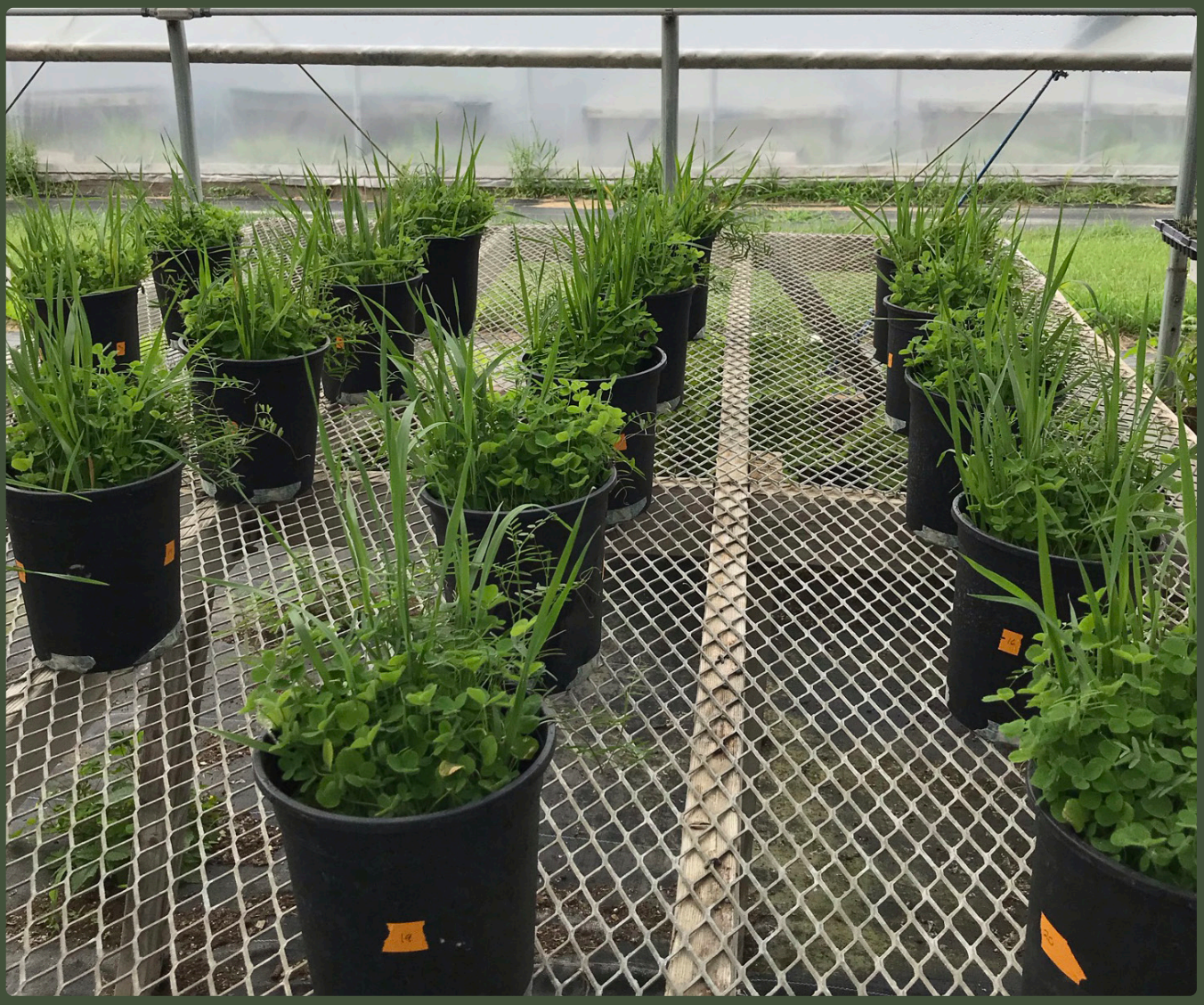
Growing Local Mycorrhizal Inoculum

A GUIDE AND INSIGHTS FROM A FIELD TRIAL



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WHAT ARE MYCORRHIZAE?

Mycorrhizae are organisms that form symbiotic associations with plant root systems. There are at least four main types of mycorrhizal fungi. Our work focuses on Aburscular Mycorrhizal Fungi (AMF) because they are the type most essential to agricultural systems[1]. AMF form symbiotic associations with more than 80% of terrestrial plant species, including many agricultural crops[2]. Unlike fungi who's fruiting bodies appear above-ground in the form of mushrooms, AMF live their entire life cycle underground as mycelium (a network of root-like threads that make up the fungus, also known as hyphae). To sustain themselves, the hyphae enter and colonize the plant root. Here they perform their symbiotic exchange: the fungi provide mineral nutrients and water to the plant, while receiving essential nutrients and carbohydrates in return.

