jove

Inoculating and Observing Arbuscular Mycorrhizal Cultures on Superabsorbent Polymer-Based Autotrophic Systems

Louis Paré[1], **Claudia Banchini**[2], **Franck Stefani**[2]

[1] Centre d'Étude de la Forêt (CEF) and Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval [2] Ottawa Research and Development Centre, Agriculture and Agri-Food Canada

Corresponding Author

Franck Stefani
franck.stefani@agr.gc.ca

Citation

Paré, L., Banchini, C., Stefani, F. Inoculating and Observing Arbuscular Mycorrhizal Cultures on Superabsorbent Polymer-Based Autotrophic Systems. *J. Vis. Exp.* (208), e66848, doi:10.3791/66848 (2024).

Date Published

June 14, 2024

DOI

10.3791/66848

URL

jove.com/video/66848

Abstract

Arbuscular mycorrhizal (AM) fungi are difficult to manipulate and observe due to their permanent association with plant roots and propagation in the rhizosphere. Typically, AM fungi are cultured under *in vivo* conditions in pot culture with an autotrophic host or under *in vitro* conditions with Ri Transfer-DNA transformed roots (heterotrophic host) in a Petri dish. Additionally, the cultivation of AM fungi in pot culture occurs in an opaque and non-sterile environment. In contrast, *in vitro* culture involves the propagation of AM fungi in a sterile, transparent environment. The superabsorbent polymer-based autotrophic system (SAP-AS) has recently been developed and shown to combine the advantages of both methods while avoiding their respective limitations (opacity and heterotrophic host, sterility). Here, we present a detailed protocol for easy preparation, single spore inoculation, and observation of AM fungi in SAP-AS. By modifying the Petri dishes, high-resolution photographic and video observations were possible on living specimens, which would have been difficult or impossible with current *in vivo* and *in vitro* techniques.

Introduction

Arbuscular mycorrhizal (AM) fungi (Glomeromycotina) are ancient plant root symbionts (~500 Ma[1,2]) that may have played an essential role in the colonization of terrestrial soils by tracheophytes. This long coevolution between AM fungi and tracheophytes places arbuscular mycorrhiza as a masterpiece of interkingdom mutualism. AM fungal hyphae significantly increase the ability of the host to forage for soil nutrients[3], including nutrient carry-over to new hosts via mycorrhizal networks[4]. The hyphal network improves soil structure, and the production of glomalin could reduce soil erosion[5]. The transfer of part of the atmospheric carbon to the fungal root symbiont increases soil carbon sequestration[6]. Overall, AM fungi improve plant resilience to both abiotic and biotic stresses and have therefore, received considerable attention in agroecology[7]. Indeed, AM fungi-friendly agricultural management practices have

the potential to reduce the use of chemical inputs for crop production and improve soil organic carbon content, which are important objectives that farmers need to integrate into their management practices in order to comply with national and international commitments regarding the transition to sustainable agricultural practices and the fight against climate change.

However, AM fungi are soil microscopic fungi, and their study is difficult due to their obligate biotrophy and rhizosphere distribution. Soil is one of the most difficult biotopes to study because of its opacity, the huge diversity of niches, and multi-trophic interactions at all scales. The isolation, propagation, and characterization of AM fungi are, therefore difficult. Until the middle of the 20th century, only AM fungal species forming sporocarps had been characterized[8]. However, the majority of AM fungal species produce non-sporocarpic spores ranging from ~20 µm to ~500 µm in diameter. The description of the soil wet sieving technique[9] opened the way to describe these AM fungal species, and the rate of species description has increased since then. Nevertheless, AM fungi represent a small group of species compared to Dikarya.

Trap cultures, i.e., the inoculation with spores or an environmental soil sample containing AM fungal spores of a pot filled with autoclaved material such as turface and vermiculite and a sterilized seed of a host (leek, plantain), is one way to propagate AM fungi under controlled conditions[10]. However, the success of the inoculation can only be assessed by looking for the presence of arbuscules in root fragments after staining or by wet sieving a subsample or the entire pot to isolate spores. It is usually recommended not to disturb the system for at least 6-12 weeks before the analysis of the pot culture. This culture technique is suitable for propagating most known AM fungal species, but live observation of the fungal symbiont is not possible, and the success of inoculation is uncertain, especially when single spore cultures are attempted.

On the contrary, the *in vitro* propagation of AM fungi can be monitored live thanks to the transparency of the culture medium[11], but this culture technique requires the availability of transformed roots and the presence of carbon in the culture medium to work in a sterile environment. The spores must be sterilized, and together with the association with a heterotrophic host, most of the known AM fungal species are not successfully propagated using this technique.

