Cooperative Extension Service
College of Tropical Agriculture and Human Resources
University of Hawai'i at Mānoa

Soil and Crop Management
July 2003
SCM-5

Manual on Arbuscular Mycorrhizal Fungus Production and Inoculation Techniques

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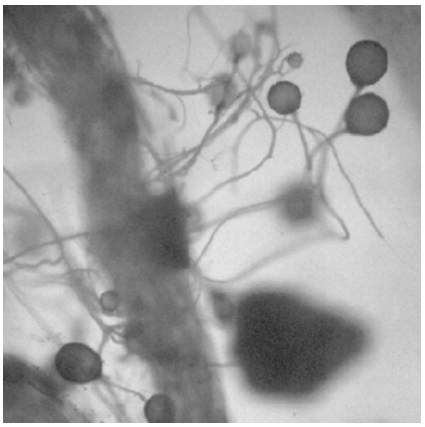
What are arbuscular mycorrhizal fungi?

Mycorrhizal associations are formed by fungi. These associations should not be confused with rhizobial associations, which are symbiotic associations with bacteria that result in nitrogen-fixing nodules.

Two major groups of fungi form mycorrhizal associations: ectomycorrhizal fungi and endomycorrhizal fungi. *Ecto*mycorrhizas associate mainly with temperate-zone trees such as pine, poplar, and willow. These fungi form sheaths around their host's root surfaces. Most ectomycorrhizas can be grown in pure culture. *Endo*mycorrhizas form associations with most plants (approximately 80 percent of all plant species). These fungi cannot be grown in pure culture but must be grown in association with plant roots. They form branched structures called arbuscules within the host's root cells, and thus they are known as *arbuscular mycorrhizal fungi*. The arbuscules are sites of nutrient exchange between the fungus and the host. This manual focuses on arbuscular mycorrhizal fungi.



Mycelia of an arbuscular mycorrhizal fungus emerge from a root; the spherical bodies are vesicles (fungal storage organs).

The associations that arbuscular mycorrhizal fungi form with plants are called symbiotic associations because they are usually beneficial to both organisms. In exchange for carbohydrates produced by the host through photosynthesis, the fungi help the plant take up water and immobile soil nutrients such as phosphorus (P), copper, and zinc. The fungus extends from the plant root and expands the volume of soil that the root system can explore by itself.

To have beneficial associations between the fungus and plant roots, a *low but sufficient* level of P in the soil or rooting medium is needed. If the soil P level is extremely low, the fungus can be parasitic (harmful to the plant) rather than beneficial, because it will compete with the plant for available P. When soil P is high (above "sufficient"), the plant can obtain enough P without the fungus, and the association will not be formed.

For a more detailed explanation of arbuscular mycorrhizal fungi, obtain the CTAHR publication *Arbuscular Mycorrhizas: Producing and Applying Arbuscular Mycorrhizal Inoculum* by M. Habte and N.W. Osorio. This book may be purchased from CTAHR; obtain an order form at *http://www.ctahr.hawaii.edu/oc/forsale/AMFflier.pdf*.

Why should plants be inoculated with arbuscular mycorrhizal fungus?

In many tropical soils, P availability is limited due to P-fixation. Plants inoculated with arbuscular mycorrhizal fungi in the nursery are better able to obtain P when they are later planted into low-P soils. In addition, the association helps the plant obtain water, which is critical to plant survival and growth under dry conditions.

The amount of P fertilizer that needs to be applied to a plant or crop is reduced when effective arbuscular mycorrhizal associations have been formed. In addition to the benefit of lowering fertilizer costs, reducing P applications can help maintain environmental water quality. Erosion of soil from fields with high P levels

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often results in P enrichment of water bodies, which causes excessive growth ("blooms") of algae.

When should plants be inoculated with arbuscular mycorrhizal fungus?

