Beneficial Microorganisms

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The web of life depends on microorganisms, a vast network of small, unseen allies that permeate the soil, water, and air of our planet. Many kinds of microorganisms existed for billions of years before any plants or animals came into being. Microorganisms created the atmosphere, turned bare rock and lava into soil, helped plants colonize land, and remain vital to the survival of plants, animals, and people today.

For people who work with plants, the greatest interest in microorganisms is in the complex living communities that are part of the soil. One gram (the weight of a small paperclip) of healthy soil can contain between 1 and 10 billion microorganisms. The living component of soil has a central role in ecosystem and plant health. Communities of bacteria, fungi, algae, protozoa, and other microorganisms make nutrients available to plants, create water and air channels, maintain soil structure, counterbalance pathogen populations, and recycle nutrients from organic matter that enable plants to grow.

This chapter focuses on two of the most important beneficial microorganisms for nurseries: nitrogen-fixing bacteria (rhizobia, the generic term for species in the genera *Rhizobium* and *Bradyrhizobium*)(figure 13.1) and mycorrhizal fungi (figure 13.2) that form mutually beneficial partnerships with their plant hosts. Scientists call this "mutualistic symbiosis." In this manual, these beneficial microorganisms are called "microsymbionts." Partnerships between beneficial microorganisms and plants are essential to plant health, as well as to healthy ecosystems and agro-ecosystems.

Facing Page: *The nitrogen-fixing bacteria rhizobia (*Bradyrhizobium*) form nodules on roots of legumes—in this case, on a native* Acacia koa *seedling in Hawai'i. Photo by J.B. Friday.*

In natural ecosystems, the root systems of most plants have microbial partnerships that enable them to survive and grow even in harsh conditions. In fact, microbial partnerships played a key role in enabling plants to first colonize land as they evolved out of the sea. Without microsymbiont partners, plants remain stunted and often die. These failures are frequently attributed to poor nursery stock when the real problem was the lack of the proper microsymbionts. In the nursery, microsymbionts can be introduced by "inoculating" the root systems of plants with the appropriate beneficial microorganisms to form effective partnerships.

Mutualistic Symbiosis

Symbiosis technically refers to two or more organisms living intimately interconnected. As a scientific term, symbiosis can be mutualistic (both organisms benefit), parasitic (one organism benefits and the other is harmed), or commensal (one benefits, the other is unaffected). In popular usage, however, "symbiosis" is considered synonymous with "mutualistic symbiosis"—both organisms benefit. In this chapter, we employ the popular usage to refer to nitrogen-fixing bacteria and mycorrhizal fungi as "microsymbionts"—microorganisms that form a mutually beneficial partnership with their plant hosts.

Figure 13.1*—Nitrogen-fixing bacteria include rhizobia, which form relationships with plants in the legume family. Pictured are native rhizobia nodules on the roots of the native Hawaiian forest tree koa (*Acacia koa*). Photo by J.B. Friday.*

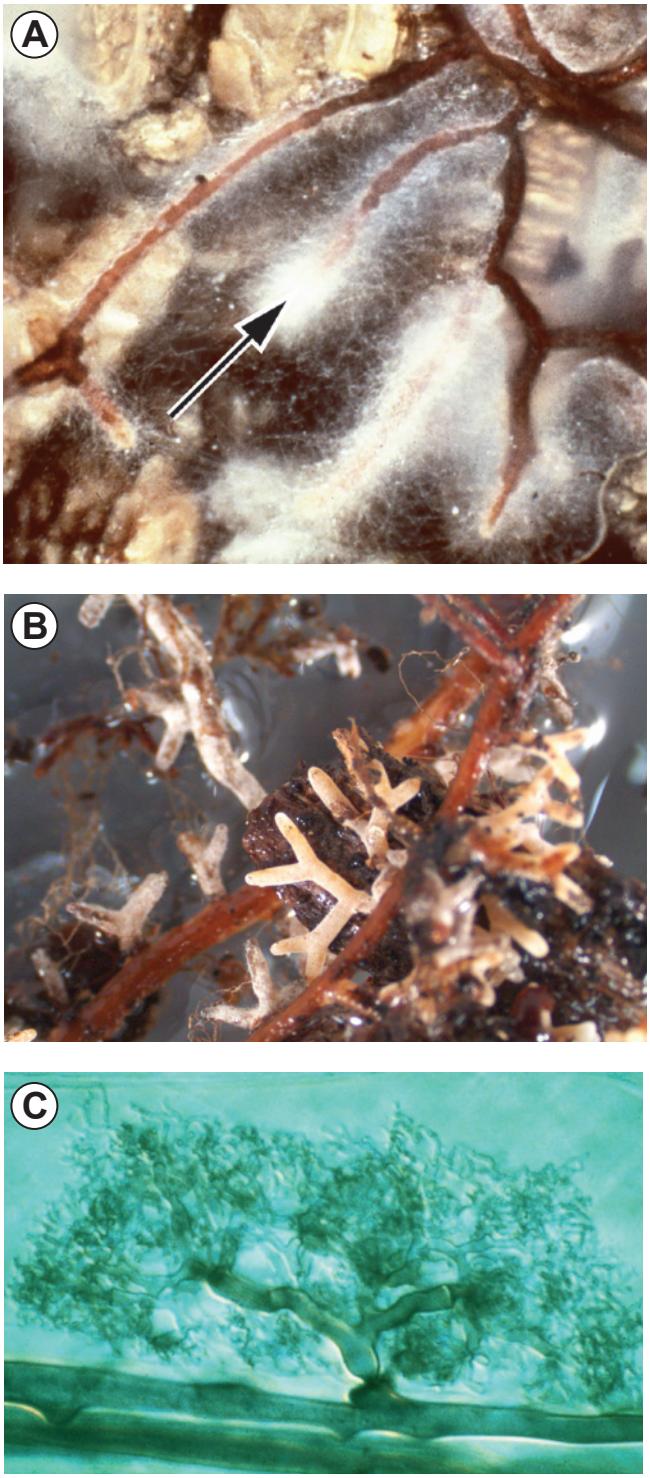

Figure 13.2*—"Myco" means "fungus" and "rhiza" means "root;" the word "mycorrhizae" means "fungus-roots." Mycorrhizal root tip (arrow) on a pine tree root (the white filaments are the fungus extending beyond the root) (A). Dichotomously branched, ectomycorrhizal roots of* Pinus elliottii *in Florida; the finest rootlets are entirely ensheathed by white fungus filaments (B). Arbuscule of an arbuscular mycorrhizal fungus (C) that serves as a nutrient exchange site between the host plant and the fungus. Photo A by Thomas D. Landis, photo B by Tania Wyss, and photo C by Mark C. Brundrett.*

The Importance of Beneficial Microorganisms in the Nursery

In natural ecosystems, the root systems of many 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 have been inoculated in the nursery will be outplanted with microbial partnerships in place and often are better able to survive in the field. Noninoculated plants, however, must "fend for themselves" and establish microbial partnerships in the field. Many plantings take place on deforested or degraded land where native microsymbiont populations may be low or nonviable (figure 13.3).

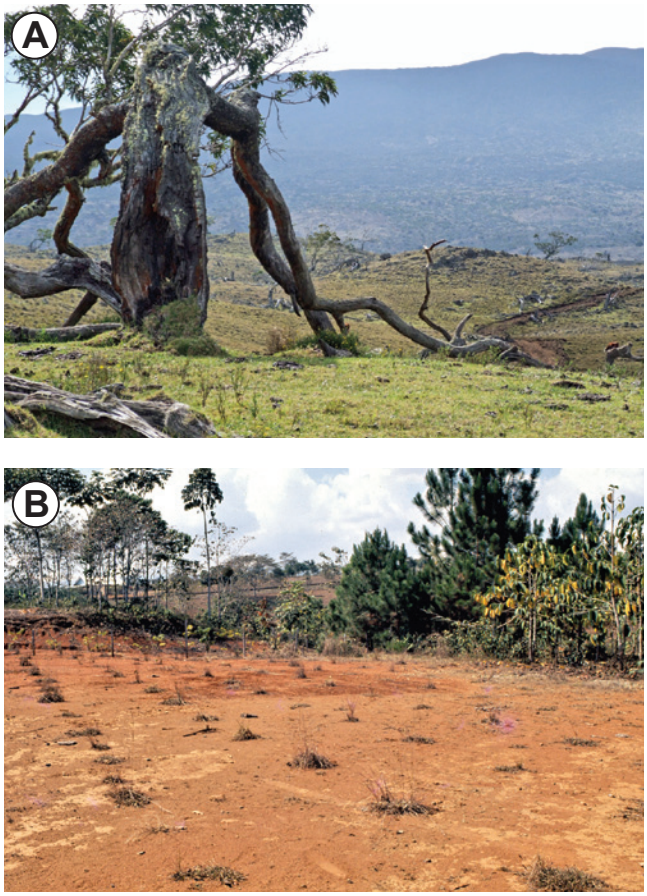

***Figure 13.3**—Microsymbionts often do not survive in the soil without their host plants. Native microsymbiont populations may be low on degraded sites, such as this formerly forested pasture in Hawai'i (A) and this test bauxite surface mine in southwestern Costa Rica—when the aluminum-rich topsoil was removed from the mine, most mycorrhizal fungi were removed with it (B.) Photo A by J.B. Friday, and photo B by David P. Janos.*

***Figure 13.4**—Plants with established microsymbiont partnerships often have a better chance of survival after outplanting. This photo shows a palm species,* Bactris gasipaes*; the plant on the right has a mycorrhizal partnership in place and the same age plant on the left does not. Photo by David P. Janos.*

Inoculating plants in the nursery is an opportunity to introduce select microsymbionts (figure 13.4). Similar to using seeds from specific seed sources, the nursery manager can match plants with optimal microsymbionts for specific site conditions. The presence of microsymbionts is often an important target plant characteristic.