Therefore, the propagation of AM fungi using current techniques, although established and widely used in most laboratories, has some limitations for the study of AM fungi. Paré et al. (2022)[12] developed an *in vivo* technique using a transparent superabsorbent polymer (SAP) in combination with whole plants to propagate AM fungi. The technique, designed as an SAP-based autotrophic system (SAP-AS), is simple and inexpensive and combines the advantages of pot culture (association with an autotrophic host, non-sterile conditions) and *in vitro* cultures (transparent medium, live monitoring of symbiosis development). Here, we present a protocol explaining how to set up the cultures with single spore inoculation and use the SAP-AS for high-magnification observation of the extraradical mycelium. Specifically, we describe how to modify two-compartment Petri dishes, prepare the nutrient solution, prepare the superabsorbent polymer (SAP), prepare the seedlings, assemble the SAP-AS and inoculate with a single spore, pregerminate the spores, and live monitor the development of the symbiosis.

Protocol

1. Modification of two-compartment Petri dishes

NOTE: The materials required for this step are listed in the **Table of Materials**.

1. Modify the Petri dish lid.
    1. Using the rotary tool and appropriate carving bit, drill two holes (~7 mm in diameter) in the top of the lid and a 1 cm opening in the side. One hole will be used to irrigate the root (vermiculite) compartment and the other for the hyphae (SAP) compartment.
2. Modify the Petri dish bottom.
    1. Drill a notch in the side of the Petri dish bottom for the plant stem using the electric rotary tool. Make sure the notch is not too deep to prevent the liquid from draining. This notch will align with the 1 cm opening in the lid (step 1.1).
    2. To replace the plastic barrier of the dual-compartment Petri dish with the nylon mesh filter membrane, use the electric rotary tool to drill a 30 mm rectangular notch down to the bottom of the Petri dish.
    3. For high-resolution observation, drill circular holes ~25 mm in diameter in the bottom of the Petri dish. Depending on requirements, make a single hole (or multiple holes) on the SAP side or one in each compartment. Ensure that the holes are not drilled directly under the irrigation holes of the lid.
3. Install nylon mesh filter membrane.
    1. Use the metal guide and paper clip to wedge the piece of the nylon mesh filter membrane in place and press it firmly onto the plastic barrier. Align the top of the membrane with the top of the plastic barrier. Make sure the membrane protrudes from the bottom of the guide.
    2. Using the pyrography kit, cut along the edge of the metal guide to melt and seal the membrane to the bottom of the Petri dish and the plastic barrier separating the two compartments.
    3. With the metal guide and paper clip still in place, carefully remove excess nylon mesh filter membrane with tweezers.
    4. Verify under the dissecting microscope that the nylon mesh filter membrane is properly sealed to the plastic to prevent root penetration into the hyphal (SAP) compartment.
4. Install coverslip.

    NOTE: The coverslip is placed under the Petri dish. This allows the immersion oil to be used and the coverslip to be cleaned.
    1. Place the silicone sealant along the edge of the circular holes (outside the Petri dish bottom).
    2. Place the coverslip on the outside of the Petri dish bottom and gently press to ensure perfect adhesion.
    3. Allow to dry, and remove excess sealant with a razor blade.
5. Inspect and clean.
    1. Use an air blast to remove any dust or pieces of plastic that may have adhered to the Petri dish.
    2. Inspect under a dissecting microscope to identify and correct any defects.

        NOTE: Wear gloves to avoid fingerprints on the Petri dishes

2. Preparation of 1 L of nutrient solution mMS-1

1. Prepare stock solutions.
    1. Macronutrients: Weigh and dissolve the following in 1 L of distilled water:
    7.31 g of $MgSO_4.7\ H_2O$, 0.80 g of KNO_3, 0.65 g of KCl, and 0.048 g of KH_2PO_4,.
    2. Weigh 2.88 g of $Ca(NO_3)_2.4H_2O$ and dissolve in 1 L of distilled water.
    3. Weigh 0.80 g of NaFeEDTA and dissolve in 0.5 L of distilled water.
    4. Weigh 0.375 g of KI and dissolve in 0.5 L of distilled water.
    5. Micronutrients
        1. Dissolve 3 g of $MnCl_2.4H_2O$ in 100 mL of distilled water.
        2. Dissolve 1.325 g of $ZnSO_4.7H_2O$ in 100 mL of distilled water.
        3. Dissolve 0.75 g of H_3BO_3 in 100 ml of distilled water.
        4. When dissolved, mix the solutions prepared in steps 2.1.5.1-2.1.5.3.
        5. Weigh 0.65 g of $CuSO_4.5H_2O$ and dissolve in 50 mL of distilled water.
        6. Dissolve 0.12 g of $Na_2MoO_4.2H_2O$ in 100 mL of distilled water.
        7. Add 5 mL of the solution from step 2.1.5.5 and 1 mL from step 2.1.5.6 to the solution from step 2.1.5.4.
        8. Adjust the volume of the solution obtained in step 2.1.5.7 to 500 mL with distilled water.
2. Prepare 1 L of mMS-1 media.
    1. Add 700 mL of distilled water to a 2 L glass bottle.
    2. While constantly stirring with a magnetic bar, add 100 mL of macronutrients solution (step 2.1.1), 100 mL of calcium nitrate solution (step 2.1.2), 5 mL of ferric EDTA solution (step 2.1.3), 1 mL of KI solution (step 2.1.4), and 1 mL of micronutrients solution (step 2.1.5).
    3. Adjust the volume of the solution to 1000 mL.
    NOTE: mMS-1 is adapted from Bécard and Fortin (1988)[14]. Sterilization at 121 °C for 15 min is optional as the SAP-AS is not sterile. pH is not adjusted because the SAP has a strong buffering effect, and Paré et al. (2022)[12] showed that the conditions on SAP were relatively neutral (6.7 to 7.4).

