Myco-Cycling of Coffee Grounds Using *Hericium erinaceus*

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Terminology:

-SCG= spent coffee grounds
-HE =Hericium erinaceus (Lion's Mane)
-GL= Ganoderma lucidum (Reishi)
-PO= Pleurotus ostreatus (Oyster)

Grain Spawn Jar naming convention:
U/T (untrained vs. trained) GL/HE/PO (species abbreviation) # +/- (with or without coffee)

Overview of Activities:

Over the course of this research we have successfully collected and processed 50+ pounds of coffee grounds into substrate for mycelium. These grounds were used to train mycelium using a regimented procedure of subculturing from media with increasing amounts of SCG added (see Research Methods).

The experience of collecting SCG from local cafés has yielded the beginnings of a case study to outline effective tactics so this practice may be replicated elsewhere using the information gathered. To understand these experiences- we have broken café interactions down into 5 categories:

Café Case Study:

1) Large Chain Cafe (eg: Starbucks):

-Daunting, intimidating due to scale
-Corporate structure makes it unlikely for individuals to act without approval from higher-ups, making it very unlikely for employees to willingly give over coffee grounds without trepidation
-unlikely to participate in any capacity beyond reluctant container filling
-larger volume of coffee ground production

+ Starbucks locations are supposed to be involved with a program to give used coffee grounds to people for use in gardens etc

(Panera Bread)

-further from campus center, therefore more difficult to pick up from

-Panera apparently is very willing to donate food waste/excess, making them a possible candidate for partnership

2) Medium Chain Cafe: (eg: Espresso Royale):

-2 state reach

- Unable to find contact info for managers etc through official means; loose corporate structure?

-Unable to partner officially due to apparent poorly connected business structure

3) Single Locations (eg: BrewLab):

-difficult to contact higher-ups, may need more establishment/visual accreditation in order to gain trust/access

-depends on culture within the cafe

-some cafes may be more welcoming to participation simply due to the work environment fostering a culture of trust and community

-LOOK FOR PLACES THAT VALUE COMMUNITY

-May need to be contacted in a very official capacity

4) Community coffee place (ETC coffeehouse):

-Welcoming, excited, very smooth implementation

-Also connected to a food bank to aid in distribution of food produced

-THIS IS AN IDEAL STARTING POINT

-be friendly, be curious, be present, people appreciate that.

5) At-home grounds collection:

-not recommended at scale, good for hobbyists

-volume produced even for 4 person household cannot compare to that of cafes

-SCG produced at home also has a greater likelihood of being composted or used as garden amendment than those produced from cafes

Research Methods:

To evaluate the efficacy/necessity of mycelial training methods on individual species' abilities to consume and produce mushrooms on mixtures of coffee grounds, we started by identifying one axenic agar plate per species tested to become a "mother plate" from which further generations of plates were all cultured to ensure initial genetic homogeneity. Following this, the mother plates were subcultured onto 2 sets of 3 agar plates, 3 regularly formulated agar plates and 3 with SCG added (include exact ratio of SCG/agar). This constituted agar phase 1. These plates were allowed to incubate for ~ 2 weeks, until fully covered with mycelium. Contaminated plates were isolated and disposed of. Of the plates remaining, 1 from each

set (2 per species) that exhibited the fastest or most vigorous growth were selected to be subcultured for the Agar 2 phase of training to maintain genetic homogeneity going forward. Speed and vigor was determined via bi-weekly check-ins in which growth was monitored and recorded from week to week by qualitatively measuring the area of growth between check-ins. The 2 selected plates were then subcultured onto normal agar and agar + (ratio SCG to agar) in triplicate. After 2 weeks, the fastest/most vigorous of these were selected from each set via the methods outlined above.

The 2 selected plates from each species (1 plate/set) were used to inoculate grain jars with a standard amount of mycelium by cutting measured 2cm x 2cm squares from each agar plate. Each plate was used to inoculate 4 half-gallon jars of grain spawn; 2 of rye grain spawn and 2 jars of rye grain spawn with added SCG. All spawn jars also included (amount) of gypsum (calcium sulfate dihydrate) as is standard in spawn preparation procedures.

The result of this phase was 2 sets of 4 jars per species, 24 in total (See fig 1.). These Jars were monitored and photographed on a weekly (sometimes bi-weekly) basis to observe and record differences in growth rates across treatments (see attached documents). Most of the jars colonized fully over the course of 1 month, with the exception of nearly all of the PO spawn jars, which seemed to stall at nearly ¾ colonization and showed some signs of bacterial infection (see Considerations section). This was also observed in a number of GL jars, however GL is sometimes known to produce yellow or red pigmentation in vitro, so some amount of these metabolites were chalked up to normal growth processes. Jars that did not reach full colonization after a month were left out of the rest of the study and were autoclaved and disposed of.

From there, each colonized jar was used to inoculate 3 treatments of 3lb spawn bags filled with substrate composed of 75%SCG/25% Sawdust, 50%SCG/50% Sawdust, and 100 Sawdust (as a control). This resulted in a batch of 24 3lbs spawn bags total per species. After removing We had initially planned to include a treatment of 90%SCG/10% sawdust, but due to the scope of production necessary for this test we are leaving that for future exploration.