Using microsymbionts in the nursery has the following benefits:

- Reduced environmental effects and fertilizer use in the nursery.
- Improved plant health and vigor.
- Improved resistance to disease.
- Better performance on outplanting sites.

Although this manual is for tropical nurseries, some tropical regions have montane habitats and species, and so a broad range of plant species and microsymbionts are mentioned in this chapter. Not all species and microsymbiont partners are present in a given region, so it is important to check about native species needs before using microsymbionts.

Nitrogen-Fixing Bacteria

Nitrogen is one of the most important nutrients for plant growth. Nitrogen (N_2) is abundant in the Earth's atmosphere, but the N_2 gas must be converted to either nitrate (NO_3^-) or ammonium (NH_4^+) ions before most plants can use it. In nature, nitrogen-fixing bacteria convert ("fix") N_2 from the air into a form usable to plants. When the growing roots of a plant capable of forming a partnership with rhizobia come in contact with a compatible strain of nitrogen-fixing bacteria in soil or growing media, the rhizobia bacteria will enter ("infect") the roots. Nodules then form on the plant's roots where the contact occurred. The bacteria live and multiply in the nodules on the host's root system, providing nitrogen from the atmosphere to their plant host (figure 13.5). Each nodule contains millions of the bacteria that convert atmospheric nitrogen.

Although plants that form a partnership with nitrogen-fixing bacteria are sometimes called "nitrogen-fixing plants" or "nitrogen-fixing trees," the plant itself is unable to obtain atmospheric nitrogen. Through the mutualistic 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 (Singleton and others 1990). When the host plant sheds leaves,

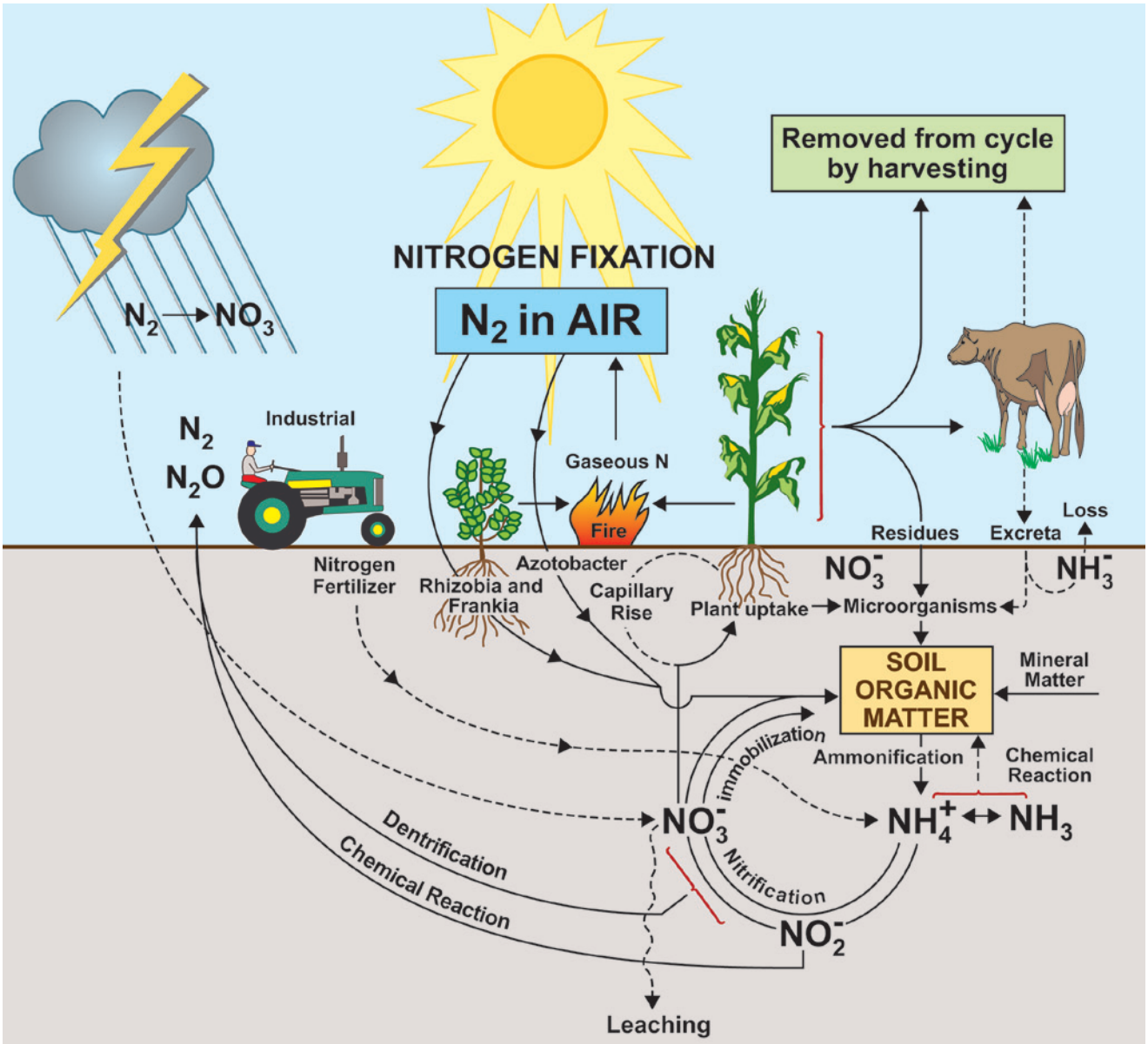

Figure 13.5—*The Nitrogen Cycle. All nitrogen in plants originates as an atmospheric gas, which is fixed by microorganisms (such as rhizobia and* Frankia*), fixed by humans in fertilizers by an energy-intensive industrial process, or to a very minor extent fixed by lightning or volcanism. The dashed lines in the diagram represent minor pathways; the solid lines represent major pathways. Adapted from Brown and Johnson (1996) by Jim Marin.*

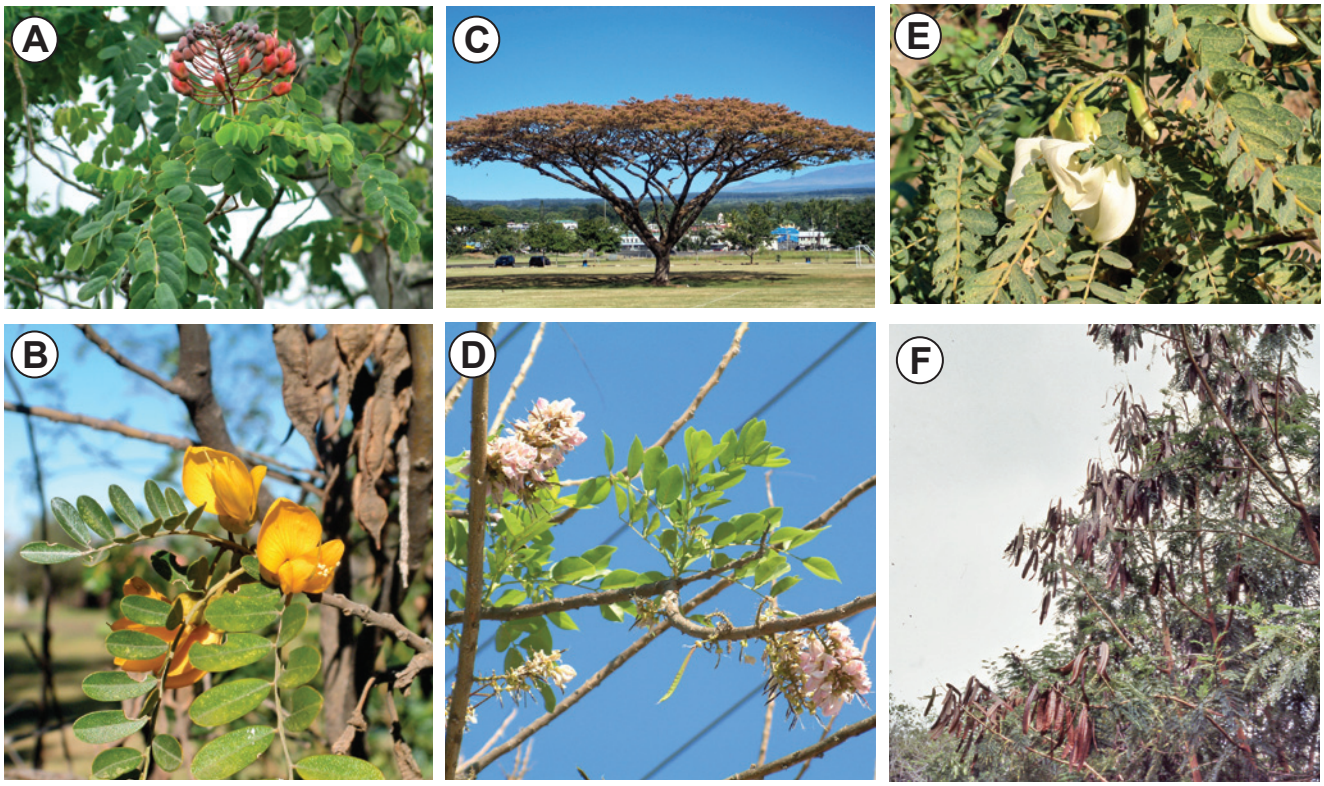

***Figure 13.6**—Because nitrogen-fixing species improve soil fertility on degraded lands, they are widely used for restoration and sustainable agriculture. Examples of native Hawaiian legume species that form relationships with nitrogen-fixing rhizobia bacteria include* Caesalpinia kavaiensis *(Uhiuhi) (A) and* Sophora chrysophylla *(māmane)(B). Other nitrogen-fixing species are known throughout much of the tropics, such as* Samanea *(C),* Gliricidia *(D), and* Sesbania *(E) species. Some nitrogen-fixing trees are considered weeds outside their native range, such as* Leucaena *species (F). Photos A through E by J.B. Friday, and photo F by Tara Luna.*

dies back, or dies, the nitrogen stored in the plant's tissues is cycled throughout the ecosystem. The process of nitrogen fixation provides the major source of nitrogen fertility in tropical ecosystems (figure 13.5).