First, the particular plant species of interest must be able to form effective associations with arbuscular mycorrhizal fungi and be dependent on these associations for nutrient exchange. A few plant families have species that do not form mycorrhizal associations (e.g., Brassicaceae, the mustard family, and Chenopodiaceae, the goosefoot family). Table 1 lists selected food and tree crops that are moderately, highly, or very highly dependent on associations with arbuscular mycorrhizal fungi.

Second, an association between an arbuscular mycorrhizal fungus and plant roots will be beneficial to the plant when it is grown under low P or dryland (i.e., low-rainfall, non-irrigated) conditions. If you are expecting to plant seedlings in a soil that is known to be low in P (e.g., volcanic ash soils along the Hamakua Coast of Hawaii, or highly weathered red clay oxisols on Oahu and Kauai) or in areas with low rainfall and no availability of irrigation, then you should consider inoculating seedlings in the nursery with an effective arbuscular mycorrhizal fungus. For further information about soil P in Hawaii, read *Predicting Soil Phosphorus Requirements* by N.V. Hue, H. Ikawa, and X. Huang in *Plant Nutrient Management in Hawaii's Soils: Approaches for Tropical and Subtropical Agriculture*. The article is available on the Web at *http://www.ctahr.hawaii.edu/freepubs* under the category Soil and Crop Management.

Third, the presence of existing populations of mycorrhizal fungi in the field soil will determine whether you need to inoculate seedlings in the nursery. If the soil contains a large population of arbuscular mycorrhizal fungi that will form effective associations with the plants you are growing, then you do not need to inoculate seedlings in the nursery. If the soil has low populations of indigenous mycorrhizal fungi, you should inoculate your seedlings in the nursery.

Low soil P can occur because the soil layers rich in P have been eroded. Low populations of indigenous mycorrhizal fungi can occur when the soil has previously supported either a predominance of plants that are non-mycorrhizal (e.g., cabbage, broccoli) or plants that are not dependent on mycorrhizal associations (e.g., kikuyugrass).

How can you tell if your plant is dependent on mycorrhizal associations or if your site contains effective arbuscular mycorrhizal fungi? One way is to inoculate half of a batch of seedlings in the nursery with mycorrhizal fungi and leave the other half uninoculated. Then, observe the growth of the seedlings after planting in the field. If there are visible differences, with inoculated seedlings showing greater growth, then in the future you should inoculate all the seedlings in the nursery. Alternatively, you could take soil samples from at least three locations within the field, mix them, place the mixture in three 6-inch pots, plant corn seeds (or seeds of another known host species, such as sorghum), and after six weeks have a laboratory determine whether mycorrhizal associations have developed. To determine the presence of mycorrhizal associations, wash a sample of fine roots from the nursery-grown plants and send it to CTAHR's Agricultural Diagnostic Service Center.

(Contact ADSC for information on service fees; call 808-956-5434 or e-mail *adsc@ctahr.hawaii.edu*. Put the sample in a plastic bag with a damp paper towel to maintain moisture, and keep it as cool as possible. On Oahu, samples can be delivered to ADSC on the UH-Manoa campus at 1910 East-West Road, Sherman Laboratory, Room 134. On Kauai, Maui, Molokai, or Hawaii, deliver samples to the nearest office of the CTAHR Cooperative Extension Service.)

Table 1. Dependency of selected trees and food crops on arbuscular mycorrhizal associations (Miyasaka and Habte 2001).

Moderately dependent	***Highly dependent***	***Very highly dependent***
Acacia koa, koa	*Allium cepa*, onion	*Leucaena leucocephala*, koa haole
Acacia mangium	*Carica papaya*, papaya	*Manihot esculenta*, cassava
Colocasia esculenta, kalo, taro	*Senna siamea*, pheasantwood	*Sophora chrysophylla*, māmane
Gliricidia sepium, madre de cacao	*Coffea arabica*, coffee	
Sesbania grandiflora, katurai	*Falcataria moluccana*, albizia	

How is arbuscular mycorrhizal fungus inoculum produced?

