On-Farm Mycorrhizal Fungal Inoculum Production

Introduction

Arbuscular mycorrhizal fungi (AMF) exist in a symbiotic relationship with over 80% of all vascular plants including most crop plants. Almost all brassicas such as canola, radish, turnips, kale, etc. are not associated with AMF.

To form the AMF relationship, a growing plant is growing exudes or releases organic (i.e. carbon-based) substances (i.e. sugars, proteins, amino acid, etc.) from its roots. These carbon substances stimulate AMF hyphae/mycelium or thread-like bodies to grow into plant roots – connecting soil to plants. Nutrients such as phosphorous, nitrogen, sulfur, potassium, and micro-nutrients transported within these hyphae are traded for more carbon substances from the plant. AMF uses this carbon to expand its body – hyphae – further into the soil and plant roots and to conduct activities – biochemical reactions including making spores.

Spores, pieces of hyphae, or colonized roots are AMF reproductive structures. These reproductive structures are used as **AMF Inoculum** to increase the amounts of AMF in soil. This protocol describes how to produce your own on-farm **AMF Inoculum** as biological amendment to plants.

In this protocol, **Inoculum Soil** is field soil or soil containing AMF spores, pieces of hyphae and colonized roots. **Inoculum Soil** is soil collected from fields or pastures where the **AMF Inoculum** is to be added, or places managed under conditions similar those being amended. This **Inoculum Soil** will be adapted to the conditions where the **AMF Inoculum** is applied. The culturing or growing process is done in pots or bags filled with **Potting Media** and grown under conditions to boost the numbers of spores, pieces of hyphae, or colonized roots in **AMF Inoculum.** This **AMF Inoculum** is applied to the field or pasture to raise the likelihood of growing in AMF roots.

See Appendices A-E for more details about AMF and the **AMF Inoculum** process.

Supplies

Cement Mixer or Buckets, optional
Cork Borer or Trowel
Greenhouse, optional
Grow Bags or Pots
Inoculum Soil
Paper – Brown or Butcher Paper or Newspaper
Plastic Bags or Buckets
Potting Mix
Sand
Seeds
Shovel
Soil Probe, optional
Tarp, optional

Collecting Inoculum Soil

1. Collect **Inoculum Soil** from the field, pasture, or range you want to treat with a shovel, soil probe or hand trowel at a depth of 3-6 inches (5-15 cm).
2. Air dry **Inoculum Soil** by manually breaking apart large clods and spreading in a thin layer (about 0.5 inch or 1.5 cm) on the paper.
3. Remove and dispose of any clods which cannot be manually broken apart and pebbles or stones larger than about 0.5 inch or 1.5 cm
4. Cut visible roots into 1-inch (2.5-cm) pieces with a scissors and mix with drying soil.
5. Let soil dry for 5-10 days.
6. Details for when and where to collect the **Inoculum Soil** are found in Appendix B.