3. Preparation of SAP

NOTE: The materials required for this step are listed in the **Table of Materials**.

1. Hydrate SAP: mix 5 g of dry SAP with 500 mL of mMS-1 nutrient solution (step 2.2).
2. Allow to hydrate for 12 h (overnight) at room temperature (RT).
3. Drain and collect the hydrated SAP grains.
    1. When hydrated SAP is used to fill the 12-well plate (section 7), do not drain the hydrated SAP and mix the hydrated grain and the leftover of mMS-1 nutrient solution.

NOTE: Three granulometries of the dry SAP are available: small, medium, and large. The medium granulometry (1-2 mm) is the most appropriate. The SAP of large granulometry swallows too much when hydrated and the Petri dish lid cannot be closed, while the small granulometry SAP leaves little space between them, which is a limitation for the observations of the fungal structure between the grains.

4. Preparation of seedlings

NOTE: The materials required for this step are listed in the **Table of Materials**.

1. Germinate the seeds of *P. lanceolata* on blotting paper moistened with mMS-1 nutrient solution in a Petri dish.
2. Incubate in the dark at RT.
3. Rehydrate as needed until rootlets are at least 2 cm long.

5. Assembly and management of SAP-AS

NOTE: The materials required for this step are listed in the **Table of Materials**.

1. Assemble SAP-AS (step 1 + step 2 + step 3 + step 4)
    1. Place the seedling stem in the notch on the side of the Petri dish with the root inward and the cotyledons/leaves and stems outward.
    2. Cover the roots with approximately 1.5-2 g of vermiculite.
    3. Hydrate the root compartment with 8 mL of mMS-1 nutrient solution. This will help stabilize the root and vermiculite.
    4. Add 5 g of hydrated SAP along the nylon mesh filter membrane on the side of the root compartment.
    5. Add 15 g of hydrated SAP to the hyphal compartment. The hyphal compartment is the compartment with no notch on the side of the Petri dish bottom.
    6. Before closing the Petri dish with the lid, make sure that the side notches of the lid and the Petri dish bottom are aligned so as not to damage the stem.
    7. Seal the Petri dish with a strip of paraffin that joins the base and lid, taking care not to crush the young stem.
    8. Weigh and record the average weight of the Petri dish to determine when the SAP-AS needs to be rehydrated.
    9. Stack the Petri dishes to optimize space. Keep the Petri dish in the dark using an opaque cover with an opening on the side for the seedlings.
2. Manage SAP-AS.
    1. Hydrate the Petri dishes with mMS-1 nutrient solution only.
    2. Use the average weight as a reference. When the Petri dish has lightened by 5-6 g, add mMS-1 nutrient solution to bring it back to the original weight. Ensure that the vermiculite and SAP are kept hydrated but not flooded. Roots must have access to oxygen.
    3. As the seedling and its fungal partner develop, irrigate them more frequently. It is not necessary to water both compartments at the same time. Irrigation requirements vary according to seedling development, but also according to the environmental conditions in which SAP-AS are incubated.

4. Reduce the irrigation requirements by removing old leaves if the plant has more than three healthy leaves. Remove dead leaves.

   NOTE: The presence of cations such as K^+, Mg^{2+}, and Ca^{2+} in the mMS-1 nutrient solution tends to decrease the expansion of the SAP network[13] through time.

6. Inoculation of the SAP-AS with a single spore

NOTE: The materials required for this step are listed in the **Table of Materials**.