Numerous studies have shown the benefit of a healthy AMF soil community to plant crops[3, 4, 5, 6, 7]. The hyphal networks essentially become extensions of the plant's root network, significantly increasing the area of soil the plant can access, and therefore available nutrients and water. Without the help of AMF, plant roots can only reach nutrients 1-2mm away, whereas hyphae attached to these roots can extend their reach by 8cm or more[8]. Due to this, AMF can decrease the need for chemical fertilizer input while simultaneously increasing crop yield[4]. AMF presence also increases resistance to stressful conditions, such as drought, heat, salinity, and soil pathogens[3,9]. Furthermore, AMF improve soil structure and aggregation, drive plant community diversity and productivity, and lower greenhouse gas emissions[10]. AMF can be a supportive tool for any farmer aiming to practice organic, regenerative agriculture to increase farm resilience in a changing climate.

Our research study and inoculum growth trial took place on unceded Abenaki territory, colonially known as Vermont, east of Lake Pitawbagok (or Lake Champlain). For us, and for many growers working in similar conditions, phosphorus uptake was a key concern. Excess phosphorus, 38% of which is agricultural runoff from farms, contributes to yearly toxic algae blooms in Lake Champlain[11]. AMF have demonstrated efficacy at increasing plant's phosphorus uptake[12, 13, 14]. Therefore, we understand AMF to be an ally in safeguarding watersheds. We encourage farmers everywhere to protect surrounding lands and water which are being overwhelmed by the effects of unsustainable farming practices; excess fertilizer application, tilling, etc.

Parsley being harvested for data collection at the research study field site.

HOW TO PROMOTE AMF ON YOUR FARM.

It's likely that the soil on your farm already has its own AMF community. However, many common farm practices negatively impact these fungal communities. Tilling breaks up mycelium. Fallow periods interrupted with frequent tilling do not leave ample time for fungal communities to develop or return[15]. Pesticides, fungicides, and herbicides also damage AMF communities. Traditional fertilizer use can be harmful, since excess use of phosphorus discourages AMF growth, inhibiting mutual symbiosis with crops. Whether or not you plan to apply mycorrhizal inoculum on your farm, limiting or eliminating these practices can nurture the microbial soil network, including the AMF community already present in the soil[16]. Other management practices which protect and encourage existing AMF communities include: consciously increasing plant diversity, incorporating native polycultures, rotating symbiotic crops, planting crops that encourage AMF, and cover cropping[15, 17, 16].

Mycorrhizal field bank planted with polyculture covercrop at Digger's Mirth Collective Farm in the Intervale, Burlington VT.

It's important to know that some plants don't form associations with AMF, they actually deter them! When trying to enhance AMF presence, don't waste time and resources inoculating these plants[19, 18, 20]:

Brassicaeceae family- broccoli, brussels sprouts, cauliflower, kale, mustard greens, collards, cabbage, rutabaga, arugula, cress, horseradish, radish, turnip, rapeseed
Ericaceae family*- blueberry, cranberry, huckleberry, lingonberry, rhododendron, azalea
Amaranthaceae family- beets, spinach, chard, amaranths, quinoa, lamb's quarters
Others- poppies (Papaveraceae), carnations (Caryophyllaceae), lupin (Fabaceae)

This is an incomplete list but includes most plants that may be of key concern in the Northeast U.S.

*Instead of AMF, plants in the Ericaceae family form associations with Ericoid mycorrhizae.

If you're ready to maximize AMF on your farm, inoculation is the way to go. There are two options for inoculating: buying commercial inoculum, or growing your own. Here's a break-down of the pros and cons of each from our experience:

	FARM GROWN	COMMERCIAL
+	• Cost effective. • Limited labor required once established. • End product is customized to your local environment, so will correspond with soil type and existing microbial community. • Minimal risks associated with introducing a foreign species as long as gathering location is vetted well. • Inoculum will represent a diverse AMF group, with more symbiotic microbes than present in commercial inoculum[21]. • Research indicates that higher numbers of AMF species in soil increases benefits to crops[23].	• Minimal added labor. • Time and resource efficient. Does not require a growing season, materials, or space to prepare. • Able to control which specific fungi species are introduced to your farm.
−	• Requires an extra season of planning, time, resources, and space to grow the inoculum. • Success of inoculum will depend on the local AMF community in the soil applied as a starter. Results can't be guaranteed.	• Can be costly • AMF are species specific. There's no guarantee that the commercial inoculum species will pair with chosen crops. • There are typically only a few AMF species in commercial inoculum. 1/3 of market products contain only one species[22]. Having a more robust and diverse AMF community provides better results[23]. • There are concerns about the impact of introducing foreign fungi to local ecosystems[21]. • Symbiosis between native AMF and the larger microbial soil community is essential to AMF function. This may not be replicated through introduced non-native commercial AMF[23, 24]. • What's on the label is not guaranteed to be in the bag[25].

Growing your own inoculum is not as intimidating as it may seem. A relatively small amount of labor can provide big rewards. The rest of this guide highlights the process of growing on-site inoculum.

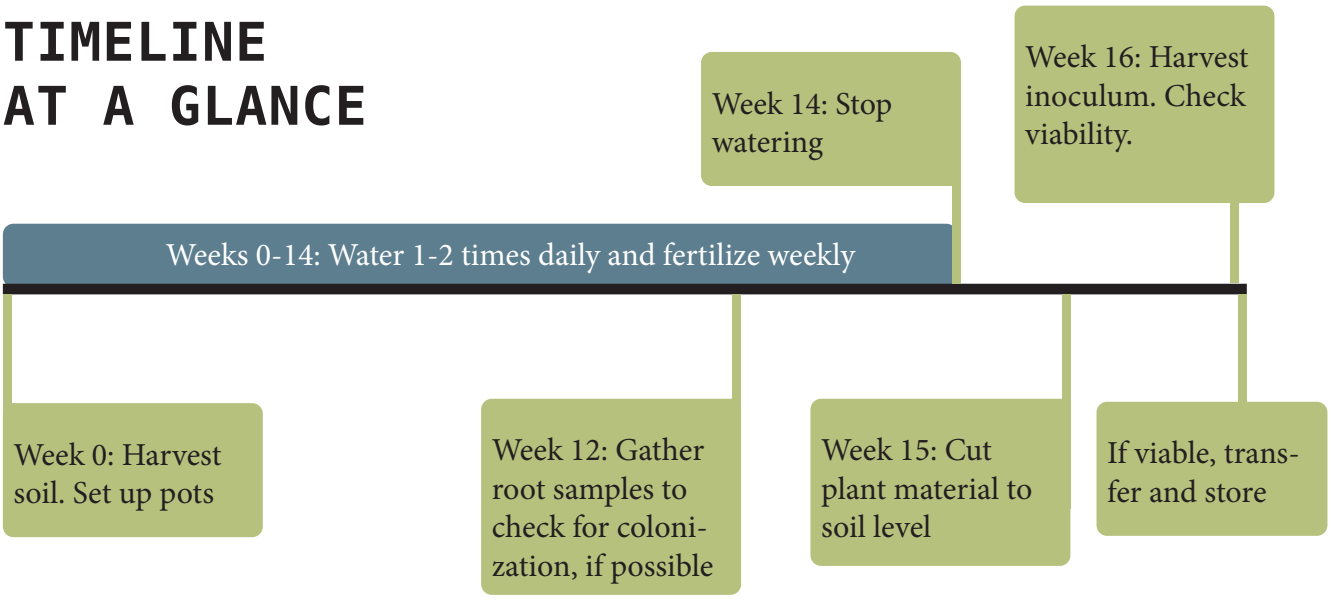
GROWING ON-SITE MYCORRHIZAL INOCULUM: 101

Materials Overview

- Reusable pots of uniform volume, cleaned and sterilized with a 30% bleach solution or 0.5% hydrogen peroxide solution.
- Growing medium, a 1:1 ratio of sand to vermiculite.
- Seeds. We used a ratio of hairy vetch, oats, and crimson clover, to replicate the covercrop mix in our field trials. You can also grow just one plant, native grasses are a good option[13].
- Soil inoculum, soil collected from a wild buffer area around your farm.
- Materials to create a modified fertilizer solution (ideally a ratio of 0.01 parts phosphorous, and 0.1 parts of all other nutrient concentrations). More detail on this later.
- A pressure cooker, or access to an autoclave.
- Gloves.
- Spray bottle with 70% ethanol solution to sterilize equipment.
- Ideally, access to a soil lab that can check inoculum viability.

