FROM SPORE TO SYMBIOSIS: HOW MYCORRHIZAL FUNGI FIND THEIR HOSTS

Britt A. Bunyard

In considering arbuscular mycorrhizal fungi, we often begin with the question of dispersal. Epigeous (above-ground) mushrooms release spores to the wind, while hypogeous forms (under-ground) rely on animals in a process known as zoochory. Yet dispersal is only the beginning of the story. What happens after a spore arrives in the soil? How does it germinate, locate its host plant, and establish the symbiosis that underpins so much of terrestrial ecology? These questions have driven decades of research, and the answers reveal a finely tuned dialogue between fungus and plant.

In the previous edition of FUNGI (vol. 18, no. 5), a review titled “How do mycorrhizal fungi get around?” discussed the spread of spores of these fungi, noting that: “Animals play a crucial role in the dispersal of fungal spores, a process that ensures reproduction, genetic diversity, and the colonization of new habitats. While wind and water are often recognized as primary agents of dispersal, the involvement of animals adds a layer of complexity and resilience to fungal life cycles. Much like plants that rely on animals for seed dispersal, fungi benefit from similar interactions, and these relationships are central to forest ecology and evolution.” In this review, I address the question of what comes next.

AFTER SPORE DISPERSAL WHAT COMES NEXT?

The life of a mycorrhizal spore after dispersal is a remarkable journey that has been illuminated by decades of research. The earliest systematic studies began in the 1980s, when Giovannetti and colleagues devised methods to observe spore germination in the presence of host plants. They showed that spores could germinate independently, producing germ tubes that explored the soil, but that the presence of roots dramatically influenced the process.

SPORES ON A MISSION

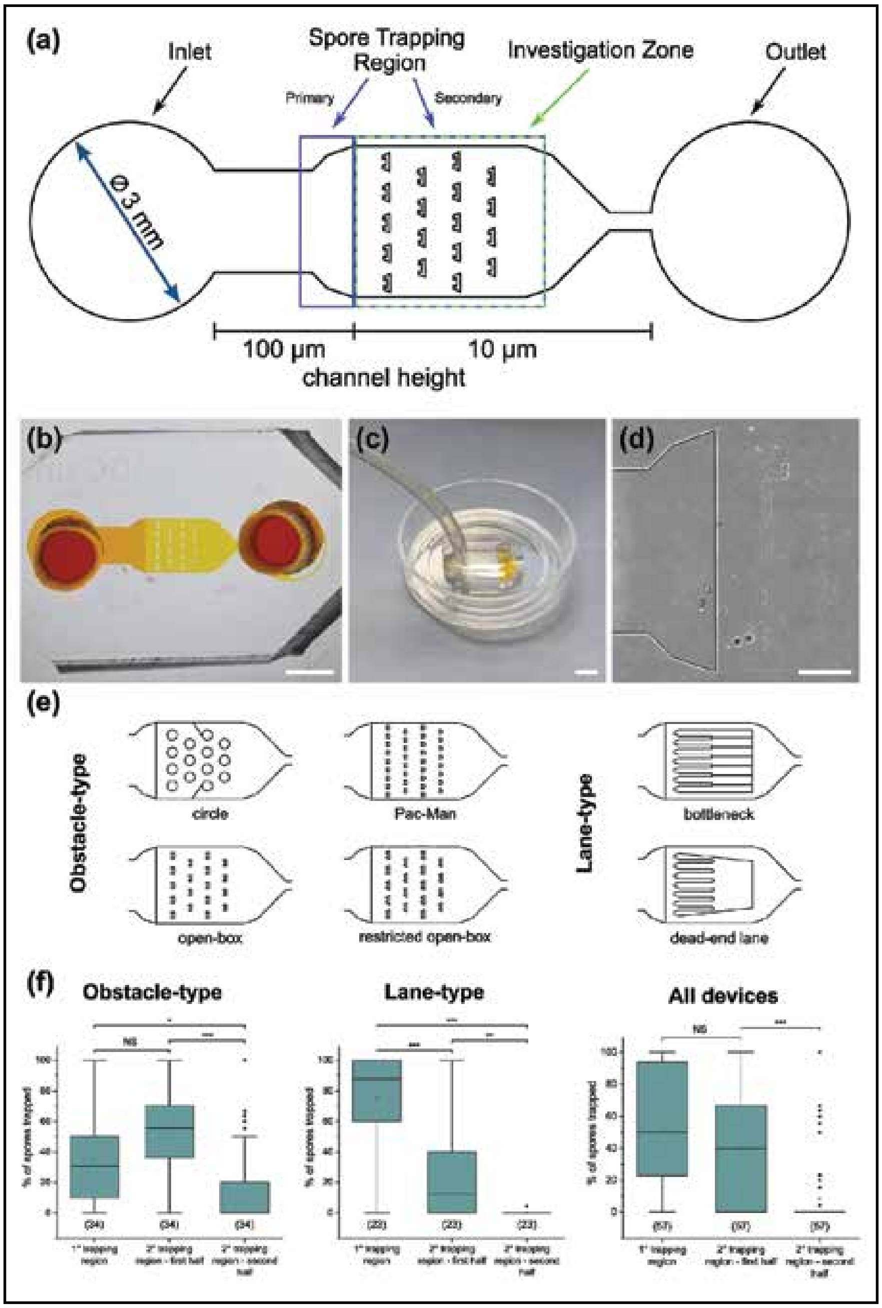
Mycorrhizal fungi don’t just scatter spores and hope for the best. Once a spore germinates, it sends out tiny hyphae that actively seek plant roots, guided by chemical signals released into the soil. Within days to weeks, the fungus makes contact and begins to colonize. Arbuscular mycorrhizal fungi form arbuscules inside root cells, while ectomycorrhizal fungi envelop roots with a sheath and extend a Hartig net between cells. In both cases, these specialized structures create the interface where nutrients are exchanged. Far from being random, this is a finely tuned partnership, a hidden dialogue between plants and fungi that sustains forests, grasslands, and farms across the globe.

