

Appendix I. Checklist of steps for the on-farm production of arbuscular mycorrhizal [AM] fungus inoculum*

The target date for setting up the system is as early as possible after the last frost. Some work is necessary before this date, and the following list is designed to help you get things started so everything is ready on time.

4 months before the predicted date of the last frost

- Germinate bahiagrass seeds in a pot of vermiculite or seed starter. Sources of seed can be found on the internet, for example: <http://agriseek.com/market/p/Pensacola-Bahiagrass-Seed.htm>
- Purchase materials
 - Conical plastic pots (we use RLC-4 Pine Cells from Stuewe and Sons, Corvallis, OR 97333)
 - 7 gallon “Grow Bags” (one source is Worm’s Way, Bloomington, IN 47404)
 - Ground cover fabric

3 months before the predicted last frost

- Collect soil from 0 to 10 cm (4 in) depth from a natural area on the farm or a field that has not been used within the past 2 years to grow the crops to be inoculated. This is to avoid introducing pathogens.
- Mix this soil with sand (1:3 soil:sand, volume basis) and use the mixture to fill the conical plastic pots. We use a coarse sand, such as swimming pool filter sand.
- Transplant bahiagrass seedlings into the pots, fertilize occasionally with a low-P or P-free fertilizer if necessary. The plants will become colonized by the AM fungi native to your soil.

As soon as possible after the last frost

- Set up the inoculum production area:
 - Cover an area with the ground cover fabric (16 bags fit nicely on a plot 1.2 m x 3.6 m (4 ft x 12 ft))
 - Mix compost with vermiculite. A good general recommendation is 1:4 compost:vermiculite (volume basis) using yard clippings compost produced by municipal composting facilities. Fill the 7 gallon bags approximately $\frac{3}{4}$ full with the mixture. Roll the lip of the bags down to just above the level of the mixture. Sixteen bags require approx. four 4 ft³ bags of vermiculite and 4 ft³ of compost.
 - Transplant 5 bahiagrass plants into each bag.

For the remainder of the growing season

- Weed and water the bags as needed. The AM fungi will proliferate as the plants grow.

Frost will kill the bahiagrass. The mycorrhizal fungi will overwinter naturally outdoors in the bags. The following spring when you are producing your seedlings in the greenhouse:

- Harvest the inoculum. Shake the compost and vermiculite mixture free from the roots into a collection bin. If you remove most of the dead leaves prior to this step, the resulting inoculum will be cleaner. Mix the inoculum from several bags together. This mix will contain spores produced by the fungi and living hyphae. You may also chop the roots into short segments (less than 1 cm [1/2 inch]) and mix these in as well.
- Mix the inoculum into your potting media. For flats with cells of 50 cm³ or less, use a 1:9 (inoculum: media, volume basis) mixture. For larger cells, a 1:19 mixture should be sufficient.
- Greenhouse fertilization regime. This aspect of the process is very important. Add too much phosphorus and the plants will resist colonization by the fungi, and all of your work to this point will have been just practice.
 - Conventional farms. Remember the discussion earlier in this article: try to achieve a P addition of 15 ppm or less for no more than three fertilizer applications per week. Apply P-free solutions at other times if necessary.
 - Organic farms. If your potting media requires additional fertilization, use a low P source. If your potting media contains all the nutrients needed during the greenhouse culture phase, no modifications are recommended at this time.

*This appendix accompanies the eXtension.org article, 'On-farm Production and Utilization of AM Fungus Inoculum', by David D. Douds Jr., USDA-ARS Eastern Regional Research Center. Go to <http://www.extension.org/> to find both the full article and this appendix.

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