1. Inoculate with a single spore.
   1. Heat the glass pipette with a flame candle or Bunsen burner. Pull as the glass melts to stretch the glass tube until it separates.
   2. Under a stereomicroscope, break the tip of the glass pipette to reduce the internal diameter (typically 100-300 µm). This will greatly facilitate the manipulation of individual spores.
   3. Open the Petri dish, locate the spot where the spore is to be placed, and clear a space among the 5 g of hydrated SAP along the nylon mesh filter membrane to expose a section of a root.
   4. Under the dissecting microscope, pick up a spore with the extruded pipette. Pipette some liquid before pipetting the spore. Avoid pipetting liquid after collecting the spore.
   5. Under the dissecting microscope, carefully deposit the spore on the root. The liquid pipetted before pipetting the spore will help to expel the spore. If the spore is not deposited in an optimal place, use an acupuncture needle or brush hair to bring the spore back into contact with the root. If necessary, take a photo of the spore deposited on the root for reference and use a marker to locate the inoculation site.
   6. Replace the SAP over the root fragment to provide a moist microenvironment for the root and spore. Wait at least 24 h before irrigating the root compartment, and be careful not to wash the spore off the root.
   7. Before closing the Petri dish with the lid, make sure that the side notches of the lid and the bottom of the Petri dish are aligned so as not to damage the stem of the seedling.

      NOTE: The SAP-AS is ready for inoculation when the roots reach the nylon mesh filter membrane, ideally a few days after irrigation, so there is no free liquid.

7. Spore germination on SAP (optional)

NOTE: The materials required for this step are listed in the **Table of Materials**.

1. Germination of spores.
   1. Blend the hydrated SAP to obtain a smooth texture (see step 3.3.1). Air bubbles usually disappear within 24 h or by autoclaving (optional) the blended SAP.
   2. Using a 20 mL syringe without a needle, pipette approximately 2 g (equivalent to 2 mL) of blended SAP into each well of the 12-well plate.
   3. Select a spore under the dissecting microscope and use the extruded pipette as described in section 6.
   4. Under the dissecting microscope, place a spore in the center of each well.

5. Incubate the 12-well plate in the dark at RT and monitor germination periodically.
6. Under the dissecting microscope, select a spore with a growing hypha (at least a few millimeters in length).
7. Add 1 mL of mMS-1 nutrient solution to the well and wait a few minutes for the medium to become more fluid.
8. If necessary, use the acupuncture needle to gently remove the spore and hyphae from the substrate.
9. Collect the spore either with a pipette or by bringing the roots of a seedling to the surface of the medium in order to stick the spore and hyphae to the roots. Then, place the seedling in the Petri dish as described in step 5.1.3.
   NOTE: Coordinate spore germination and seedling growth. This method has only been tested with *R. irregularis* spores.

8. Live monitoring of symbiosis development

NOTE: The materials required for this step are listed in the **Table of Materials**.

1. Routinely observe with a dissecting, upright, or inverted microscope.
   1. Observe the top of each compartment at 20x – 40x using a dissecting microscope with white illumination and dark field.
   2. If an inverted microscope is not available, observe the bottom of each compartment with a dissecting or upright microscope by inverting the Petri dish. Before inverting the Petri dish, allow the SAP-AS to dry for 2 days so that the SAP adheres slightly to the bottom of the Petri dish.
   3. Make detailed observations at 40x through the bottom of the Petri dish and up to 100x (oil immersion) through the coverslip.
2. Observe spores and hyphal networks.
   NOTE: The fungal structures developing inside the SAP grains can be stained.
   1. Prepare an ink and vinegar solution according to Vierheilig et al. (1998)[15]. Do not heat.
   2. Extract a SAP grain containing hyphae and spores and place it in the ink at RT. The ink will penetrate the SAP grain and stain the hyphae and spores within a few minutes.
   3. Remove the SAP grain and place it in mMS-1 nutrient solution or tap water. The ink will come out of the grain within 12 h, but the spores and hyphae will remain stained.
   4. Press the SAP grain between two microscope slides to flatten it for photography and spore counting.
3. Observe arbuscules.
   1. Extract a few roots from the root compartment and stain using the ink and vinegar protocol according to Vierheilig et al. (1998)[15]. Be careful not to immerse the roots in the KOH solution for more than 2-3 min; otherwise, the roots will become too fragile for the following steps and disintegrate into unusable debris.
   2. Under a dissecting microscope, select a root fragment approximately 5 mm long that may contain arbuscules and place it on a slide in a drop of water.
   3. Using acupuncture needles or extremely thin tweezers, tear off the roots in layers a few cells thick.

4. Absorb the water and check under the microscope that the segments with arbuscules are still present and of satisfactory quality, and correctly placed on the slide. At this point, the arbuscules must be easily visible within the root cell layers.
5. Allow drying for 2-3 min so the root layers adhere to the slide. Add a drop of polyvinyl alcohol-lactic acidglycerol (PVLG) buffer, and add the coverslip. Observe by standard microscopy methods.

Representative Results

The SAP-AS is a simple and inexpensive technique for culturing AM fungi and observing the development of intraradical and extraradical fungal structures. Here, we provided a detailed protocol to help users set up SAP-AS and we introduced two modifications compared to the original description of the SAP-AS.

First, the presence of SAP on the root compartment along the Nitex membrane facilitates the inoculation of the system with a single spore (**Figure 1**).

