

# DEVELOPING MICROBIAL INOCULANTS FOR NATIVE HAWAIIAN TREES

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## Key Words

Reforestation, afforestation, Rhizobia, mycorrhizal fungi

Our nursery grows tree seedlings mainly for nonindustrial projects, usually for people who are reforesting their own property. Our clients generally share 3 characteristics:

- Most are planting on land that is somewhat marginal or degraded: former pasture, old sugar cane land, eroded hillsides, exposed ridges, and so on;
- Most operate on a limited budget, planning for minimal maintenance after establishment; and
- Most have a larger vision for their project than simply creating a stand of trees; they want their trees to survive independently and to reestablish some of the ecosystem value of a forest.

We use certain microbial inoculants in the nursery as a part of supporting the objectives of accelerating rehabilitation of degraded land and ecosystem function, as well as reducing costs in establishment and maintenance of forest plantings. Microbial inoculants re-create natural partnerships between plants and some of the beneficial microorganisms that support plants. Using microbial inoculants is a way to reintroduce some naturally occurring microorganisms that support the productivity of plants in nature. In our nursery, we work with both rhizobia and mycorrhizal inoculants.

The use of rhizobia inoculants for nitrogen-fixing trees, including the native Hawaiian koa (*Acacia koa*) will be discussed in this paper, along with how nitrogen fixation works, why inoculation in the nursery is advantageous, and how to use

rhizobia inoculants in the nursery. Information is given about mycorrhizal fungi, whose use has, to date, been limited in nurseries in Hawaii.

For the end user, the tree planter, there can be some tremendous economic and ecological benefits of rhizobia and mycorrhizal inoculants having been used in the nursery. In the case of Hawaiian koa, for example, inoculation can result in faster early growth, with reduced use of chemical fertilizers. Faster early growth also means faster shading of understory and quicker canopy closure, which reduces weed control expenses. Inoculating tree seedlings in the nursery stage can save the people who plant the trees a lot of money in tree establishment, while at the same time reducing the use of some chemical fertilizers and herbicides. Using microbial inoculants can help make it more sustainable, and also more affordable, for people to reforest with species such as the native Hawaiian koa.

## THE IMPORTANCE OF RHIZOBIA

Rhizobia inoculants are used for nitrogen-fixing legumes. They are important here because they form partnerships with the koa, which is the most widely used native tree for forestry in Hawaii, as well as a number of other important forestry species including rosewoods (*Dalbergia spp.*), narra (*Pterocarpus spp.*), monkeypod (*Albizia saman*), and other species of Acacias and Albizia.

Rhizobia are also essential for nitrogen fixation, a key process for restoring productivity to marginal or degraded land, where most of our nonindustrial

forestry takes place. Although the terms "nitrogen fixing plants" and "nitrogen fixing trees (NFTs)" are widely used, the plants themselves do not have the ability to make use of the nitrogen gas in the air—it is only through the symbiotic association with rhizobia bacteria that the process takes place. Simply planting leguminous "nitrogen fixing" trees will not ensure that nitrogen will be accumulated; the process of biological nitrogen fixation depends on the plant forming a partnership with the correct rhizobia bacteria.

### **BIOLOGICAL NITROGEN FIXATION**

Nitrogen is usually the most limiting element in production, and one of the most expensive to purchase as fertilizer (NifTAL 1984). There is an abundant supply of nitrogen in the air (the air is 80% nitrogen gas, amounting to about 8,000 pounds of nitrogen in the air over every acre of land, or 6,400 kilograms above every hectare). However, the nitrogen in the air is a stable gas, normally unavailable to plants. Many leguminous plants are able to utilize this atmospheric nitrogen through an association with rhizobia, bacteria that are hosted by the root system of certain nitrogenfixing plants. Rhizobia are able to convert the nitrogen gas in the atmosphere into amino acids, which are the building blocks of proteins. The legume is then able to use this for its nitrogen needs. Rhizobia exchange nitrogen for carbohydrates from the plant. As the plants drop organic matter, or when the plants die, the nitrogen from their tissues is made available to other plants and organisms. This process of accumulating atmospheric nitrogen in plants and recycling it through organic matter is the major source of nitrogen in tropical ecosystems. Various land rehabilitation and agroforestry practices exploit this natural fertility process by using nitrogen-fixing trees.

### **WHY USE RHIZOBIA INOCULANTS?**

The process of biological nitrogen fixation depends on the plant forming a partnership with the correct rhizobia strain. There are many strains of rhizobia, and each will work only with certain legumes. Likewise, each legume species will only associate with the proper rhizobia. Unless the strain of rhizobia suited to the legume species is present in the soil, no biological nitrogen fixation will take place. In some cases, 1 strain of rhizobia

may provide some nitrogen fixation, but will be less effective than another.

For example, with *Acacia koa*, of all the rhizobia strains in the world, only a very narrow range will form an effective partnership with koa. Within that narrow range, there is a tremendous amount of variation in effectiveness: some rhizobia strains form mediocre or poor partnerships with koa, not fixing much nitrogen for the tree. A few strains are highly productive partners for koa, forming abundant nodules, fixing a lot of nitrogen for the tree, and really contributing to its growth. A tremendous amount of research has been done over the past few decades by the University of Hawaii NifTAL Project, matching species not only for compatibility, but also selecting strains to maximize nitrogen fixation and nodulation. Inoculation in the nursery is about ensuring that nitrogen-fixing plants form the optimal partnerships with select rhizobia that will fix the most nitrogen for that species. Rhizobia inoculants are live bacteria cultures that are applied to seeds or young seedlings, infecting their root systems with the beneficial bacteria.