In the early 20th century, human beings also learned to convert atmospheric nitrogen gas into fertilizers through an energy-intensive industrial process called the Haber-Bosch process. The process requires a source of hydrogen gas to react with nitrogen from the air under heat and high pressure. The most common sources for the hydrogen are fossil fuels, and additional energy is expended to power the reaction. Growers who use synthetic nitrogen fertilizers such as ammonium nitrate and urea are using products generated by this process. In contrast to industrial/synthetic nitrogen fixation, biological nitrogen fixation by bacteria associated with green plants is powered by the sun, and thereby is renewable and effectively inexhaustible.

Nitrogen-fixing trees and plants are usually outplanted to help restore fertility, nutrient cycling, and organic matter to the ecosystem. Soils at the outplanting site, however, may not contain a viable strain of bacteria to form an effective partnership with the plant. Inoculating plants in the nursery ensures that an effective partnership is formed to enhance plant survival and growth and to accelerate rehabilitation of degraded land. Unlike mycorrhizal fungi, which affect most trees and plants, only a fraction of plants can form partnerships with nitrogen-fixing bacteria; however, nitrogen-fixing plants play a vital role in nutrient cycling.

Two types of nitrogen-fixing bacteria form symbiotic partnerships with plants: rhizobia (consisting of several genera) and the genus *Frankia*. Rhizobia nodulate many (but not all) members of the legume family (Fabaceae, sometimes called Leguminosae) (figure 13.6). The legume family is made up of three subfamilies (Mimosoideae, Caesalpinioideae, and Papilionoideae [Faboideae]). Rhizobia also nodulate species of the genus Parasponia in the elm family (Ulmaceae). *Frankia* are a genus of filamentous bacteria (in a group called "actinomycetes" because of their somewhat fungus-like appearance) that form partnerships with about 200 different plant species distributed across eight families (figure 13.7). The species affected by *Frankia* are called "actinorhizal" plants (table 13.1).

Figure 13.7*— Non-leguminous species that form relationships with nitrogen-fixing* Frankia *bacteria include* Casuarina *species. Photo by J.B. Friday.*

Benefits of Inoculating With Nitrogen-Fixing Bacteria

Applications of inoculant for nitrogen-fixing bacteria can have some direct benefits in the nursery. If an effective partnership is formed, most of the plant's requirements for nitrogen will be met, thereby reducing or eliminating the need to apply nitrogen fertilizer and decreasing the nursery's need to manage pollution from fertilizer runoff.

In the field, however, is where the benefits of the partnership are most apparent. Nitrogen-fixing trees and plants sent from the nursery with their root systems already nodulated have faster early growth than plants that were not inoculated. Nursery inoculation can reduce costs in establishment and maintenance. The benefit from a few dollars' worth of inoculant applied in the nursery not only offsets the need for purchased nitrogen fertilizer but is also much cheaper than replacing a tree that dies from nitrogen deficiency. Also, instead of providing spurts of fertilizers in the field (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 early field growth can lead to faster canopy closure, which in turn shades the soil and understory, reduces weed management expenses, and leads to faster restoration of the natural nutrient cycling and fertility role of nitrogen-fixing species in the ecosystem.

Inoculating in the nursery ensures both the effectiveness and the timeliness of the nitrogen-fixing partnership. Noninoculated plants may eventually form a partnership in the field with a *Frankia* or rhizobia strain if some of the bacteria are present on the outplanting site. This partnership does not guarantee, however, that the plant will benefit. Some

Table 13.1*—Plants that form partnerships with nitrogen-fixing bacteria. Adapted from NFTA (1989) and Wall (2000).*

Bacteria	Plant family	Subfamily (notes)	Examples (genus)
Rhizobia	Legume (Fabaceae)	Caesalpinioideae (about 1,900 species; about 23% fix nitrogen)	*Cassia* and *Senna*
		Mimosoideae (about 2,800 species; about 90% fix nitrogen)	*Enterolobium, Leucaena, Pithecellobium, Acacia, Albizia, Prosopis,* and *Mimosa*
		Papilionoideae (about 12,300 species; about 97% fix nitrogen)	*Sesbania, Cajanus, Erythrina, Gliricidia,* and *Robinia*
Frankia	Birch (Betulaceae)		*Alnus*
	She-oak (Casuarinaceae)		*Casuarina, Allocasuarina,* and *Gymnostoma*
	Coriariaceae		*Coriaria*
	Datiscaceae		*Datisca*
	Buckthorn (Rhamnaceae)		*Ceanothus* and *Rhamnus*
	Myrtle (Myricaceae)		*Myrica, Comptonia,* and *Myrtus*
	Oleaster (Elaeagnaceae)		*Elaeagnus* and *Hippophae*
	Rose (Rosaceae)		*Cercocarpus, Chamaebatia, Cowania, Purshia,* and *Chamaebatiaria*

***Figure 13.8**—Some legumes can nodulate with a broad range of rhizobia strains, but not all strains are equally effective at fixing nitrogen. These tropical legume seedlings were inoculated with different rhizobia strains. Some partnerships were very effective (a green, thriving plant such as the one labeled 18b indicates that adequate nitrogen is being fixed at a low cost to the host plant) and others were not (a plant such as 4b is a little green, but not thriving and a plant such as 8b has no green). Careful selection of microsymbiont partners is important to ensure a productive partnership. Photo by Harold Keyser.*

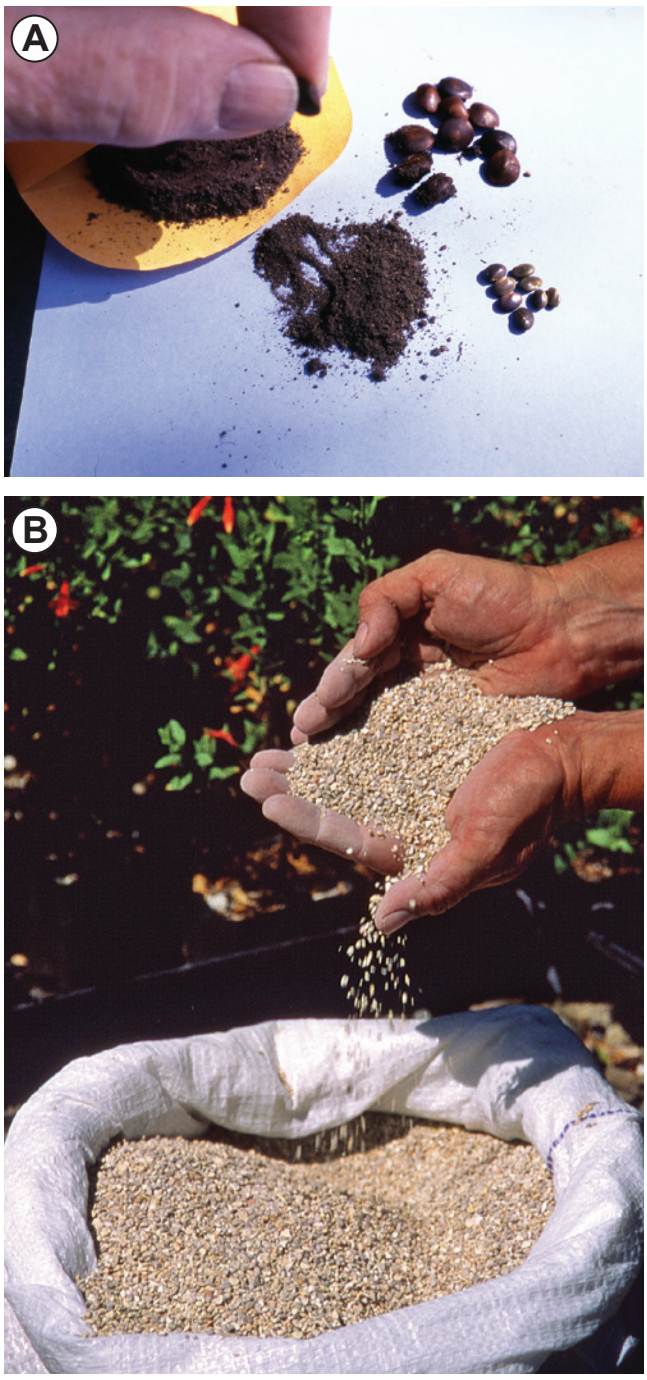

***Figure 13.9**—Nitrogen-fixing bacteria are commercially available as pure-culture inoculant (A), often in a carrier (B). Photo A by Tara Luna, and photo B by Mike Evans.*

plants will nodulate with a broad range of rhizobia strains, and not all strains are equally effective at fixing nitrogen (Keyser 2002). Some strains are very effective and productive, supplying plenty of nitrogen at a low cost to the host plant. Other strains are not productive, requiring a great deal of energy from the host plant with little return of nitrogen (figure 13.8). In other words, partnerships can range from mutually beneficial to parasitic (Evans 2002, Schmidt 2007, Baker and others 2009). Selecting microsymbionts that form healthy, productive partnerships is believed to warrant as much mindfulness as selecting seed sources (Schmidt 2007). In addition to source, time is also a factor for noninoculated plants after outplanting. It can take months or even years for effective partnerships to form if 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, noninoculated plants will grow very slowly, and sometimes are outcompeted by weeds.