1. Surface-sterilize seeds of a host plant, such as corn or sorghum, using a 10% solution of household bleach: 1 volume of bleach (containing 5% sodium hypochlorite) plus 9 volumes of water. Soak the seeds in the solution for approximately 5–10 minutes.

2. Germinate the seeds by placing them between moistened paper towels on a plate placed in a loosely sealed plastic bag. Corn seeds will germinate within two to three days.

3. ***It is essential to select a medium with low phosphorus***. An appropriate nursery medium for producing arbuscular mycorrhizal inoculum contains a low level of P. Do not use a nursery medium that contains P fertilizer. If there is much P available in the planting medium, the fungi will not form associations with the seedlings. Our recommended medium is crushed basaltic rock with a diameter less than ⅛ inch (available from some concrete or aggregate suppliers). This medium was selected because it contains very low levels of available plant nutrients (including P). Alternative media are quartz sand (not coral sand), available from some agricultural suppliers, or a mixture of 1 volume of peat moss with 3 volumes of either vermiculite, perlite, or volcanic cinder.

4. ***It is critical that the medium be sterilized to ensure good mycorrhizal infection***. If it is not sterilized, there is a chance that microorganisms present in the medium will inhibit the formation of mycorrhizal associations. In an autoclave or pressure cooker, heat the medium for 60 minutes. In a steam-sterilizer, heat it at 200°F for 60 minutes. Let the medium cool and use it immediately.

5. Place the medium in 6–10-inch diameter pots. To prevent escape of medium, seal the large bottom holes with tape, but poke small holes to allow drainage of water.

6. Obtain a starter-culture of arbuscular mycorrhizal inoculum from a commercial source or researchers working on mycorrhizal fungi. A starter culture of *Glomus aggregatum* may be obtained from Dr. Mitiku Habte, CTAHR, but this is subject to availability. Thoroughly mix the culture into the medium at 1 volume of inoculum to 20 volumes of medium. Or, put ½ ounce (5–10 grams) of inoculum into each planting hole when planting the germinated seeds.

7. Plant 2–6 germinated seeds in each pot. The object is to maximize root development in the pot so that the medium is completely filled with a mass of roots.

8. Place the pots in a greenhouse or in a well lighted area under a rain shelter to minimize contamination from microorganisms carried by rain or wind.

9. Water the plants once a day until water begins to drip from the bottom of the pot.

10. Apply 1 teaspoon per pot of a low-P, slow-release fertilizer, such as 19-5-12 Apex® Foliage Fertilizer or another low-P (e.g., 17-6-10), slow-release fertilizer. Or, make your own low-P fertilizer using the following recipe (adapted from a manual by J.N. Gemma and R.E. Koske, available on the Web at *http://www.hawaii.edu/scb/scinativ_mycor.html*). Mix into 2 gallons of water 1⅓ teaspoons (6 g) of Peters Professional® 15-0-15 fertilizer, ⅕ teaspoon (0.9 g) of epsom salts ($MgSO_4$), and 1.5 milliliters of a concentrated phosphate fertilizer solution called Quick Start™ (4-12-4) made by Miracle-Gro®. (These materials are available from some garden shops and fertilizer companies). You can measure milliliters using a simple measuring spoon for liquid medicines obtainable at most drug stores.) Apply 1 cup of fertilizer solution once a week to each pot.

11. Associations between the host plant roots and the arbuscular mycorrhizal fungi should form in approximately six weeks, depending on temperature and sunlight. To confirm that mycorrhizal associations have formed, excavate several roots containing fine branches after six weeks and send to CTAHR's Agricultural Diagnostic Service Center for determination of mycorrhizal associations (see instructions for sending on page 2).

12. To produce a mycorrhizal inoculum containing spores, continue growing the host plant for 16 weeks. Withhold water beginning at 14 weeks after planting. At 16 weeks, remove the plant tops and discard them. Dump the medium and roots from the pots onto a clean tray. Cut up the dried roots with scissors, mix the fragments with the medium, and store this as a "crude inoculum" in a refrigerator or a cool, dry location. It can be stored for up to one year.