Warning! Watch out for soil pathogens
While the purpose of growing mycorrhizal inoculum is to increase the quantity of fungi in the soil, we want to ensure we are only doing this for the beneficial AMF and not any potential soil pathogens, which can include fungi, bacteria, and nematodes.
Here are links to a few companies that provide soil pathogen testing services: https://all-cropsolutions.com, https://microbe.creativebiomart.net.
This step is optional, but it's always a good idea to be safe, especially if you're planning on applying your inoculum to a large number of crops.

TIMELINE AT A GLANCE



UTILIZING A LAB FOR VIABILITY CHECKS: HOW AND WHY

Because mycorrhizae cannot be seen with the naked eye, the only way to guarantee your inoculum has enough fungi to be viable is through lab analysis. In the lab, scientists can check the roots of the plants grown in your pots to see approximately what percent of them were colonized by mycorrhizae. They can also extract spores from the medium of your inoculum and quantify those, another important signifier of adequate mycorrhizal presence. Ideally you want a minimum of 60% root colonization and 50 spores for every gram of inoculum.

Unfortunately since this is an emerging field, there are not many labs that offer these services yet, especially in the U.S. We are aware of these that do:

- **INVAM** (The International Collection of (Vesicular) Arbuscular Mycorrhizal Fungi) https://invam.ku.edu/ordering
- **MycoRoots** https://mycoroots.com/services-and-fees/
- **University of Florida Soil Microbiology Extension Program** https://soils.ifas.ufl.edu/soil-microbial-ecology/mycorrhizal-services/soil-biology-tests/

We recognize there are accessibility barriers here, both in the number of labs available and in associated costs. If sending your inoculum to a lab for viability tests is not feasible currently, we still encourage you to try growing your inoculum! Consider testing the viability yourself by setting up a small field trial. Create two plots near each other where the environmental conditions are as similar as possible. Plant the same crop into each plot, applying your inoculum to the crop in one of the plots and leaving the other plot as the control. Let both plots grow, applying the same amount of water and nutrients. Observe any differences you notice. Do some plants seem more robust than others? Did one plot have a higher yield on average? Did plants in one plot grow more quickly? Ideally, you would do at least 3 trials of this in different locations to control for variables. Even just a simple trial like this can reveal a lot about how well your inoculum may be working.

Additionally, check out this guide: Mycorrhizae Observations Made Simple by Ben Waterman at https://projects.sare.org/information-product/mycorrhizae-observation-made-simple/ to learn about an on-farm technique to stain roots and check for colonization levels yourself. To learn more check out his NESARE FNE12-770 Report in 2015.

GROWING STEPS

1) **Gather pots of uniform volume.** These can be any size, and the number depends on how much inoculum you would like to grow. Help with estimating the amount of inoculum required is in the next section, *Utilizing your Inoculum*. If the drainage holes on your pots are relatively large, we recommend covering them with tape on both sides and then poking small holes. You want ample drainage, but don't want the medium to leave the pots.
   - Clean the pots with soap and water
   - Spray and wipe them down with a 30% bleach solution or hydrogen peroxide to ensure no airborne microbes or fungal spores will contaminate your inoculum.
   - Let them air dry completely.

GROWING STEPS CONTINUED

2) **Gather soil inoculum starter.** Find a relatively wild and untouched area of your farm, where perennial herbaceous species known to partner with AMF grow. A wild buffer zone around or between fields is a great option. A helpful chart to reference can be found at https://mycorrhizae.com/mycorrhizal-status-of-plant-families-and-genera/, look in the first column for plants that form endomycorrhizal associations. Once you've found a promising area, you're ready to sample. Using a clean trowel and bucket, sample from the top 10 cm, which is where most AMF live. Gather a trowel full every 2 feet apart along the chosen area. Mix soil in the bucket gently to create a composite sample. The highest concentration of AMF will be within and around plant roots so shake the soil off roots into the bucket as you go. You will need 50 grams of soil for each pot.


These are the wild buffers to the north and south of the Diggers' Mirth field site where we gathered our inoculum soil starter in the early spring

3) **Prepare the medium.**

- Calculate the amount of medium you will need based on the volume and number of pots, knowing you will fill the pots with sand and vermiculite at a 1:1 ratio.
- Mix together calculated amount of sand and vermiculite in equal parts.