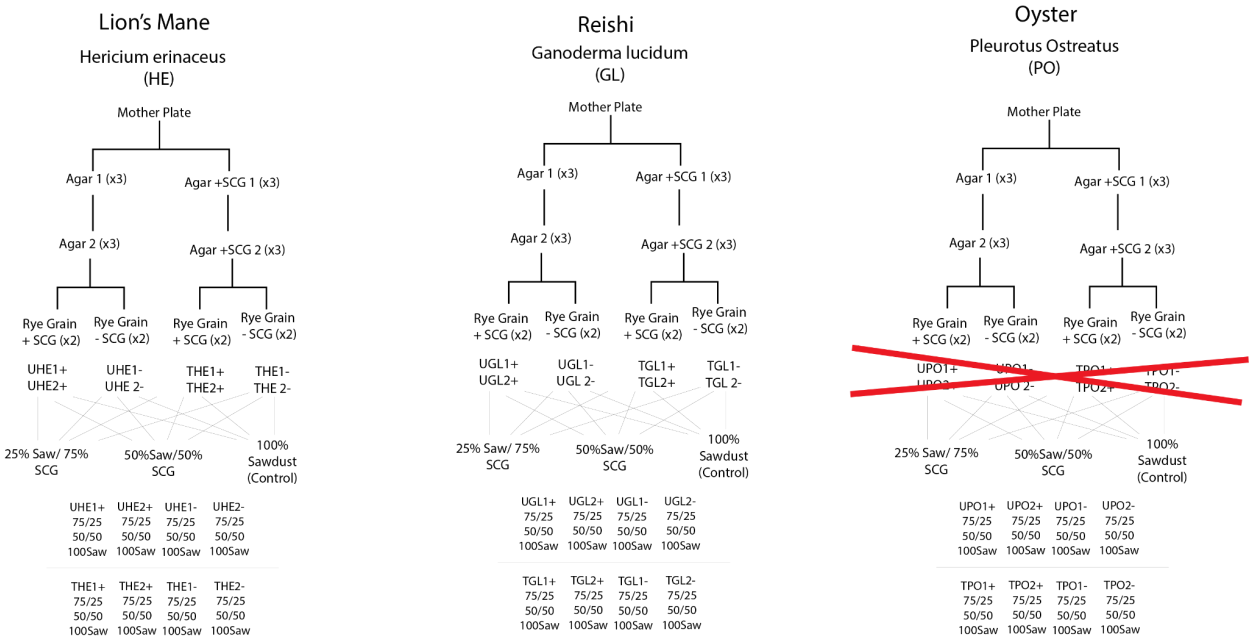

Fig 1: Diagram of Cultures and Subcultures, Illustrating Training Regiment

The failure of the PO jars poses a very confusing question as Pleurotus spp. are the only species tested in this experiment that have been tried and proven to take very well to coffee grounds as substrate across numerous research studies (cite). Therefore, the failure of these jars is most likely due to issues with culturing technique, or the specific genetics used. There is also a possibility that the mycelium began to senesce, losing some of its enzymatic activity.

Considerations:

It's possible the failure of the Oyster mushroom grain spawn was due to excessive subculturing leading to early senescence. To avoid this in the future, we recommend limiting the agar training step to 1 combined step.

The presence of bacterial contamination may indicate an overabundance of water in the spawn stage, which will be tweaked in future research.

New, vigorous cultures will be obtained in future research to ensure that culture's are viable and fresh for training.

Ideally, these cultures would be obtained via spore germination from fruitbodies arising from SCG substrate.

The end result of this project will be one of two final products:

1. A zine laying out best practices for implementing a myco-cycling initiative in one's own community
2. A research paper outlining the efficacy/ necessity of mycelium training methods
3. Partnerships with local cafes to make visible the myco-cycling initiative and to raise public awareness of sustainable coffee ground waste practices.

While the research is running, we will be running an informational Instagram account (@Sporecycle) to raise awareness and generate interest in myco-cycling initiatives. Occasionally, we will produce and disseminate stickers to participating coffee shops.

Results from First Growing Cycle:

100% Sawdust	Yield		75% SCG/ 25% Sawdust	Yield		50%SCG/50 %Sawdust	Yield
THE 1-	64		THE 1-	289		THE 2+	83
THE 2-	54		THE 2-	195		THE 1+	318
THE 1+	44		THE 1+	135		THE 1-	279
THE 2+	72		THE 2+	162		THE 2-	231
UHE 2-	75		UHE 1-	187		UHE 1+	163
UHE 1+	35		UHE 2-	240		UHE 2+	26
UHE 1-	130		UHE 2+	97		UHE 1-	219
UHE 2+						UHE 2-	292
Total Yields:	474		1305			1611	
Trained Yield:	234		781			911	
Untrained Yield:	240		524			700	

Discussion:

While these results did not derive from a large enough sample size to conduct effective statistical analysis, they do illustrate the viability of growing HE fruitbodies from majority SCG based substrates. This is demonstrated very well by the volume of fruitbody material arising from 75% SCG based substrate, which was found to be comparable to the yields from primarily sawdust based substrates. Further testing will need to be done to compare SCG enriched substrate with sawdust substrate amended with common commercial enrichments such as soy hull pellets or wheat bran.

Next Steps/ Final Culmination:

Greenhouse automation processes were developed and tested in the initial trials. Every bag inoculated (24 HE, 8 GL) fully colonized. Further testing will involve scaling up the number of HE substrate bags, as those were found to be the most successful. This will then result in a more in depth result collection and analysis stage building off of this preliminary aptitude study.

Food and nutrition tests down the line will aim to identify whether or not mushrooms grown on substrates predominantly composed of SCG differ from those grown under standard conditions.

Possible future PCR testing to determine if any genetic modifications were produced through the training process.

Comparison testing between SCG enriched substrate and pure sawdust substrate amended with soy hull or wheat bran material.