How are plants inoculated in the nursery?

1. Surface-sterilize the seeds. For example, for koa seeds, soak them in a 10% solution of household bleach (1 volume of bleach plus 9 volumes water) for 5–10 minutes. For other plant species, you may need to try more dilute concentrations of bleach solution or a shorter soaking duration to ensure surface-sterilization without harming the seeds. Test a few seeds before treating large batches to be sure that the treatment will not inhibit their germination.

For seeds that have seed-coat dormancy, additional treatment may be needed to ensure good germination. For example, koa seeds can be immersed in hot water removed from the stove after it reaches boiling, and soaked overnight.

2. Germinate the seeds between moistened paper towels on a plate enclosed in a loosely sealed plastic bag. Alternatively, if you have developed an effective surface sterilization technique, you could plant ungerminated seeds directly into the potting media.

3. ***Selection of a medium with low P is critical to successful development of mycorrhizal fungus associations with plant roots***. We have successfully used a mixture of 1 volume of volcanic ash soil (a highly P-fixing soil collected along the Hamakua Coast) with 1 volume of Sunshine® Mix #2 from Sun Gro™, or a mixture of 1 volume of volcanic ash soil with 1 volume of Sunshine Mix #2 and 1 volume of crushed basaltic rock. Or, use 1 volume of peat moss (for example, Sunshine Mix #2) mixed with 3 volumes of either vermiculite, perlite, or volcanic cinder.

4. ***Sterile medium is critical to success in developing mycorrhizal fungus associations with plant roots***. To prevent pathogen problems or competition from other microorganisms, sterilize the nursery medium in an autoclave or pressure cooker for 60 minutes. Or, use a steam-sterilizer and raise the temperature of the medium to 200°F for 60 minutes. In the case of soil, let it cool and aerate for 2–7 days to allow release of possible toxins generated by heating.

5. Inoculate the nursery medium with arbuscular mycorrhizal inoculum at a rate of 1 volume of inoculum to 20 volumes of medium.

6. Plant surface-sterilized seeds in pots containing the sterilized medium. Or, put ½ ounce (5–10 grams) of inoculum into each planting hole before sowing the seeds.

7. Water daily until water begins to flow out of the bottom of the pot.

8. Apply a low-P, slow-release fertilizer such as 19-5-12 Apex® Foliage Fertilizer or another low-P (such as 17-6-10), slow-release formulation. Alternatively, make your own low-P fertilizer as described earlier (Step 10 under *How is arbuscular mycorrhizal fungus inoculum produced?*). The amount of fertilizer applied depends on your plant species and pot size. You will need to determine the proper amount through careful observation of plant growth. For example, for koa seedlings grown in Super Cell tubes (10 cubic inches, #SC-10, Steuwe and Sons, Inc.), we applied 10 milliliters per tube per week.

9. After six weeks, confirm presence of arbuscular mycorrhizal fungus associations by submitting root samples to ADSC as described on page 2.

References

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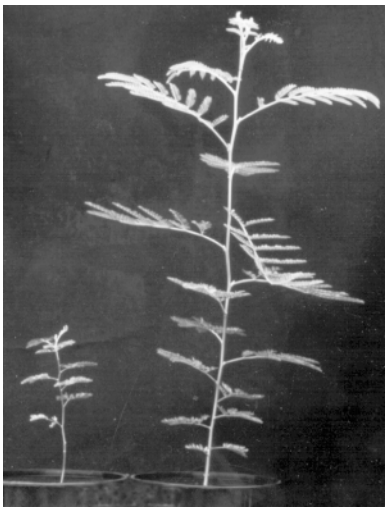
Corn in a 10-inch pot ready to be harvested for inoculum.



The koa at right grew better in a low-P soil when inoculated with fungus.