Acquiring Inoculants for Nitrogen-Fixing Bacteria

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. Inoculants for nitrogen-fixing bacteria tend to be very specialized. In other words, they are not "one size fits all." Care must be taken to select appropriate and effective nitrogen-fixing partners for specific plant species. Two forms of inoculant can be used for nitrogen-fixing plants in the nursery: pure-culture inoculant is purchased from commercial suppliers, seed banks, or sometimes, universities (figure 13.9) and homemade (often called "crude") inoculant is made from nodules collected from the roots of healthy nitrogen-fixing

plants of the same species to be inoculated (figure 13.10). 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 need to be maintained to ensure the viability of inoculant during storage, handling, and application.

Using 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 considered if they can be obtained; these inoculants contain optimal partners for the host species to which they are matched, 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 more than 40 percent better growth (Schmidt 2000). Not all manufactured inoculants are selected and matched to native species, however, so be sure to check the source. If a match cannot be found, use the crude inoculant method instead. Manufactured products usually come with application instructions; these directions need to be followed. In general, about 3.5 oz (100 g) of cultured inoculant is sufficient to inoculate up to 3,000 plants, usually exceeding the recommended 100,000 bacteria per plant. Because they contain living cultures of bacteria, these inoculants are perishable and need to be kept in cool, dark conditions, such as inside a refrigerator.

Peat-based inoculants are added to chlorine-free water to create a slurry. (If the nursery's water supply is chlorinated, allowing a bucket of water to stand uncovered for 24 hours is a good way to let the chlorine evaporate.) A blender or electric mixer is recommended to blend the inoculant with water to ensure the bacteria are evenly mixed in the solution. If a blender is not available, a whisk can be used. After plants begin to nodulate, nodules from their roots can serve as the basis for making crude inoculant to use on future crops. This way, inoculant need be purchased only once for each plant species and can thereafter be perpetuated in the nursery.

Preparing Crude Inoculant

Nodules, the small root structures that house the bacteria, are used to make crude inoculant. Nodules can be col-

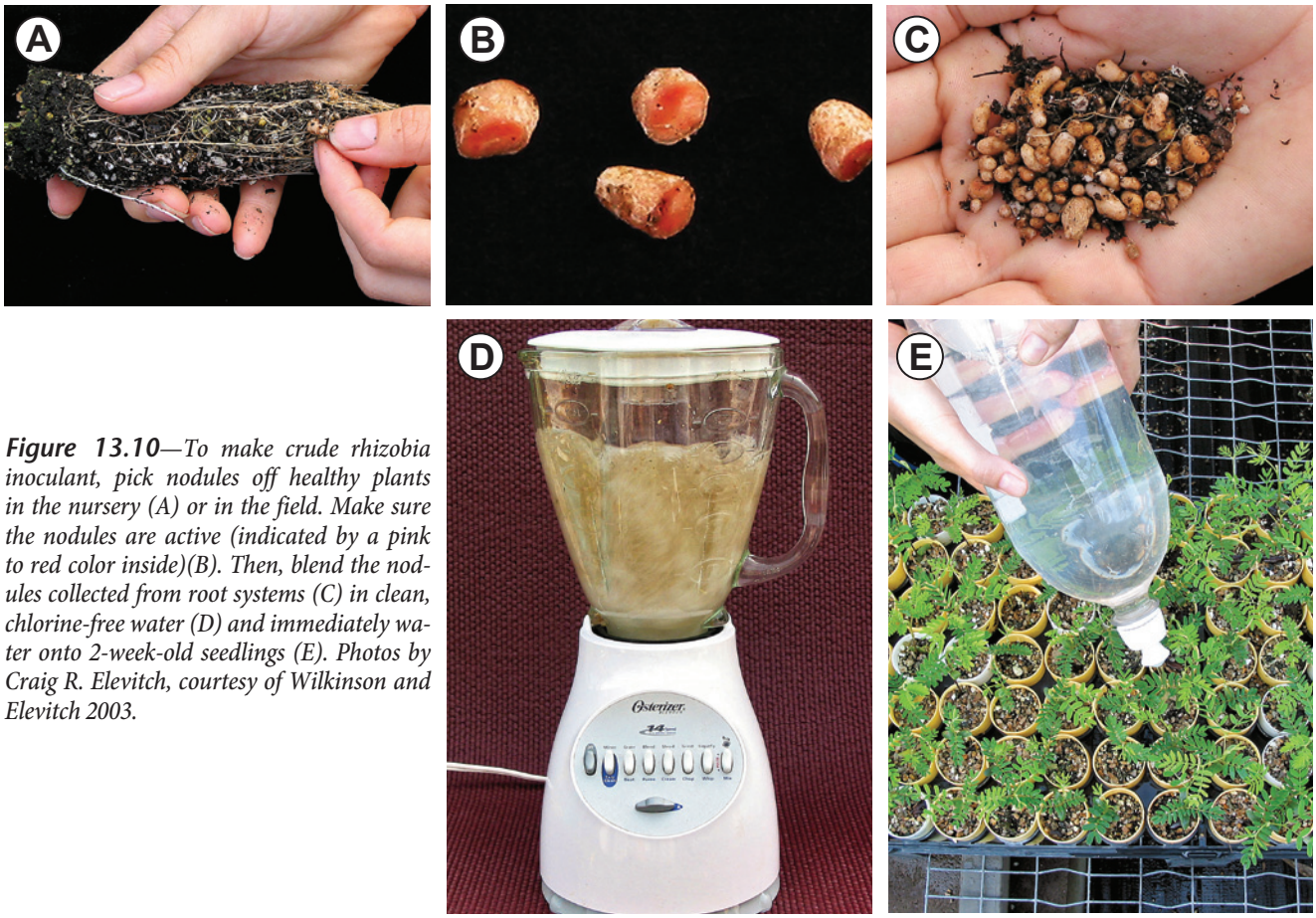

Figure 13.10—*To make crude rhizobia inoculant, pick nodules off healthy plants in the nursery (A) or in the field. Make sure the nodules are active (indicated by a pink to red color inside)(B). Then, blend the nodules collected from root systems (C) in clean, chlorine-free water (D) and immediately water onto 2-week-old seedlings (E). Photos by Craig R. Elevitch, courtesy of Wilkinson and Elevitch 2003.*

lected from the roots of nursery stock that were previously inoculated with cultured, select inoculant, or nodules can be collected from healthy, established host plants. For rhizobia, 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. Grey or green nodules should be avoided, because they likely are inactive.

To make crude inoculant, choose healthy, vigorous plants of the same species as the plants to be inoculated. Dig shallowly around the base of the nodulated plant to expose some of its root system. Young roots often contain the most active nodules. Search for nodules with the proper color and pick them off cleanly (figures 13.10A, 13.10B). Collect nodules from several healthy plants of the same species to ensure diversity (figure 13.10C). Put the 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 (figure 13.10D). About 50 to 100 nodules blended in about 1 qt (1 L) of water are enough to inoculate about 500 plants. This solution is a homemade liquid inoculant, ready to apply in the same way as cultured inoculant (figure 13.10E).

Applying Inoculant

Inoculant for nitrogen-fixing bacteria is commonly applied when seedlings are emerging, usually within 2 weeks of sowing, or just after cuttings have formed roots. This helps ensure successful nodulation and maximizes the benefits of using inoculants. The liquefied inoculant, made from either nodules or cultured inoculant as per the instructions in the previous sections, is then watered into the growing media or soil in which seedlings are growing (figure 13.10E).

Verifying the Nitrogen-Fixing Partnership

After 2 to 6 weeks, the noticeable signs in the following list should appear and are indications 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 13.11A).
- Root systems give off a faint but distinctive ammonia-like scent.
- Nodules are visible on the root system.
- When a nodule is broken open, its inside is pink, red, or brown (for rhizobia) (figure 13.11B), or yellow or white (for *Frankia*).