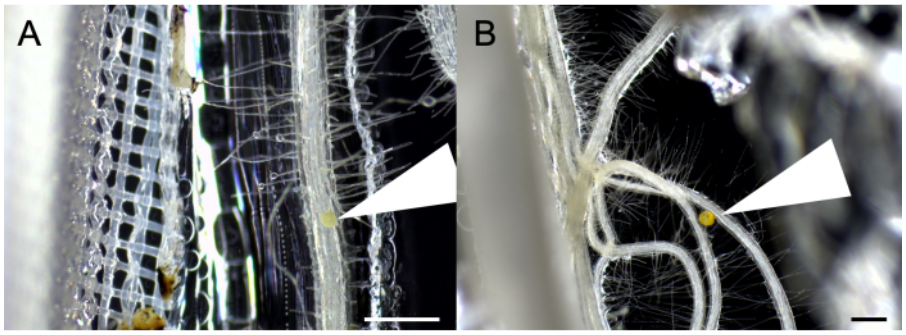

Figure 1: Single spore inoculation of the SAP-AS. (**A**) Inoculation with a single spore of *Rhizophagus irregularis.* (**B**) Inoculation with a single spore of *Funneliformis mosseae*. The white arrows indicate the point of inoculation. Scale bars: 500 µm. Please click here to view a larger version of this figure.

It is easy to identify an area with a few roots where the spore can be deposited. The exact location of the spore can be marked on the surface of the Petri dish lid and checked periodically for germination and colonization of the roots. The presence of hydrated grains of SAP provides a moist environment conducive to spore germination. The inoculation on the roots next to the Nitex membrane allows rapid development of the symbiosis with the extraradical mycelium to quickly access the hyphal compartment and forage for nutrients (**Figure 2**). The ability to observe whether the single spore placed at the inoculation point germinates or not also allows us to identify and remove unsuccessful cultures and replace them.

Second, the coverslips at the bottom of the Petri dish allow high-resolution live imaging of the AM-fungal symbiosis (**Figure 2B**,**C**) and, in particular, of the cytoplasmic flow within the extraradical mycelium (**Figure 2D**).

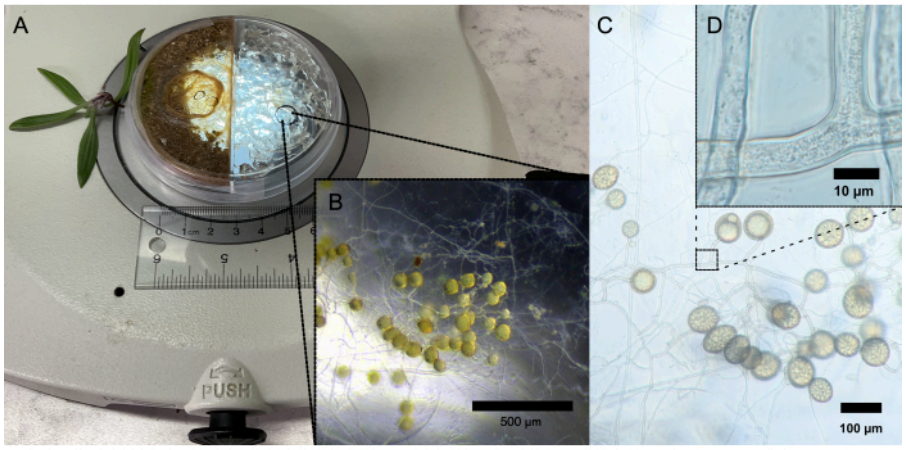

Figure 2: Inoculated SAP-AS. (**A**) SAP-AS inoculated with a single spore of *Rhizophagus irregularis* (DAOM 197198). The host plant is *Plantago lanceolata*. (**B**) Cluster of spores observed through the coverslip in the hyphal compartment under a stereomicroscope. Scale bar: 500 µm. (**C**) High-resolution photograph of the spore cluster observed through the coverslip under a light microscope at 10x magnification. Scale bar: 100 µm (**D**) High-resolution photograph of hyphae observed through the coverslip under a light microscope at 100x magnification. Scale bar: 10 µm. Please click here to view a larger version of this figure.

Users can attempt to germinate AM fungal spores on SAP hydrated with mMS-1 nutrient solution to guarantee the SAP-AS are inoculated only with viable spores (**Figure 3**). However, this was tested only with commercial spores of *R. irregularis*, and the success of spore germination on SAP hydrated with mMS-1 nutrient solution may vary significantly with other AM fungal species.