Culturing/Growing AMF Inoculum

1. Make a **Potting Mix** from sand, potting soil, compost, sterilized field soil, perlite, or vermiculite. Details for this mix are in Appendix C.
2. Fill grow bags or pots about ¾ full of **Potting Mix**.
3. Mix **Potting Mix** and **Inoculum Soil** from step above together in a plastic bag or bucket by shaking or mixing with a shovel or trowel. The concentration of **Inoculum Soil** to **Potting Mix** should be a minimum concentration of 1:30 (1 part inoculum soil in 30 parts potting mix) to a maximum concentration of 1:5 (1 part inoculum soil in 5 parts potting mix). See Appendix D.
4. Place bags or pots on concrete or tarp or in a greenhouse to prevent roots from outside of the pots or bags growing into the ground or other pots or bags. The number of bags or pots needed is calculated according to the amount of inoculum needed. See Appendix E.
5. Seed pots with annual or perennial seeds. Choose your seeds and seeding density according to Appendix D.
6. Water the pots/bags with tap or well water only adding as needed. Add fertilizer if you are growing in sand only, high concentrations of perlite and/vermiculite, or if needed to keep the plants from dying. See Appendix C for further details.
7. Check the status of the **AMF Inoculum** by removing a sample containing some roots from the pot/bag with a cork borer or trowel and accessing AM density by measuring rhizosheaths, root colonization, and/or spore density after about 6-8 weeks of growth. See details in Appendix E.
8. Continue growing the plants to build the amounts of spores, pieces of hyphae, and roots for a total of 10 to 14 weeks depending on the results from the step above.
9. Prepare the pots/bags to harvest the **AMF Inoculum** (see Appendix E) by cutting the plants at the surface.
10. Quit watering to dry.
11. Once the soil is dry remove the **AMF Inoculum** or all the materials from the pots/bags.
12. Manually break apart large (>1-2 inches) clods.
13. Remove visible roots and cut into 1-inch (2.5-cm) pieces.
14. Mix cut roots back into the **AMF Inoculum** using plastic bags, buckets, or a sand mixer to create the **AM Inoculum** for field or pasture application (see Appendix E).

Observations/What's Happening

1. AM fungi need living plants to grow.
2. AM fungi reproductive structures are spores, pieces of hyphae in the soil, and pieces of hyphae and spores in both living and dead roots.

3. Stressful conditions such as low nutrient levels typically increases AMF activity, growth, and spore production. Therefore, if the plants appear to be slightly stressed, it is likely there will be high amounts of roots colonized with AM hyphae, hyphae in the soil, and spores.
4. If the **Inoculum Soil** does not appear to have many AMF spores, hyphae, or roots with AMF, it may still be used because the culturing/growing process is designed to largely boost the amounts of AMF.

References

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The International Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) - https://invam.ku.edu/

Appendix A. Arbuscular Mycorrhizal Fungi Details

Arbuscular mycorrhizal fungi (AMF) exist in a symbiotic relationship with over 80% of all vascular plants including most crop plants. The major plant exceptions to this relationship are almost all brassicas such as canola, radish, turnips, kale, etc. In this relationship, AMF trade plant nutrients and water from the soil for carbon (i.e. food) from the plant. AMF do this very efficiently (i.e. require less work or energy and have little loss than if the plants address all their water and fertility needs directly). The bodies of AMF are hyphae or mycelium (i.e. fine threads that are shaped like microscopic fine roots). These fine threads will grow throughout the soil accessing small, microscopic areas (i.e. microsites) where AMF sense nutrients are located. In these microsites, the shape and size of the hyphae allow AMF to maximize nutrient and/or water absorption, because the hyphae have a greater surface area (i.e. direct contact with soil) to volume (i.e. diameter or width of hyphae). The higher surface area to volume ratio is replicated inside plant roots where AMF hyphae penetrate the root cell wall and branch out around the cell membrane to maximize contact with the cell membrane along with nutrient and water delivery. The growth of AMF hyphae inside plant root cells along with the formation of AMF spherical spores and vesicles in roots is referred to as colonization.

If AMF are able to enter and colonize plant roots early in seedling growth, they can help reduce plant pathogens particularly soil borne pathogens like *Fusarium* sp. and *Rhizoctonia* sp. **AMF inoculum** (i.e. growing media containing AMF colonized roots along with fungal hyphae and spores in the media) may be applied to seeds or seedlings at planting or mixed with soil in the field (i.e. inoculated). Commercial inoculum is available, but producing on-farm inoculum may be more effective. On-farm inoculum is adapted to edaphic factors (i.e. soil conditions such as texture, pH, nutrient concentrations, temperatures, plant types – annual or perennial, etc.) with favorable epigenetics (i.e. heritable changes in gene expression influenced by various environmental factors) to enhance inoculum efficacy and plant growth.