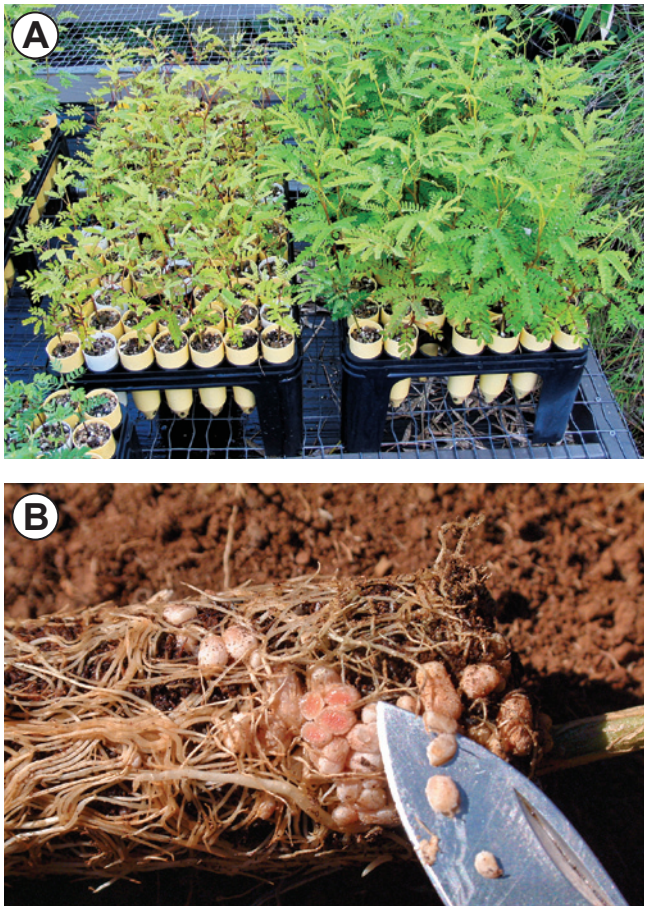

Figure 13.11*—The 6-week-old native* Acacia koa *seedlings (right) were inoculated with rhizobia at 2 weeks of age; the seedlings on the left were not inoculated (A). Nodules from an* Acacia koa *seedling showing pink inside, signifying nitrogen is being fixed (B). Photo A by Craig R. Elevitch, and photo B by J.B. Friday.*

Management Considerations

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

- **Timing**—Ensure the inoculant is applied when seedlings have just emerged or when cuttings have formed new roots to ensure successful nodulation and maximize the benefits of using inoculants.
- **Fertilization and Micronutrients**—The use of nitrogen-fixing bacterial inoculant requires some adjustments in fertilization. Excessive nitrogen fertilizer will inhibit formation of the partnership. If an optimal partnership is formed, the application of nitrogen may be eliminated from nitrogen-fixing plants and they may need to be isolated from nonnitrogen-fixing species to implement this change in fertilization.

Some nutrients, including calcium, potassium, molybdenum, and iron, are necessary to facilitate nodulation. These nutrients need to be incorporated into the growing medium. Phosphorus is also necessary for nodulation, supplied from the growing medium or, better yet, through mycorrhizal partnerships.

- **Water Quality**—Excessive chlorine in water is detrimental to rhizobia 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. As an alternative, chlorine will evaporate if clean water is left to stand uncovered in a container for 24 hours before use.
- **Sourcing Inoculants**—Locating appropriate sources of viable inoculants (either cultured or obtained as nodules) matched to native species may require some research and time but benefits of successful inoculation are well worth the effort.
- **Client Education**—Make sure nursery clients and people outplanting the plants understand the nitrogen-fixing bacteria so they appreciate the presence of nodules and are careful not to expose root systems to full sun. Also, educate clients so they know that only some species of plants can form this partnership—otherwise some might think all species can fix nitrogen.
- **Outplanting Site Considerations**—After nodules form, rhizobia are enclosed and usually less affected by soil conditions such as pH or aluminum toxicity than are other beneficial microorganisms such as mycorrhizal fungi. In very harsh outplanting conditions (such as extremely low pH in some rehabilitation sites), however, the rhizobia are not likely to spread in the soil, and so might not be available to spread to subsequent cohorts of the outplanted species. Therefore, inoculating subsequent crops in the nursery before outplanting on the same site is advisable.

Tripartite Symbiosis

Most plants that form partnerships with nitrogen-fixing bacteria also require mycorrhizal partners. When a nitrogen-fixing plant has effective partnerships with both nitrogen-fixing bacteria and mycorrhizal fungi, this is called "tripartite symbiosis" because three partners exist (a host plant and two microsymbionts) (figure 13.12). When working with both types of microsymbionts, simply apply each inoculant separately, as described in the sections of this chapter.

Several studies have shown that legumes may need to first form arbuscular mycorrhizae before they can form rhizobia nodules. This may occur because nitrogen fixation is an energy-demanding process, and the plant's energy metabolism is highly phosphorus-dependent. In other words, a grower who applies only rhizobia inoculant may have difficulty getting good nodulation if the mycorrhizal partnership is not in place (or if adequate phosphorus is not available in the growing medium). The next section describes how to introduce mycorrhizal fungi to nursery crops—they often are incorporated in the growing medium before the rhizobia inoculant is applied.

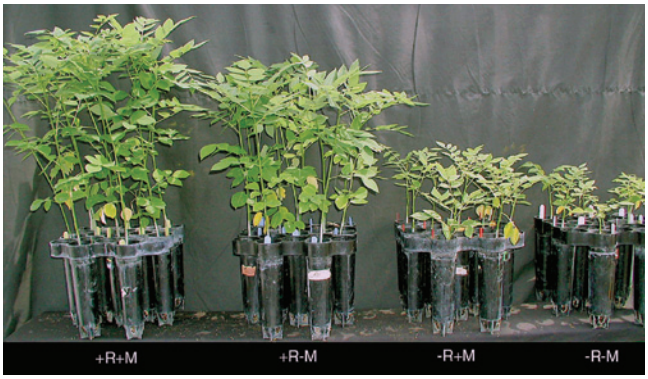

Figure 13.12—*The* Gliricidia sepium *plants on the far left were inoculated with both rhizobia (R) and mycorrhizal fungi (M) and show tripartite symbiosis: a beneficial partnership among the plant host, rhizobia bacteria, and arbuscular mycorrhizal fungi. Photo by Kenneth W. Mudge*

Mycorrhizal Fungi

Unlike nitrogen-fixing bacteria, mycorrhizal fungi form partnerships with nearly all plant families and forest trees. "Myco" means "fungus" and "rhiza" means "root;" the word "mycorrhizae" means "fungus-roots." Most of the world's plants depend on their partnership with mycorrhizal fungi to grow and thrive. The host plant's roots provide a substrate for the fungi and supply food in the form of simple carbohydrates. In exchange, the mycorrhizal fungi offer the following benefits to the host plant:

- **Increased Water and Nutrient Uptake**—Mycorrhizal fungi help plants absorb mineral nutrients, especially nitrogen, phosphorus, and several micronutrients such as zinc and copper. The fungal hyphae extend out into the soil far beyond the host's roots, expanding the mineral- and water-absorbing surface area for the host plant. Researchers estimate that mycorrhizal fungus hyphae can explore volumes of soil hundreds to thousands of times greater than roots can alone.
- **Stress and Disease Protection**—Mycorrhizal fungi protect the plant host in several ways. With ectomycorrhizal fungi, for example, a fungus sheath (called a "mantle") completely covers fragile root tips and acts

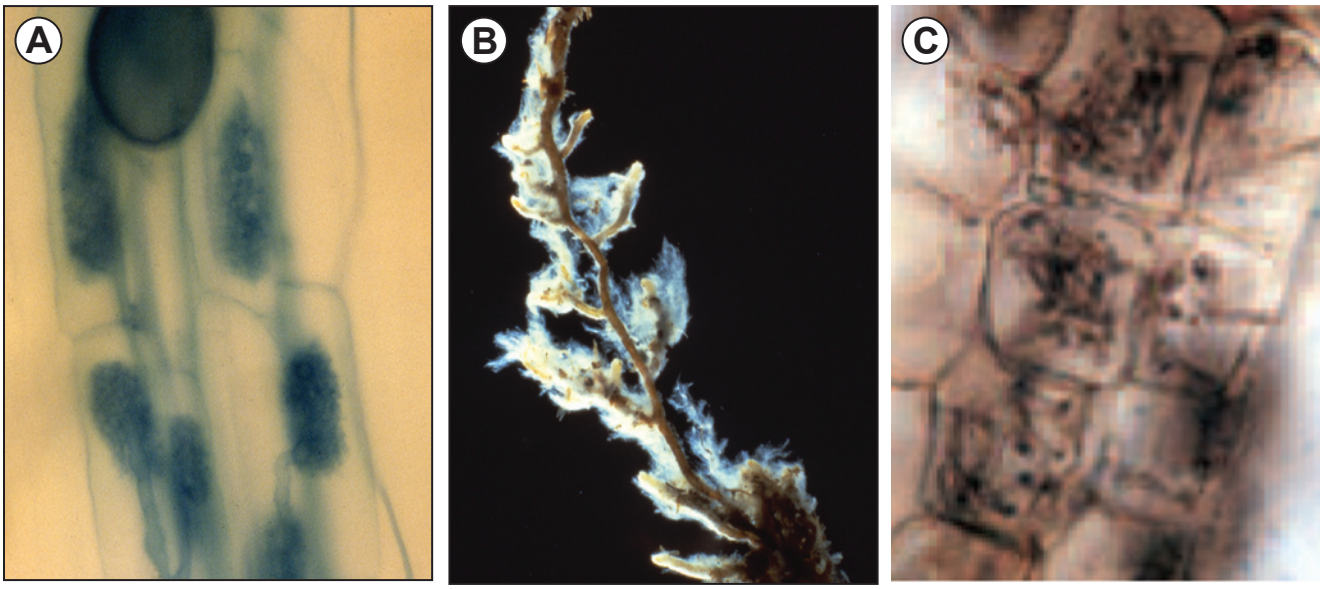

***Figure 13.13**—The three types of mycorrhizal fungi. Arbuscular mycorrhizal (AM) fungi (A). Ectomycorrhizal (ECM) fungi (B)—the mantles of ECM may be visible to the unaided eye, although ECM on* Eucalyptus *can be inconspicuous. Ericoid mycorrhizal (ERM) fungi (C). Both AM and ERM can only be seen on plant roots with the aid of a microscope. Photos A and B by Michael A. Castellano, and photo C by Efren Cazares.*

as a physical barrier to dryness, pests, and toxic soil contaminants. Other mycorrhizal fungi may produce antibiotics, which provide chemical protection.