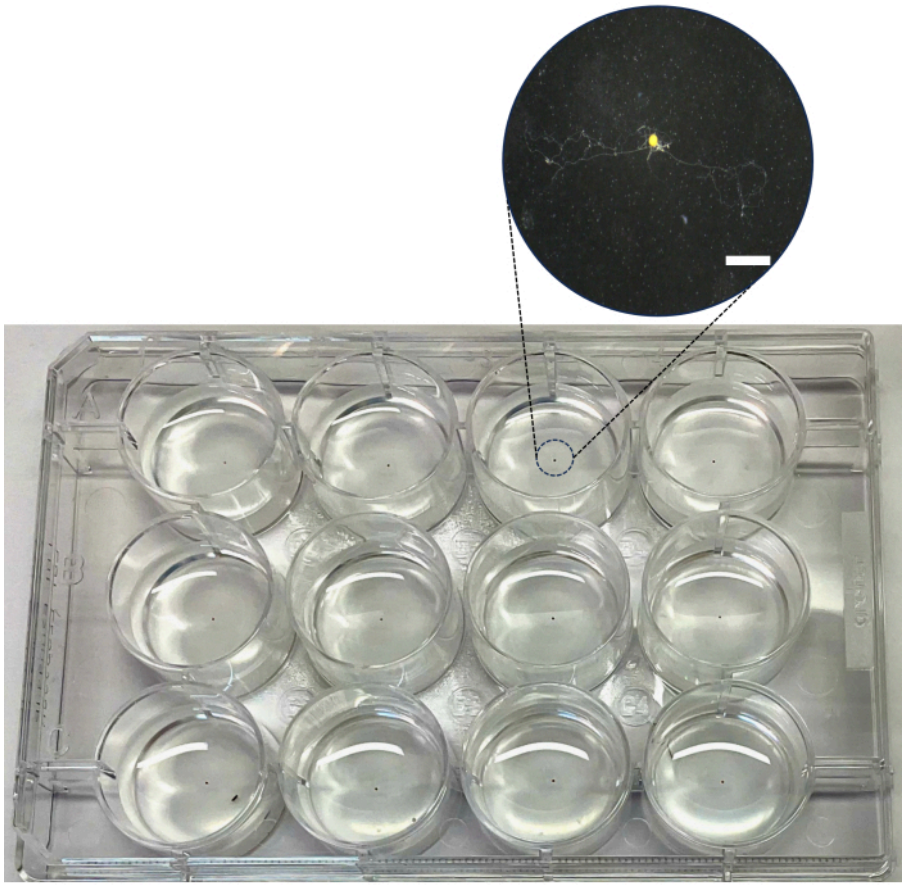

Figure 3: Germination of *R. irregularis* spores in 12-well plate. Scale bar: 500 µm. Please click here to view a larger version of this figure.

Finally, because SAP-AS is an *in vivo*, non-sterile technique for propagating AMF, the Petri dish can be manipulated on the bench and opened to sample colonized roots, free spores, or colonized grains of SAP (**Figure 4**). Intraradical and extraradical fungal structures can be stained in a manner similar to AMF propagated in pot or root organ cultures (**Figure 4C**).