Because AMF need a living plant to grow, inoculum production of AMF is referred to as trap culturing. While a plant is growing, it will exude organic (i.e. carbon-based) substances (i.e. sugars, proteins, amino acid, etc.) which trigger AMF reproductive structures (i.e. propagules) such as spores, pieces of hyphae, or colonized roots to germinate (i.e. form a small threadlike germ tube). Germ tube growth is limited by the amounts of available resources (i.e. food/carbon). If a root from a living plant is not encountered before these resources run out, the spores or hyphae dies. A germ tube from pieces of AMF hyphae has the least amount of carbon and is not a good inoculum source. Spores, because of their larger sizes and lipid composition, may form a longer germ tube to explore the soil for living roots. However, spores and hyphae inside roots (i.e. colonized roots) provide the best source of AMF inoculum since their germ tubes have access to carbon/food in the AMF structures along with the resources inside the roots themselves.

Not all spores, pieces of hyphae, or colonized roots in field soil will be viable (i.e. healthy and able to germinate). Viability typically declines with age so using field soil which has living plants growing in it is ideal followed by soil which has just had active plant growth. Over-drying soil or heating soil prior to using it as an inoculum source in trap culturing reduces viability of spores, hyphae, and even colonized roots. Hyphae and spores inside roots also will be protected from adverse growing conditions (i.e. temperature and moisture extremes). Therefore, it is critical to have colonized roots in the **inoculum soil** when trap culturing.

Although trap culturing is the only mechanism for producing **AMF inoculum** to inoculate seeds, seedling roots, or field/potting soil, the amounts of viable propagules in the **AMF inoculum** may fluctuate. To improve results, a plant that is strongly associated with AMF such as a warm-season grass should be used in the trap cultures. In arid and semiarid environments, sporulation may be low and 2-3 successive culturing rounds may be needed. The roots may be highly colonized and increasing spore density may not be

necessary, but it is important to have both spores and colonized roots in the **AMF inoculum**. Repeated culturing and increasing spore density is important if you are trying to identify AMF species but are not as critical as having more colonized roots if you are trying to generate on-farm **AMF inoculum**.

Appendix B - Inoculum Soil

Inoculum soil should be soil collected from fields or pastures managed under similar conditions rather than undisturbed soil. If there are pest – weed, pathogen, or disease – issues, soil from a different field should be selected. Soil from undisturbed areas may be used as a last resort rather than using soil from a different ecoregion. Undisturbed soil may have similar edaphic and climatic conditions as field, pasture, or rangeland soil but the fungal diversity and function may not match agricultural fields. AMF are capable of adapting to new conditions but this may take some time. Soil should be collected towards the end of the growing season when plants are beginning their senescence. If soil is not to be used within 1-2 weeks, soil should be air-dried by spreading it out on paper for about 5 days with coarse roots chopped by a scissor or knife to similar sizes and soil clods broken up manually or with a hammer. Before mixing with potting media, fresh or air-dried inoculum soil should be stored in a refrigerator or a cold room at around 40^{o}F (4^{o}C). Inoculum soil may be stored air-dried in the cold for up to 10 months.

Appendix C - Potting Media

The potting media should be free from pests – viable weed seeds or pathogens. If compost or high fertility **inoculum soil** or potting soil is used in the **potting mix,** this media should be mixed with higher amounts of regular soil, sand, perlite, or vermiculite (i.e. a 1:10 mix – one part high fertility to 10 parts low fertility) to keep fertility levels in the containers low. Caution is needed when using compost in the **potting mix** depending on compost qualities such as pH, pathogens, and nutrient concentrations. When determining what to use to make the **potting mix**, it is critical to identify how the field, pasture, range, seeds, or seedlings are going to be inoculated with the **AMF inoculum.**

Appendix D - Growing Conditions

On-farm AMF inoculum is produced by mixing **inoculum soil** with **potting mix** in grow bags or pots with several (i.e. 5-20) seeds or seedlings depending on bag or pot size. The number of seeds or seedlings per bag or pot depends on the container size and type of plant. For annual grasses such as sorghum, sorghum-sudan, wheat, oats, etc.; annual or perennial forages; or tropical grasses, plant density should be one plant per 2.5 cm^2 (1 in^2), while for corn, plant density should be one plant per 10 cm^2 (4 in^2). Grass plants, especially corn, sorghum, and warm seasons, are chosen because these plants are typically strongly associated with AMF while annual or tropical (i.e. plants which do not grow well in your climate) plants reduce any potential 'weed issues from seeds present in the **AMF inoculum**.