Like the pots, your medium will need to be sterilized to ensure there are no pathogens present that could interfere with mycorrhizal association formation. We use an autoclave since we have access (autoclaving the medium at 121 C ° for 20 minutes), however a pressure cooker will work just as well. If you don't have one, this is the most significant piece of equipment to invest in or borrow for this process. It is worth it since it guarantees sterilization of your medium.

- Heat the medium at 15 PSI (equivalent to 121 C °) for 60 minutes. This can be done in clean jars or heat tolerant spawn bags in the pressure cooker.
- After sterilization, let the medium cool down completely. Use immediately.

4) Now that the media and soil are ready, it's time to set up your pots!

- Add sterilized media up to 5 cm below the pot tops.
- Add 50 grams of soil inoculum starter to each pot. Gently stir in with a sterilized tool.
- Add additional sterilized media up to 3 cm below the pot tops. Water thoroughly until a small amount of water drains through the bottom of the pot.
- Add seeds. We calculated seed number based on the ratio of seeds per acre applied as a cover crop. We then converted this to the pot surface area. Feel free to estimate, ensuring uniform quantity across all pots. It is crucial to use enough seeds to achieve high germination and plant growth across the entire pot.
- Finally, top off pots with a handful of sterilized medium to cover the seeds.

Above: Luca setting up pots.
Right: Pots in the Diggers' Mirth greenhouse the day of setup.

5) Place the pots somewhere with full sunlight, ideally a greenhouse. Optimum environmental conditions for mycorrhizal growth are 16 hours of sunlight at 25 C °. For the next 3-4 months water the pots once or twice daily (depending on climate, sun exposure, and humidity) to encourage healthy plant growth. Weed as needed, and fertilize weekly (starting the week after setup).

6) Fertilizing: Phosphorus is central in nutrient exchange between plants and AMF. Lack of phosphorus will encourage AMF growth since plants rely heavily on AMF to meet their phosphorus needs. Hence, a low phosphorus fertilizer should be applied to the pots each week. Low phosphorus slow release fertilizers can be applied in small amounts, or you can mix your own to create the optimum ratio of nutrients. Here is our recipe for a modified fertilizer:

Into 2 gallons of water add:

- ***1/2 ounce of North Country Organics 6-0-6***
- ***1/4 teaspoon epsom salts***
- ***1 heaping teaspoon of Neptune's Harvest 2-3-1***

Mix thoroughly, and add 240ml (1 cup)* to each pot weekly.

**We used half gallon pots, you may need to adjust for different sized pots.*

GROWING STEPS CONTINUED

7) **Maintain pots each week and monitor growth until week 12.** If you found lab access, this is a good time to check plant root AMF colonization levels. Gently harvest a few roots from multiple pots using a sterile tool. Put them in sterile bags and submit to the lab to analyze. If roots are over 60% colonized on average, continue with next steps. If they're not, maintain plant growth for 2-4 additional weeks before continuing to next steps.

8) **At week 14,** stop watering and fertilizing the pots. Let them dry.

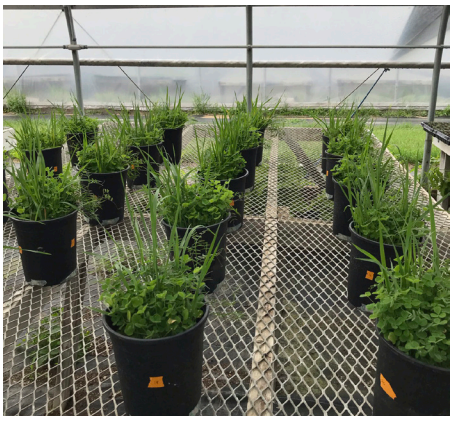
9) **At week 15,** cut the plants at soil level, composting or reusing them as mulch. Continue to let the medium dry.

10) **At week 16,** gather samples for final viability checks if you have lab access. Again, gently gather some roots from multiple pots (ideally ones you did not gather from before) with sterile tools. Submit to lab for root colonization checks. If the lab can conduct spore quantification, gather some medium as well and submit in sterile bags. After that, or if unable to check for viability, harvest your inoculum. Pour the medium onto a sterilized surface. Cut up the roots into small pieces with sterilized scissors and mix them gently into the medium with a sterilized tool. Ensure there is no above-ground plant material in this, only roots!