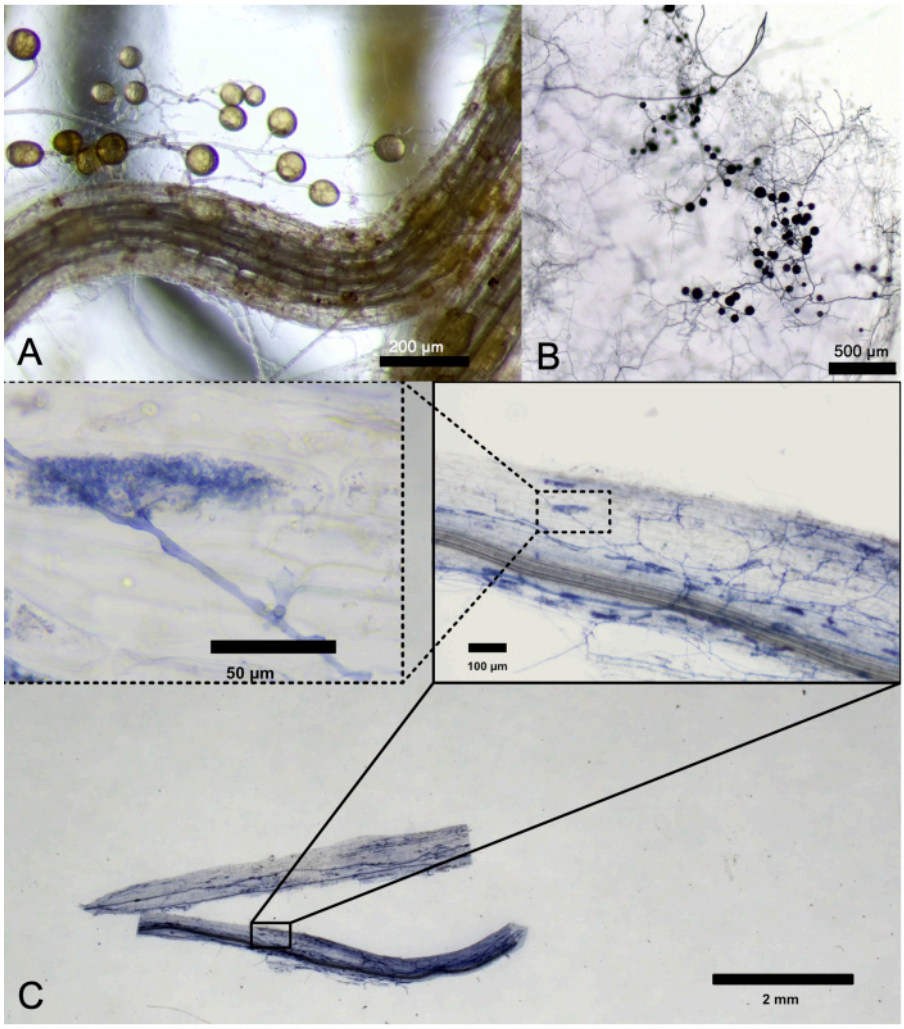

Figure 4: Extraradical and intraradical AM fungal structures extracted from the SAP-AS. (**A**) Free spores of *R. irregularis* observed in the root compartment. Scale bar: 200 µm. (**B**) Spores of *R. irregularis* stained in SAP. Scale bar: 500 µm. (**C**) Stained root fragments showing the intraradical hyphae and arbuscules. Scale bars: 50 µm, 100 µm. Please click here to view a larger version of this figure.

Supplementary Figure 1: Comparison of the thickness of the various tools available to manipulate AM fungal spores. Please click here to download this File.

Supplementary Figure 2: Stacked SAP-AS. Please click here to download this File.

Discussion

Inoculation is the most critical step in the protocol, and the extruded glass Pasteur pipettes proved to be an excellent tool for accurately manipulating single spores of AM fungi while preserving their integrity. The extruded glass Pasteur pipettes are easily shaped using the flame of a candle or Bunsen burner, and the opening can be adjusted under

the stereomicroscope to match the size of the spore being pipetted. It is important to manipulate the spores with tools adapted to the size of AM fungal spore (**Supplementary Figure 1**) and to inoculate the SAP-AS when the host plant roots are long enough to reach the Nitex membrane where the spore is deposited.

The SAP-AS are easy to adapt to the experiment requirements. Larger Petri dishes can be used, with multi-compartments, to monitor, for example, the interaction between closely relative strains or between different species of AMF or to modify the chemical (pH) or biological environment (introduction of nematodes, bacteria, fungi). Various mycotrophic host plants can also be used to provide the AM fungi with photosynthates. The nutrient solution mMS-1 was derived from the minimal (M) medium recipe described by Bécard and Fortin (1988)[15] minus the sucrose, vitamins, and bacto agar to limit carbon sources. However, the SAP-AS can be supplemented with various nutrient solutions, depending on the experiment objectives.

The propagation of AM fungi in SAP-AS requires regular watering. The limited volume of vermiculite and SAP exposes the roots and the AM fungus to fluctuations in humidity, especially in standard two-compartment Petri dishes (10 cm diameter). The ability to expand and, therefore, the transparency of the SAP grains decreases over time. In fact, the presence of cations from the nutrient solution progressively limits the expansion of the acrylate network and requires the replacement of the SAP after months. In addition, green algae and mold may grow over time if Petri dishes are not properly protected from light or overwatered.

To date, seven AM fungal species have been successfully cultivated in SAP-AS, which is far below the number of AM fungal species that can be propagated in pot cultures. However, both the biotic and abiotic conditions in SAP-AS are very similar to those in pot cultures, and it is likely that other AM fungal species should be able to propagate in SAP-AS. Direct inoculation of spores in SAP-AS probably provides environmental conditions closer to those of the rhizosphere in a natural soil due to the presence of root exudates and/or bacteria, if non-sterile spores/seeds are used for inoculation. This should be preferred to inoculation with germinated spores. In addition, the conditions that trigger spore germination within AMF are still poorly understood, therefore SAP hydrated with mMS-1 nutrient solution may not be adapted to germinate spores of other AM fungal species. Spore germination on SAP hydrated with mMS-1 nutrient solution was tested to specifically select viable spores for the inoculation step using only *R. irregularis* inoculum.

Inoculation and monitoring of the development of the AM symbiosis are easily performed in SAP-AS. The modified two-compartment Petri dishes allow the cultivation of different AM fungal species. Paré et al.[12] have propagated seven different AMF species from six genera and three families. Modification of two-compartment Petri dishes is easily done with inexpensive tools. The cost and quantity of materials (vermiculite, SAP, Pasteur pipette, etc.) and reagents (mMS-1) required to prepare and maintain the SAP-AS are limited, allowing a large number of SAP-AS to be managed at minimal cost. The ability to stack the SAP-AS also significantly reduces the footprint of AM fungal cultures compared to pot cultures. For example, 50 SAP-AS (5 stacks of ten) can fit on a 1 m long shelf (**Supplementary Figure 2**). These features make the SAP-AS a simple and inexpensive technique that is compatible with teaching AM symbiosis in high school lab courses or at the undergraduate level in universities.

