSIDERATIONS

As with any nursery practice, becoming familiar with the application and management of nitrogen-fixing microsymbionts is a learning process. Several factors, listed below, are of primary concern to the nursery manager when using inoculants for nitrogen-fixing bacteria.

- **Timing.** The nursery manager should be mindful that inoculant is applied in a timely fashion, when seedlings are just emerging or cuttings are just forming new roots. This timing helps ensure successful nodulation and maximizes the nursery benefits of using inoculants.
- **Fertilization.** The use of nitrogen-fixing bacterial inoculant requires some adjustments in fertilization. Excessive nitrogen fertilizer will inhibit formation of the partnership. The application of nitrogen should be eliminated from nitrogen-fixing plants, and they might need to be isolated from non-nitrogen-fixing species to achieve this result.
- **Water Quality**. Excessive chlorine in water is detrimental to *Rhizobium* and *Frankia*. The water supply may need to be tested and a chlorine filter obtained if excessive chlorine is a problem in the water supply.
- **Micronutrients**. Some nutrients, including calcium, potassium, molybdenum, and iron, are necessary to facilitate nodulation. These nutrients should be incorporated into the growing media.
- **Sourcing Inoculants**. Locating appropriate sources of viable inoculants (either cultured or obtained as nodules) may require some research and time. The assistance of a specialist is invaluable. The benefits of successful inoculation are well worth the effort.

Most plants that form partnerships with nitrogen-fixing bacteria also require mycorrhizal partners; it is fine to inoculate plants with both microsymbionts as needed. Simply apply each inoculant separately, as described previously.

OTHER BENEFICIAL MICROORGANISMS

In natural soil, communities of bacteria, fungi, algae, protozoa, and other microorganisms make nutrients available to plants, create channels for water and air, maintain soil structure, and cycle nutrients and organic

Figure 14.12—*(A) Alder seedlings, inoculated with* Frankia *at 4 weeks of age, grow well the next 2 months as (B) the partnership forms and the bacteria survive and multiply on the root system, (C) creating nodules that can be easily seen.* Photos by Tara Luna.

matter. A healthy population of soil microorganisms can also maintain ecological balance, preventing the onset of major problems from viruses or other pathogens that reside in the soil. The practice of introducing beneficial microorganisms dates to ancient times. As a science, however, the use of beneficial microorganisms is in its infancy. Although thousands of species of microorganisms have been recognized and named, the number of unknown species is estimated to be in the millions. Almost every time microbiologists examine a soil sample, they discover a previously unknown species (Margulis and others 1997). Nursery staff should keep an eye on developments in this field and see how their plants can benefit from new insights into the roles of microorganisms. Working with these small and unseen allies is an important link to the greater web of life.

SUMMARY

Using mycorrhizal fungi and nitrogen-fixing bacteria in the nursery involves working with living organisms and adjusting the environment to foster them. In many cases, nurseries shifting from chemical fertilizer regimes into the use of beneficial microorganisms will have to make many adjustments to their management practices and schedule. The process of introducing microsymbionts does not involve a simple, direct replacement of chemical fertilizers. The results, however, in terms of stronger plants better able to survive independently makes taking up the challenge well worth the effort. In all, it is not as complicated as it may sound, and support can often be found through a local specialist or soil scientist.

By far, the greatest benefits of using microsymbionts in the nursery are seen in the field, with faster early growth and higher survival rates. The results in the field are the ones the nursery should focus on.

It is recommended to start small and try various sources of inoculants and options for their application. Work with a specialist or advisor if possible. Keep records and monitor results in the nursery. Verify that symbiotic partnerships are forming. After outplanting, follow up with clients to monitor the effectiveness of the microsymbionts. Also, teach clients about the importance of beneficial microorganisms: the more they understand the functions of these microsymbionts, the more they will appreciate the high quality of plant materials that have their microbial partnerships in place.

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APPENDIX 14.A. PLANTS MENTIONED IN THIS CHAPTER

acacia, *Acacia* species
albizia, *Albizia* species
alder, *Alnus* species
American licorice, *Glycyrrhiza lepidota*
ash, *Fraxinus* species
azalea, *Rhododendron* species
beech, *Fagus* species
birch, *Betula* species
bitterbrush, *Purshia tridentata*
blacklocust, *Robinia pseudoacacia*
blueberry, *Vaccinium* species
buffaloberry, *Shepherdia* species
cascara buckthorn, *Frangula purshiana*
cassia, *Cassia* species
ceanothus, *Ceanothus* species
cherry, *Prunus* species
cliffrose, *Purshia* species
clover, *Trifolium* species
cranberry, *Vaccinium* species
crowberry, *Empetrum* species
Douglas-fir, *Pseudotsuga menziesii*
elm, *Ulmus* species
fernbush, *Chamaebatia* species
fir, *Abies* species
heath, *Erica* species
hemlock, *Tsuga* species
honeylocust, *Gleditsia triacanthos*
huckleberry, *Gaylussacia* species and *Vaccinium* species
Indian breadroot, *Pediomelum* species
indigobush, *Psorothamnus arborescens*
juniper, *Juniperus* species
Kentucky coffeetree, *Gymnocladus dioicus*
kinnikinnick, *Arctostaphylos uva-ursi*
larch, *Larix* species
leadplant, *Amorpha canescens*
locoweed, *Oxytropis* species
lodgepole pine, *Pinus contorta*
lupine, *Lupinus* species
maple, *Acer* species
mesquite, *Prosopis* species
milkvetch, *Astragalus* species
mimosa, *Mimosa* species
mountain mahogany, *Cercocarpus* species
mountain-avens, *Dryas* species
myrtle, *Myrtus communis*
nicker, *Caesalpinia* species
oak, *Quercus*
pine, *Pinus* species
pipsissewa, *Chimaphila umbellata*
plum, *Prunus* species
poplar, *Populus* species
prairie clover, *Dalea* species
redbud, *Cercis* species
redwood, *Sequoia* species
rhododendron, *Rhododendron* species
rushes, *Juncus* species
sedges, *Carex* species
sesbania, *Sesbania* species
silverberry, *Elaeagnus* species
spruce, *Picea* species
sweetgum, *Liquidambar styraciflua*
sweetvetch, *Hedysarum* species
sycamore, *Platanus* species
tuliptree, *Liriodendron tulipifera*
vetch, *Vicia* species
walnut, *Juglans* species
western redcedar, *Thuja plicata*