Soon after, Bécard and Piché (1989) demonstrated that root exudates stimulated fungal growth, providing the first clear evidence that plants actively signal to fungi. They showed this using a clever dual culture system in which fungal spores and plant roots were physically separated but shared the same growth medium. Root-released exudates could diffuse across the barrier, allowing the researchers to observe the fungus’s response without any direct contact. Spores exposed to these exudates germinated faster, produced longer and more highly branched hyphae, and even grew directionally toward the source of the chemical cues. It was the first demonstration that plants initiate the conversation—chemically recruiting their fungal partners long before they ever touch. This discovery shifted the paradigm: the relationship was not passive, but a dialogue.

Another breakthrough came in 2005, when Akiyama et al. identified strigolactones as key signaling molecules. These sesquiterpenes, released by plant roots, induce hyphal branching in arbuscular mycorrhizal fungi (AMF), effectively guiding them toward the host. They showed that the fungus, once germinated, does not wander blindly, but actually responds to chemical cues that direct its growth. Within days to weeks, hyphae form appressoria on the root surface, penetrate the epidermis, and establish arbuscules inside cortical cells. These arbuscules are the structures through which nutrients are exchanged, establishing the functional symbiosis.

Biotrophic fungi (including AMF), by necessity, must find a living host to partner with. Thus, as soon as the spore germinates, the race is on! The timeline of this process is relatively short. Spores can germinate within a few days under favorable conditions, and hyphae can survive for a limited period without a host. Once they detect root signals, however, colonization accelerates. Within one to two weeks, the fungus has typically established itself inside the root, and the partnership is fully functional.

Modern research has added layers of detail to this picture. Lanfranco et al. (2018) synthesized the molecular and ecological dimensions of the