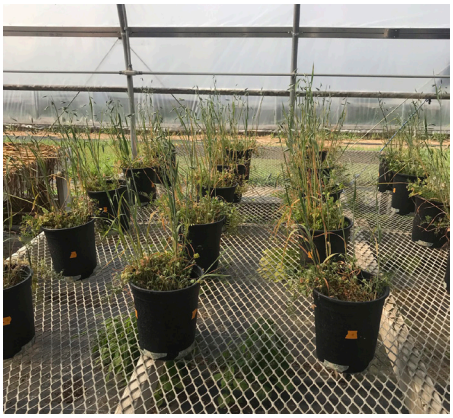
11) **Congratulations, your inoculum is now complete!** If you sent samples to a lab, you're aiming for average root colonization levels over 60%, and spore counts of at least 50 spores/gram of medium. This will confirm that your inoculum is viable, meaning there is enough AMF presence in the mix to effectively inoculate crops. The inoculum can be used immediately, or stored. If you perform this process in the fall to prepare for the next growing season and temperatures have already dropped sufficiently, inoculum can be left to overwinter in the cold. Alternatively, you can store the inoculum at 4° C, fridge temperature, for up to two years. We stored ours at 4° C in sterilized bins with holes. We covered the holes with breathable tape to prevent spore contamination but allow for respiration (fungi respirate!) (see image on page 11).

WEEK 1

WEEK 3



WEEK 6



WEEK 13

UTILIZING YOUR INOCULUM

There are a few options for how to utilize your AMF inoculum. One way is to mix the inoculum into potting mix to germinate seedlings. The recommended ratio of inoculum to potting soil is 1:9 for growing cells 50 cm^3 (1.7 fluid ounces) or smaller, and 1:19 for larger cells[17]. You can also sprinkle inoculum (about a teaspoon) onto the rootball of the plant, or into the hole in the field you're transplanting into. Ensure the soil is well packed before watering to avoid washing away the inoculum. The first option may provide better results because it allows the plants to form relationships with the AMF from the beginning of their growth. Hence by the time they're transplanted into the field, with minimal transplanting shock, their roots will already be colonized by mycorrhizae, and nutrient transfer from the field soil can begin immediately. Either way, you are now successfully implementing your farm-grown, local mycorrhizal inoculum. Congratulations!

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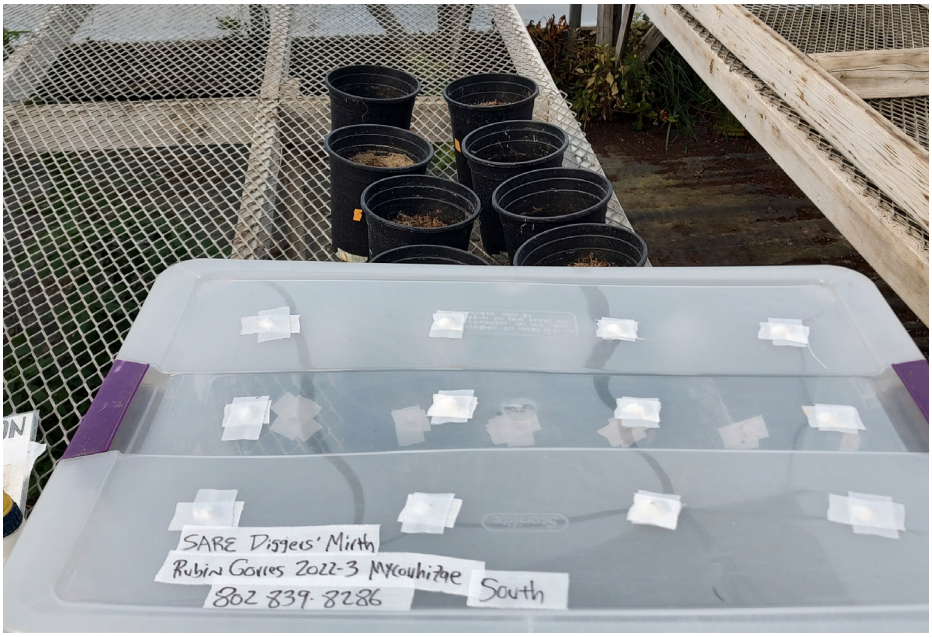
We acknowledge that this research took place on unceded Abenaki territory and that endemic mycorrhizae were abundant in their ancestral territory before colonial land practices [26, 27, 28].



Finished inoculum being transferred into sterile, breathable bins to be placed in a cooler for storage.

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All photos were taken by either Luca Kolba or Jess Rubin.

FARMER NOTES