- **Increased Vigor and Growth**—Plants with mycorrhizal roots may have an improved hormone status, and they survive and grow better than noninoculated plants after they are planted out on a project site. Studies show that establishing a partnership with mycorrhizal fungi while the plants are in the nursery results in improved field growth (Habte and others 2001, Baker and others 2009).

The following three types of mycorrhizae are important to tropical native plant nurseries (table 13.2):

- **Arbuscular Mycorrhizae (AM)**—Formed by the most ancient and predominant type of mycorrhizal fungi. AM fungi are found on the roots of most tropical plants and many of the world's food crops (including rice, corn, and legumes), associating with more than 80 percent of the world's plant families (figure 13.13A).
- **Ectomycorrhizae (ECM)**—Partnerships with many temperate forest trees and a few abundant tropical trees including pines (*Pinus*), eucalypts (*Eucalyptus*), poplars (*Populus*), and dipterocarps (*Dipterocarpus*) (figure 13.13B).
- **Ericoid Mycorrhizae (ERM)**—Partnerships with plants in the heath or heather (Ericaceae) family, including the genera of blueberries, cranberries, azaleas, and rhododendrons (figure 13.13C).

Mycorrhizal fungi are not "one size fits all," but they often are "one size fits many." Also, one plant can partner simultaneously with several species of mycorrhizal fungi, and a plant may change partners over time as it grows and adapts to its environment (Amaranthus 2010).

***Table 13.2**—Plants and their mycorrhizal partners. Adapted from Castellano and Molina (1990) and Wang and Qiu (2006).*

Mycorrhizal fungi	Plants
Arbuscular mycorrhizal (AM)	More than 80% of the world's plant families including most tropical trees, herbs, and ferns
Ectomycorrhizal (ECM)	Fewer than 10% of plant families, including the genera pine (*Pinus*), oak (*Quercus*), eucalyptus (*Eucalyptus*)
AM and ECM	Some *Allocasuarina, Acacia, Eucalyptus,* juniper (*Juniperus*), poplar (*Populus*), and willow (*Salix*)
Ericoid mycorrhizal (ERM)	Heather or heath family (Ericaceae in the broad sense, including the former Epacridaceae and Empetraceae), including blueberry (*Vaccinium*) and *Rhododendron* (including azaleas)

Because most plants are associated with a particular type of mycorrhizal fungus, however, different plant species have different fungal partners that must be matched appropriately to be effective (table 13.2).

Mycorrhizal fungi may be obtained either from commercial suppliers or from roots around a healthy host plant of the species being propagated. In all cases, mycorrhizal inoculum must physically contact living roots of the plant to colonize most effectively. Ways to acquire and successfully apply mycorrhizal fungi are explained in subsequent sections. Although the fungi are similar in how they function and in their benefits to host plants, they appear differently on roots. Also, each mycorrhizal fungi type has a unique application method that must be described separately. Management practices in the nursery are similar and will be discussed together at the end of this section.

Arbuscular Mycorrhizal (AM) Fungi

AM fungi are essential for most tropical trees and other plants and for many annual crops and grasses. AM fungi are not visible on plant roots to the unaided eye and must be observed under a microscope. The large spores of AM fungi are not easily disseminated by wind, unlike the wind-dispersed microscopic spores of ECM fungi.

Inoculant for AM fungi is sometimes collected from root systems of AM host plants or soil underneath them and incorporated into growing media. This method can work well because the fungi collected are likely to be adapted to prevailing site conditions (Janos and others 2001). Freshly collected and chopped mycorrhizal roots must be used within six days of collection or their efficacy will decline. This method is often discouraged, however, because of damage to plants and natural ecosystems, variable effectiveness, and the risk of introducing pests and pathogens along with the soil or roots. The two main sources of AM fungi inoculant for nurseries are "pot culture" made from a known fungus species, and commercially available cultures. Because AM spores are relatively large, it is critical to ensure that spores come in direct contact with the root systems. Spores will not pass easily through irrigation injectors or nozzles. Therefore, for all inoculant types, thorough incorporation into growing media is the best practice.

Pot Culture Inoculant

In pot culture inoculant, a specific AM fungus species is acquired either commercially or from a field site as a starter culture and then incorporated into a sterile growing medium. A host plant such as corn, sorghum, clover, or an herbaceous native plant, is grown in this substrate. As the host plant grows, the AM fungi multiply in the medium

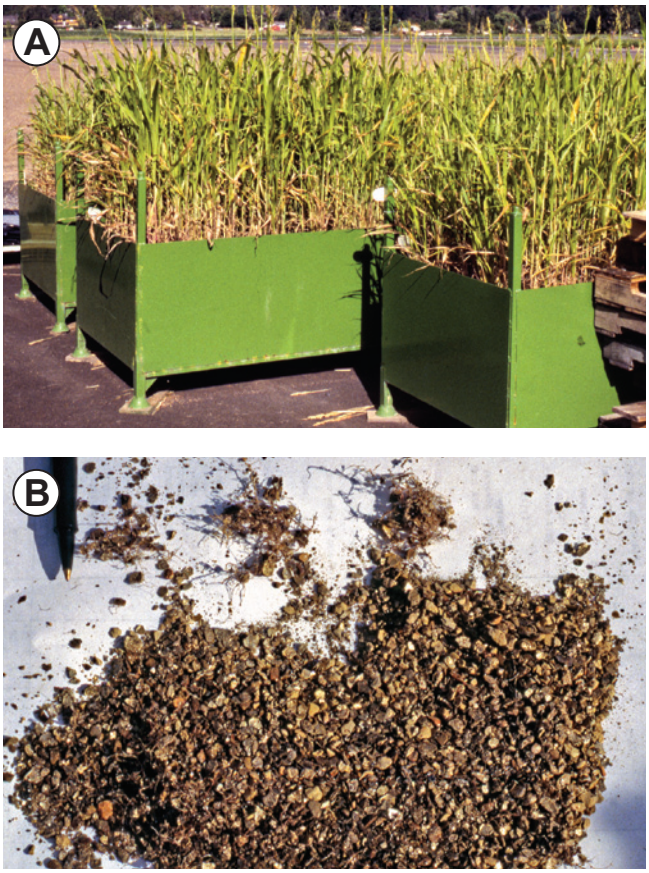

Figure 13.14*—In pot culture inoculant, a specific arbuscular mycorrhizal fungus species is acquired as a starter culture and added to a sterile growing medium with a fast-growing host plant (A). The shoots of host plants are later removed, and the substrate, now rich in roots, spores, and mycelium, is chopped up (B) and incorporated into the growing medium before containers are filled. Photos by Thomas D. Landis.*

(figure 13.14). After the host plant roots have spread throughout the medium, their shoots 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 further details on how to use this method, consult the publications in the References section of this chapter (particularly Habte and Osorio 2001, Miyasaka and others 2003).

Commercial Inoculant Sources

Commercial sources of AM fungi inoculant are also available, usually containing several species or strains. Because AM fungus spores are fragile, they are usually mixed with a carrier such as vermiculite or calcined clay to aid in application. These products are thoroughly incorporated into the growing medium before filling containers.

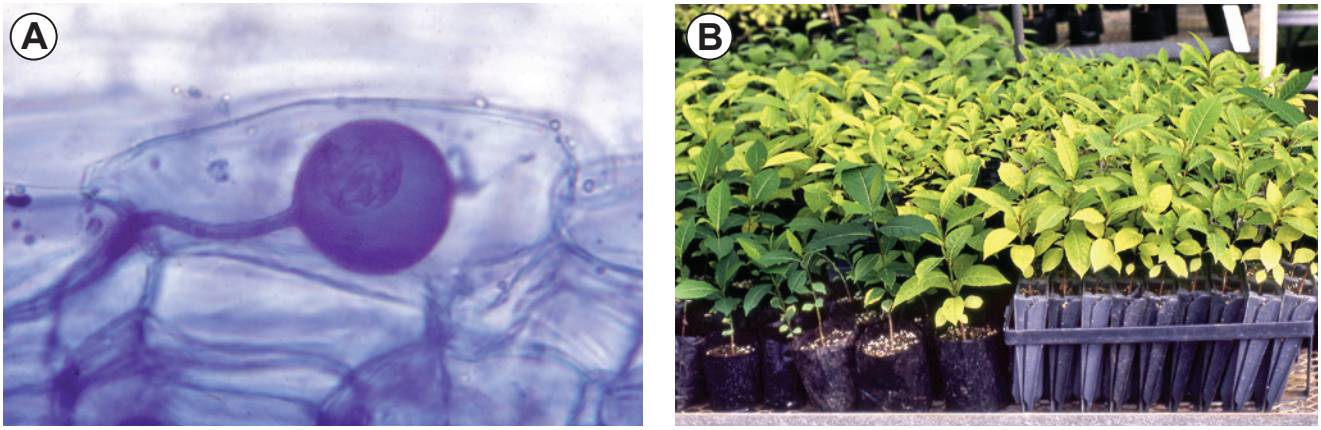

***Figure 13.15**—In this microscope photo (400 times magnification), a tropical tree root has been clarified and stained with a blue dye so that an AM fungus "vesicle" within the walls of a root cortical cell is clearly visible (A). Vesicles are places where AM fungi store excess energy-rich materials, such as lipids. Vesicles always are attached to AM fungus filament (a hypha), which distinguishes them from the spore-bearing structures of some root-parasitic fungi. Although AM fungi are visible only under a microscope, nursery workers may observe differences in plant growth of inoculated versus noninoculated plants. A mosaic pattern of nutrient deficiencies as shown by these mahogany (*Swietenia *species) seedlings, may indicate that some plants have formed successful partnerships while others have not (B). Photo A by David P. Janos, and photo B by Tara Luna.*

Inoculation effectiveness has been shown to differ 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 vigor.