To effectively produce **AMF inoculum** with high concentrations of AMF reproductive structures – spores, hyphal fragments, and roots containing spores or hyphae, the container used for growth should not be too large (<30 cm or 12 inches in diameter) and maybe as small as cone-tainers (~1 inch (2.5 cm) in diameter). The fresh or air-dried **inoculum soil** from the field should be mixed with **potting mix** at a minimum concentration of 1:30 (1 part **inoculum soil** in 30 parts **potting mix**) to a maximum concentration of 1:5 (1 part **inoculum soil** in 5 parts **potting mix**). The concentration depends on if the **inoculum soil** is fresh or if the roots of the plants in the field where the **inoculum soil** was collected had rhizosheaths. Lower concentrations of **inoculum soil** is appropriate if fresh soil or soil containing plants with roots with a high density of rhizosheaths.

AMF inoculum soil is produced by placing containers outside, typically on gravel, concrete, or a tarp. or in a greenhouse. Containers should be watered with tap water from a hose, watering can, or fertigation system. Keeping the fertility in the containers low is critical to increasing AMF inoculum density. Plants should be grown until senescence begins, or if forages or perennials are being used, the plants may be clipped. Annual grasses may be clipped or containers reseeded if the goal is to keep plants growing for longer periods of time.

Appendix E - AMF Inoculum

The quality of the **AMF Inoculum** should be checked prior to plant senescence or terminating the plants and collecting the **AMF Inoculum**. Collect a soil sample from the container using a ½ inch (1-cm) cork borer or small trowel at least halfway to the bottom. If there are more than five plants growing in the container, use a trowel or cork borer to remove a plant keeping most of the roots intact. If there are less than five plants, carefully separate the roots from the soil. Assess if the roots appear to have rhizosheaths or soil remains attached with shaking. If no or few rhizosheaths are present, examine the roots for the presence of AMF following the *Measuring Mycorrhizal Colonization* protocol. Mycorrhizal spores may also be separated from the soil following the *AMF Spore Isolation* protocol. If indicators of AMF are found, the plants should be terminated by clipping at the soil surface and ceasing to water. If AMF indicators are not found in the samples or there is concern about the amount of AMF structures – spores, hyphae, and/or colonized roots, the containers should be reseeded or the plants should be allowed to grow for at least 6-10 more weeks.

The **AMF Inoculum** is allowed to air-dry in the containers and transferred to a cement mixer, barrel, or other equipment to mix together all the inoculum. The mixed inoculum is applied to seeds as a dry applicant or a slurry may be created and applied to seeds or seedlings. The application rate for the dry inoculum is 2-4 ounces (50-100 grams) per 1000 seeds or 1000 seedlings while for the slurry should be mixed at a rate of 2-4 ounces per quart (50-100 grams per liter) for 1000 seeds or 1000 seedlings depending upon the quality. Alternatively, the **AMF Inoculum** may be placed in furrow like a dry fertilizer or spread on the soil prior to or at planting using a manure or compost spreader. A slurry also may be applied using a Y-drop or liquid fertilizer system. To be most effective, application needs to be on seeds or as close as possible to germinating seeds or seedlings for the roots to be colonized by AMF as early as possible. Application rates depend on **AMF Inoculum** quality with high quality application rates being about 40-50 pounds per acre (0.05 tonnes per hectare) and low quality at 450 pounds per acre (0.5 tonnes per hectare).