Colonized grains of SAP can be used to inoculate new SAP-AS or pot cultures.

The presence of a coverslip at the back of the bottom of the Petri dish allows high-resolution photography and video of the extraradical fungal structures. The cytoplasmic flow can be easily studied under living conditions that are very close to natural conditions. This is of great importance for studies of AM fungi functions related to their mycelia (nutrients, water transport, soil structure, etc.) and for studies of hyphal morphogenesis.

AMF complete their biological cycle in plant roots and within the rhizosphere. The study of soil microorganisms is complex due to the inherent difficulty of observing the soil environment. The main objective of the SAP-AS is to recreate an environment as similar as possible to the rhizosphere for the propagation of AM fungi while maintaining the ability to observe the development of AM fungi in great detail. Because this implies non-sterile conditions, the amount of available carbon should be limited to avoid the proliferation of saprotrophic microorganisms. The knowledge of the behavior of AM fungi in the rhizosphere is still extremely limited, and the SAP-AS offers the possibility to make detailed comparisons between species regarding their ability to forage in the extraradical environment, their spore production, and their root colonization. This can be further complicated by adding interactions with other soil species such as bacteria, nematodes, protists, and root fungal pathogens, and the knowledge of soil microbiota interactions can be improved thanks to the SAP-AS.

Disclosures

The authors have nothing to disclose.

Acknowledgments

We would like to thank the two anonymous reviewers for their suggestions. Funding for this research was provided by Agriculture and Agri-Food Canada (AAFC) under project J-002295 (Management and enhancement of AAFC's biological collections).

References

1. Dotzler, N., Krings, M., Taylor, T. N., Agerer, R. Germination shields in *Scutellospora* (Glomeromycota: Diversisporales, Gigasporaceae) from the 400 million-year-old Rhynie chert. *Mycol Prog.* **5** (3), 178-184 (2006).
2. Redecker, D. Glomalean fungi from the ordovician. *Science.* **289** (5486), 1920-1921 (2000).
3. Olsson, P. A., Jakobsen, I., Wallander, H. *Foraging and Resource Allocation Strategies of Mycorrhizal Fungi in a Patchy Environment. Mycorrhizal Ecology. Ecological Studies.* Springer, Berlin, Heidelberg (2003).
4. Zheng, C., Chai, M., Jiang, S., Zhang, S., Christie, P., Zhang, J. Foraging capability of extraradical mycelium of arbuscular mycorrhizal fungi to soil phosphorus patches and evidence of carry-over effect on new host plant. *Plant Soil.* **387** (1-2), 201-217 (2015).
5. Singh, A.K. et al. The role of glomalin in mitigation of multiple soil degradation problems. *Crit Rev Environ Sci Technol.* **52** (9), 1604-1638 (2022).
6. Wilson, G. W. T., Rice, C. W., Rillig, M. C., Springer, A., Hartnett, D. C. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. *Ecol Lett.* **12** (5), 452-461 (2009).

7. Gianinazzi, S., Gollotte, A., Binet, M. -N., Tuinen, D. van, Redecker, D., Wipf, D. Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza.* **20** (8), 519-530 (2010).
8. Stürmer, S. L. A history of the taxonomy and systematics of arbuscular mycorrhizal fungi belonging to the phylum Glomeromycota. *Mycorrhiza.* **22** (4), 247-258 (2012).
9. Gerdemann, J. W., Nicolson, T. H. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans Br Mycol Soc.* **46** (2), 235-244 (1963).
10. Walker, C., Vestberg, M. A simple and inexpensive method for producing and maintaining closed pot cultures of arbuscular mycorrhizal fungi. *Agr Sci Finland.* **3**, 233-240 (1994).
11. Fortin, J. A. et al. Arbuscular mycorrhiza on root-organ cultures. *Can J Bot.* **80** (1), 1-20 (2002).
12. Paré, L., Banchini, C., Hamel, C., Bernier, L., Stefani, F. A simple and low-cost technique to initiate single-spore cultures of arbuscular mycorrhizal fungi using a superabsorbent polymer. *Symbiosis.* **88**, 61-73 (2022).
13. Saha, A., Sekharan, S., Manna, U. Superabsorbent hydrogel (SAH) as a soil amendment for drought management: A review. *Soil Tillage Res.* **204**, 104736 (2020).
14. Bécard, G., Fortin, J. A. Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. *New Phytol.* **108** (2), 211-218 (1988).
15. Vierheilig, H., Coughlan, A. P., Wyss, U., Piché, Y. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Appl Environ Microbiol.* **64** (12), 5004-5007 (1998).