Verifying the Effectiveness of AM Fungi Inoculation

To verify the effectiveness of AM fungi inoculation, roots must be stained and examined under a microscope (figure 13.15A). This verification can often be done through a soil scientist at a local agricultural extension office. After some practice, nursery staff may get a feel for when inoculation is successful, because noninoculated plants often grow more slowly and may have higher incidence of root rot issues. The plants also may exhibit signs of phosphorus deficiency (a frequent consequence of lack of mycorrhiza), indicated by purple coloration of leaves or other symptoms (figure 13.15B).

Ectomycorrhizal (ECM) Fungi

Many recognizable mushrooms are fruiting bodies of ECM fungi. These fruiting bodies are a small portion of the total organism; underground, the amount of fungus covering the short feeder roots of plants may be enormous. ECM fungi extend the volume of the feeding area of roots by many times and protectively coat the feeder roots. On some species, such as pines (*Pinus*), the mantles of ECM are visible on the roots of the host plant. For other species, such as many *Eucalyptus* ECM, the mantles can be inconspicuous. ECM are important to many temperate forest species, especially evergreens. In the tropics, far fewer plant species partner with ECM fungi than with AM fungi. In Hawai'i, for example, no native ECM are known, although some strains may have been introduced along with introduced trees (Amaranthus 2010). ECM only affect a small percentage of tropical species, including pines, eucalypts, poplars, oaks, dipterocarps, and some legumes (table 13.3).

Four sources of ECM fungi inoculant have been used in nurseries. Nurse plants and soil spores have been used historically while spores and pure culture inoculant are usually recommended for nurseries.

Nurse Plants as Inoculum Sources

In the early days of trying to establish pines in the tropics where they were not native, "nurse" plants were sometimes used. Conspicuously vigorous mycorrhizal seedlings were transplanted to nursery beds at 3- to 6-ft (1- to 2-m) intervals and allowed to become established, maintaining the ECM fungi on their roots. Seeds were then sown or germinants transplanted around and between these mycorrhizal nurse plants. After becoming colonized by ECM fungi spreading from the nurse plants, the seedlings were transplanted to the outplanting site. Some plants were left in the beds to serve as nurse plants for the next crop of seedlings. Sometimes the spread of mycorrhizal fungi from the nurse seedlings was slow (roughly 2 ft per year), which suggested that some unfavorable soil property (such as high pH) should have been remedied. The mycorrhizal fungi usually spread fastest among seedlings in sterilized soil. This method was laborious, and, as with all bareroot crops, care had to be taken not to cause damage when lifting seedlings (Mikola 1973).

Table 13.3—*Tropical families and genera with ectomycorrhizal associations. Adapted from Brundrett (2009).*

Family	Genera
Gnetaceae	*Gnetum*
Pinaceae	*Cedrus, Keteleeria, Larix, Picea,* and *Pinus*
Nyctaginaceae	*Guapira, Neea,* and *Pisonia*
Polygonaceae	*Coccoloba*
Myrtaceae	*Allosyncarpia, Agonis, Angophora, Baeckea, Eucalyptus, Leptospermum, Melaleuca, Tristania,* and *Tristaniopsis*
Fabaceae: Caesalpinioideae	*Afzelia, Anthonotha, Aphanocalyx, Berlinia, Brachystegia, Cryptosepalum, Dicymbe, Didelotia, Eperua, Gilbertiodendron, Gleditsia, Intsia, Isoberlinia, Julbernardia, Microberlinia, Monopetalanthus, Paraberlinia, Paramacrolobium, Pellegriniodendron, Tetraberlinia,* and *Toubaouate*
Fabaceae: Papilionoideae	*Aldinia, Gastrolobium, Gompholobium, Jacksonia, Lonchocarpus, Mirbelia, Oxylobium,* and *Pericopsis*
Fabaceae: Mimosoideae	*Acacia* and *Calliandra*
Casuarinaceae	*Allocasuarina* and *Casuarina*
Fagaceae	*Castanea, Castanopsis, Fagus, Lithocarpus,* and *Quercus*
Phyllanthaceae (Euphorbiaceae)	*Uapaca* and *Poranthera*
Salicaceae	*Populus* and *Salix*
Rhamnaceae	*Cryptandra, Pomaderris, Spyridium,* and *Trymalium*
Dipterocarpaceae	*Anisoptera, Dipterocarpus, Hopea, Marquesia, Monotes, Shorea, Vateria, Vateriopsis,* and *Vatica*
Sarcolaenaceae	*Leptolaena, Sarcolaena,* and *Schizolaena*

Soils as Inoculum Sources

Topsoil, humus, or duff from beneath ECM fungi host trees has historically been used to inoculate nursery plants. This practice is more common in bareroot nurseries in temperate regions than in container nurseries. Because sterilization would kill these beneficial fungi, unsterilized soil and organic matter are incorporated into the growing medium, up to 10 percent by volume. Today, this practice is generally discouraged for ECM fungi because (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. If soil is used, inoculum should be collected from plant communities near the outplanting site. Small amounts should be collected from several different sites, then thoroughly mixed, and care should be taken not to damage the plants during soil collection.

Spores as Inoculum Sources

Nurseries can make their own ECM inoculum from spores. Collected from the fruiting bodies (figure 13.16) of mushrooms, puffballs, and especially truffles, 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 (figure 13.17). Two applications 2 to 3 weeks apart are recommended to ensure even inoculation.

Pure-Culture Inoculum

ECM fungi are available commercially as pure cultures, usually in a peat-based carrier (figure 13.18). The quality of commercial sources varies, however, so it is important to verify vigor by testing formation of mycorrhizae. Most commercial sources contain several different species of

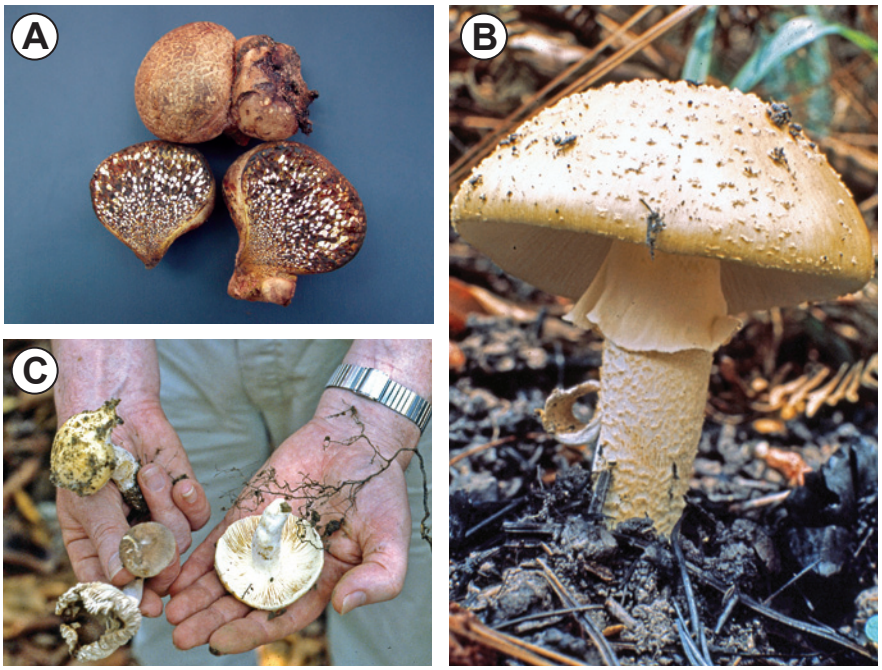

***Figure 13.16**—Fruiting bodies of ectomycorrhizal fungi. A puffball of* Pisolithus tinctorius*, showing the cavities where the spores are located (A). An* Amanita *from pine forest in Guatemala (B), and gilled ectomycorrhizal mushrooms and roots from dipterocarp forest in Malaysia (C). Photo A by Michelle M. Cram, and photos B and C by David P. Janos.*

***Figure 13.17**—Inoculating tree seedlings with ectomycorrhizal fungi. Two applications 2 to 3 weeks apart are recommended to ensure even inoculation. Photo by Michael A. Castellano.*

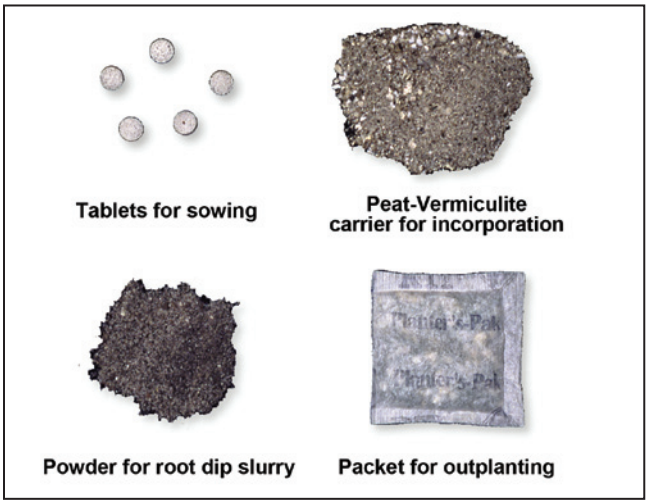

***Figure 13.18**—Forms of commercial ectomycorrhizal inoculants available for nurseries. Photo by Thomas D. Landis.*

ECM fungi. Commercial inoculum can be purchased separately and mixed into the growing medium as per the instructions on the product and before filling containers. In some areas, bales of growing medium with inoculum already premixed may be purchased. It is important to inquire if selected strains to match site needs are available through suppliers.