If we don't inoculate nitrogen-fixing trees with rhizobia in the nursery, and uninoculated plants go out in the field, they are on their own to form a rhizobia partnership. Especially on marginal or degraded land, there are a lot of unknowns. Whether an uninoculated plant will spontaneously associate with rhizobia in the field depends on several factors: 1) if the correct rhizobia for that plant species are present in the soil; 2) if the rhizobia are available in sufficient quantity to infect roots; and 3) if the rhizobia in the soil are healthy and still able to fix nitrogen (researchers have found that over a period of years, rhizobia in the soil can and do lose the ability to fix nitrogen) (Keyser 2000).

However, even if the correct rhizobia is present in the soil, its nitrogen fixing effectiveness (compared to the recommended and tested inoculant strain applied in the greenhouse prior to transplanting) will be unknown—the soil population may consist of mediocre strains. Also, if the bacteria is present in the soil, it may take several weeks, months, or even years for the association to develop, and this delay will cost the tree planter unnecessary time and money in early maintenance.

Using rhizobia inoculants in the nursery ensures that the nitrogen-fixing trees form a highly

effective partnership to support their growth, and also that the partnership forms early in the tree's life so that its early growth and establishment can be accelerated. This can give seedlings a tremendous jump-start when they get to the field.

#### **How Is RHIZOBIA INOCULANT APPLIED?**

Inoculation (infecting the plant roots with the rhizobia) should take place as early in the tree's life as possible, when the tree will most readily form the association. Rhizobia inoculant can be applied to seeds or to young plants. Usually, rhizobia bacteria come in a peat-based inoculant, with billions ( $10^9$ ) of cells per gram. The inoculant is usually coated onto the seed immediately before planting, or dispersed in clean water and soaked into the planting medium. One hundred grams of inoculant is usually sufficient for 20,000 to 100,000 seeds. The cost is very small per tree inoculated—a fraction of a penny worth of inoculant can replace a hundred or more dollars worth of nitrogen fertilizer over the life of the tree.

If necessary, after select rhizobia strains have been obtained, a crude inoculum can be made in the future from nodules taken from the seedling roots, without the need to purchase additional rhizobia inoculants.

#### **How CAN THE EFFECTIVENESS OF RHIZOBIA INOCULATION BE VERIFIED?**

When rhizobia are present and nitrogen is being fixed, nodules can be seen on the roots of the plants. Each one of the nodules houses millions of rhizobia bacteria. When a nodule is opened, a pink or red color inside is usually a good indicator that it is active, that biological nitrogen fixation is taking place. Thus the effectiveness of inoculation can be verified easily with the naked eye. The rhizobia will survive and multiply as the plant grows.

#### **MYCORRHIZAL FUNGI**

There are other symbiotic microorganisms that can make a significant difference in tree growth. Of these, mycorrhizal fungi are probably the most important. Mycorrhizal fungi form a symbiotic association with many kinds of plants, acting as a kind of conduit for nutrients from the soil to the root. While only a narrow group of plants can form symbiotic associations with rhizobia, 80% to

90% of the world's plant families associate with mycorrhizal fungi, including most subtropical and tropical trees and many native Hawaii species such as koa, ohia (*Metrosideros polymorpha*), and native *Hibiscus* species. It is also a much more generalized association than those formed by rhizobia, because 1 species of mycorrhizal fungus can successfully associate with many plant families.

The mycorrhizal association is known to improve nutrient uptake, particularly of phosphorus. With the help of mycorrhizal fungi, a plant can often take up many times more of certain nutrients than would be possible in the absence of the fungi. Mycorrhizal associations can also improve a tree's drought resistance, salt tolerance, and resistance to both soil pathogens and adverse conditions prevailing on degraded lands. Research with mycorrhizal inoculant and koa has established that inoculation does have a beneficial effect on early koa establishment, giving the trees a "jump-start" in their early growth (Miyasaka 2000).

The use of mycorrhizal inoculants is becoming standard in many areas for forestry and conservation practices, but until recently their use has been very limited in Hawaii. Due to quarantine restrictions and restrictions on the use of imported microorganisms, commercial mycorrhizal inoculants have not been available here. We recently obtained a permit to bring in a commercial inoculant, and have started testing it for effectiveness.

We are fortunate in Hawaii that Dr. Mitiku Habte, one of the world's foremost experts on mycorrhizal fungi, is based at the University of Hawaii. He has developed a method for nurseries to produce their own "crude inoculum" by multiplying inoculum from a starter culture (of *Glomus aggregatum*) in the nursery. A workshop for nurseries interested in learning how to produce mycorrhizal inoculants is planned for the summer of 2001.

#### **CONCLUSION**

Inoculating plants in the nursery allows them to associate with beneficial microorganisms in their early stages of development, when they are most receptive to inoculation. Even if the appropriate beneficial microorganisms such as rhizobia bacteria or mycorrhizal fungi are present in the soil at the field planting site, the unknown quality and the delay in forming this association can cost

the person who is planting the tree in the field hundreds of dollars in establishment costs, while contributing to unnecessary fertilizer and/or herbicide use. In contrast, inoculation in the nursery is very cheap.

When a seedling is supported at the outset by beneficial microorganisms, it is better prepared to survive independently. Using microbial inoculants in the nursery can play an important role in improving both the economic and ecological viability of forest planting in Hawaii.

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