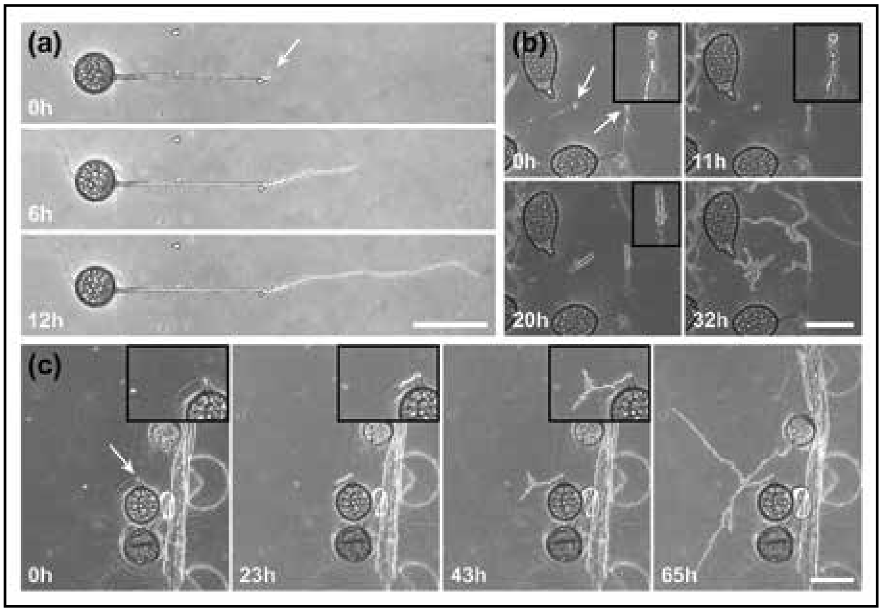


FIGURE 1 *(top left)*. **Design of the AMF-SporeChip. (a) Two-dimensional schematic of the microfluidic device showing the dimensions and structure of the spore trapping region and investigation zone containing micron-sized obstacles. (b) Real life image of the device filled with fluorescein dye solution to highlight the channels and the change in channel height. (c) The AMF-SporeChip fitted with tubing at the device inlet for the introduction of spores. The outer diameter of the Petri dish is 35 mm. (d) Brightfield image of the transition zone (microchannel height step-change) with trapped spores of *Rhizophagus irregularis*, strain MUCL41833. (e) Different geometries of the investigation zone of the AMF-SporeChip, including obstacle-type and lane-type structures.**

FIGURE 2 *(bottom left)*. **AMF germination on chip. Time-series illustrating the germination process of (a) *Rhizophagus irregularis* MUCL 41833, (b) *Rhizophagus irregularis* MUCL 43194 and (c) *Rhizophagus intraradices* MUCL 49410. In (c), debris from the root-organ culture can be observed. White arrows indicate the germination site, with inset boxes in (b) and (c) illustrating an enlarged region of the germination event. Scale bars: 100 µm.**

interaction, emphasizing the role of nutrient signaling alongside chemical cues. More recently, Claire E. Stanley and her colleagues at Imperial College, London, in 2024 introduced microfluidic technology with their “SporeChip” (see Richter et al., 2024). The SporeChip is a microfluidic “lab-on-a-chip” platform that allows researchers to watch AMF (*Rhizophagus* species, in this case) spores germinate, extend hyphae, and even fuse (anastomose) in real time (Figs. 1 and 2; used here courtesy of the authors). It was developed specifically to overcome the challenge of studying arbuscular mycorrhizal fungi, which normally grow hidden belowground in dark, densely packed soil where their early developmental stages are nearly impossible to observe. These advances have confirmed and refined the earlier observations, showing that the process is highly orchestrated and responsive to environmental conditions. Resources such as the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM) continue to provide practical methods and long-term data, ensuring that the study of spore germination and colonization remains grounded in both laboratory and field contexts.

What emerges from these studies is a portrait of a finely tuned ecological relationship. Spores germinate into exploratory hyphae, plants release chemical signals that direct fungal growth, and within a matter of days to weeks, a functional symbiosis is established. Far from being random, the process is a dialogue between partners, each actively engaged in recognition and adaptation. From a microscopic spore in the soil arises one of the most important ecological relationships on Earth, a partnership that underpins the health of forests, grasslands, and agricultural systems. The journey from spore to symbiosis is thus not only a biological process but a cornerstone of terrestrial life, sustaining ecosystems and shaping global nutrient cycles.

> **“FROM A MICROSCOPIC SPORE IN THE SOIL ARISES ONE OF THE MOST IMPORTANT ECOLOGICAL PARTNERSHIPS ON EARTH.”**

REFERENCES CITED

Akiyama, K., K. Matsuzaki, and H. Hayashi. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435(7043): 824–827.

Bécard, G., and Y. Piché. 1989. Funga growth stimulation by exudates of host roots in vesicular–arbuscular mycorrhizal symbiosis. *Applied and Environmental Microbiology* 55(6): 1480–1483.