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 and often involve conspicuous morphological changes of the finest roots. During the hardening phase, short feeder roots need to be examined for a cottony white appearance on the root surface or a white or brightly colored mantle or sheath over the roots (figure 13.19A). Unlike pathogenic fungi, mycorrhizae never show signs of root decay. Sometimes, mushrooms or other fruiting bodies will appear in containers alongside their host plants (figure 13.19B). Although 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 likely can assist with this process.

Figure 13.19—*Nursery staff can learn to recognize the presence or absence of ectomycorrhizal fungi by examining plants. Cottony white ectomycorrhizae may be visible on roots of some species (A), fruiting bodies may be growing from containers (B). Photo A by William Sayward, and photo B by Michael A. Castellano.*

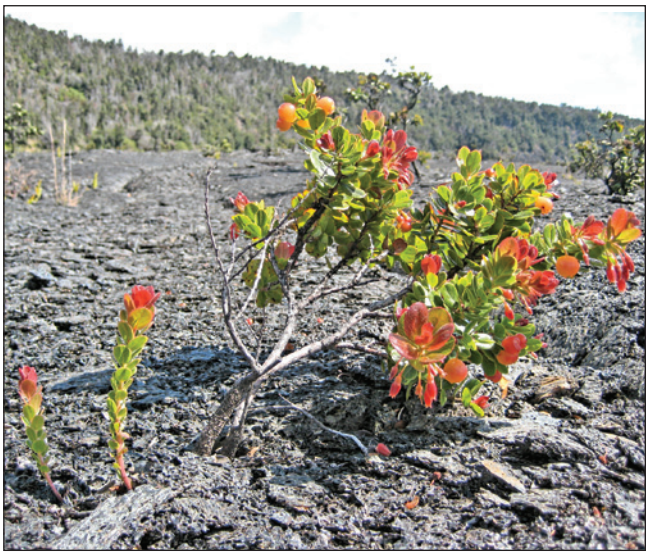

Figure 13.20—*A native Hawaiian Ericoid,* Vaccinium reticulatum*, ʻōhelo, growing on a recent lava flow. Partnerships with ericoid mycorrhizal fungi enable these plants to survive and thrive in harsh conditions. Photo by Kim M. Wilkinson.*

Ericoid Mycorrhizal (ERM) Fungi

Plants that form partnerships with ERM fungi are able to grow in exceptionally nitrogen-poor soils and harsh conditions, including bogs, alpine meadows, tundra, and even in soils with high concentrations of certain toxic metals (figure 13.20). ERM fungi form partnerships in the plant order Ericales in the heath (Epacridaceae), crowberry (Empetraceae), and most of the rhododendron (Ericaceae) family (table 13.4). Similar to ECM fungi and AM fungi, ERM fungi must come in contact with the host plants roots 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 "hair roots" (the fine, ultimate rootlets of the plants that are only a few cells

Table 13.4—*Genera known to associate with ericoid mycorrhizal fungi. Adapted from Read (1996) and Smith and Read (1997).*

Family	Genera
Ericaceae	*Acrotriche, Andersonia, Astroloma, Brachyloma, Cassiope, Calluna, Ceratiola, Conostephium, Corema, Cyathodes, Dracophyllum, Empetrum, Epacris, Erica, Gaultheria, Kalmia, Ledum, Leucopogon, Lissanthe, Lysinema, Melichrus, Monotoca, Needhamiella, Oligarrhena, Pentachondra, Richea, Rhododendron, Rupicola, Sphenotoma, Sprengelia, Styphelia, Trochocarpa, Vaccinium, Woollsia*

wide), infecting the outer cells. As with AM fungi, nutrients are shared through the membranes that form 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

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 need to be modified to culture mycorrhizal roots. In some cases, working with a manufactured product of known quality may be the easiest way to begin; the nursery can then expand into collecting and processing its own inoculant sources. Monitor the effectiveness of inoculation and keep records of crop development. See Chapter 20, Discovering Ways to Improve Nursery Practices and Plant Quality, to learn more about how to create some small trials and experiments.

Although mycorrhizal fungi are not very specialized, different strains of mycorrhizae are believed to perform differently for given site challenges. 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. For example, some strains may be more beneficial if lack of nutrients is the main challenge while others may be particularly helpful to their hosts in withstanding soil pathogens or even heavy metals. If possible, working with several strains for diversity in the field may be a good safeguard, especially because plants can partner with multiple strains simultaneously and can change partners if necessary to adapt to site conditions. The nursery can do a little research or work with a specialist to help with the following tasks:

- Select optimal mycorrhizal partners for the species and outplanting sites.
- Determine the most appropriate sources of inoculant and evaluate their effectiveness in the nursery.
- Design outplanting trials to evaluate plant vigor and survival and modify the inoculant sources if improvements are needed.

***Figure 13.21**—Four-month-old guava seedlings (*Psidium guajava*) in the nursery in a low fertility (about 8 ppm available phosphorus) lowland tropical acid clay soil; the plant on the right has a mycorrhizal partnership in place while the plant on the left does not (A). Even with abundant phosphorus fertilization, lack of mycorrhizal fungi can slow plant growth. The photo shows lychee (*Litchi chinensis*) air layers grown for 16 months in 25 gal pots of a soil-free medium after cutting from the source trees (B). Although phosphorus fertilization did not affect growth, AM fungus inoculation with field-collected inoculant improved shoot growth by 39 percent (see Janos and others 2001). Photos by David P. Janos.*

Inoculation with mycorrhizal fungi affects plant growth (figure 13.21). Fertilization practices will need to be adjusted to support the formation of mycorrhizal partnerships in the nursery. An excessive amount of phosphorus inhibits formation of the partnership; therefore phosphorus must be reduced. Enough phosphorus must be present to keep the mycorrhizal fungi from competing with its host plant for the nutrient, however, and potentially becoming parasitic instead of symbiotic. The recommendation is to use "low but sufficient" levels of phosphorus to facilitate the partnership (Miyasaka and others 2003). In some cases, the overall quantity of fertilizer may need to be reduced by half or more because of the efficiency of nutrient uptake by mycorrhizal fungi. Fertilizer type and form is also important. If nitrogen is applied, ammonium-nitrate is better used by a wide variety of plants than nitrate-nitrogen alone (Castellano and Molina 1990). In general, controlled-release fertilizers may be better than liquid fertilizers for inoculated plants because they release small doses of nutrients gradually rather than sudden high doses periodically. Excessive or inadequate water will inhibit the presence of mycorrhizal fungi and the formation of the partnership, so watering schedules need to 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 decision makers in terms of modifying fertilization and watering regimes to support the microsymbionts.

Other management adjustments may be necessary because of improved survival and growth. Improved survival percentages will affect estimates and oversow rates. Scheduling also may be affected; inoculated plants may be ready for outplanting sooner than noninoculated plants. Applications of certain fungicides are detrimental to mycorrhizal fungi; susceptibility varies by species and pesticide applications need to be assessed and adjusted.

Some plants form partnerships with both AM fungi and ECM fungi. These plants include some of the *Allocasuarina, Acacia, Eucalyptus, Juniperus,* and *Populus* species. In these cases, trials should be done to see which inoculant produces the best results in the nursery, and if those results persist after outplanting. For some species, additional inoculation with the second type of mycorrhiza (usually ECM fungi following AM fungi) may be necessary before outplanting.

Also, most plants that form partnerships with nitrogen-fixing bacteria also require mycorrhizal partners. For example, many leguminous trees partner with both rhizobia and AM fungi; in these cases, it may be beneficial to apply the AM fungi before the rhizobia (as discussed previously). Trees such as alders (*Alnus*) partner with both *Frankia* and ECM fungi. In these cases, inoculants can be applied separately to the same crop of plants.

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 matter. A healthy population of soil microorganisms helps to maintain ecological balance, preventing the onset of major problems from soil viruses or other pathogens. Realizing that soil is alive, and reducing or eliminating practices that may be harmful to soil microlife is important. As Aldo Leopold advised, "The first rule of intelligent tinkering is to keep all the parts." Using compost, mulch, and organic matter is important for soil life. Protecting soil from erosion and unnecessary disturbances, eliminating pollution from soluble fertilizers, and minimizing the use of fungicides, disinfectants, and other chemicals that may kill microlife are all key practices. Introductions of mycorrhizal fungi and nitrogen-fixing bacteria in the nursery can support the balance of beneficial soil microorganisms.

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