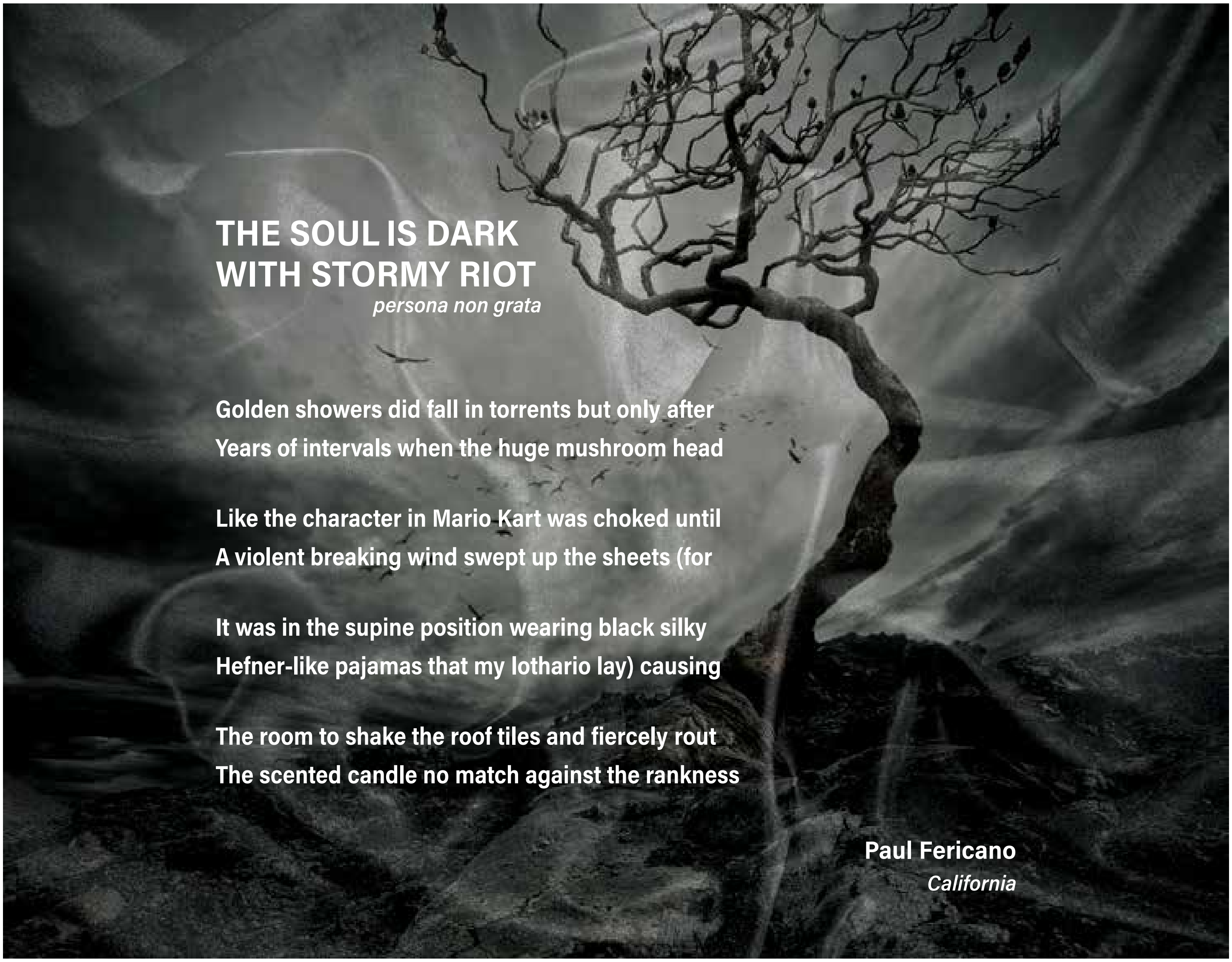
Giovannetti, M., D. Azzolini, and A.S. Citernesi. 1983. Methods for studying spore germination of vesicular–arbuscular mycorrhizal fungi in the presence of host plants. *Transactions of the British Mycological Society* 80(2): 419–423.

Giovannetti, M., and L. Avio. 2002. Biology of arbuscular mycorrhizal fungi. In: M.G.A. van der Heijden and I.R. Sanders (Eds.), *Mycorrhizal Ecology* (pp. 47–69). Springer, Berlin.

INVAM (International Culture Collection of Arbuscular Mycorrhizal Fungi). Online resource: https://invam.wvu.edu.

Lanfranco, L., V. Fiorilli, and C. Gutjahr. 2018. Partner communication and role of nutrients in the arbuscular mycorrhizal symbiosis. *New Phytologist* 220(4): 1031–1046.

Richter, F., M. Calonne-Salmon, M.G.A. van der Heijden, S. Declerck, and C.E. Stanley. 2024. AMF-SporeChip provides new insights into arbuscular mycorrhizal fungal asymbiotic hyphal growth dynamics at the cellular level. *Lab Chip* 24: 1930-1946; DOI: 10.1039/D3LC00859B.



THE SOUL IS DARK WITH STORMY RIOT

persona non grata

Golden showers did fall in torrents but only after
Years of intervals when the huge mushroom head

Like the character in Mario Kart was choked until
A violent breaking wind swept up the sheets (for

It was in the supine position wearing black silky
Hefner-like pajamas that my lothario lay) causing

The room to shake the roof tiles and fiercely rout
The scented candle no match against the rankness

Paul Fericano
California