Lab #5. Nematophagous Fungi

Ecology is the study of the interactions of organisms with their environment and with other organisms in it. Since there are perhaps 1.5 million (or more) species of fungi and they exploit almost every imaginable carbon-based resource as food (including organisms from every Kingdom), fungal ecology is an almost limitless research field. It is also an area in which many good and important questions have not been answered, so there are many opportunities for fungal ecologists: in plant pathology, medical mycology, terrestrial and aquatic ecology, biodegradation and bioremediation, and brewing!

In today's lab, you will study one very small (and yes, possibly obscure) area of fungal ecology: a guild of fungi that subsists by attacking and consuming nematodes. These fungi provide an excellent example of some fundamental principles in fungal ecology:

a. Fungi have been very successful at exploiting 'obscure' sources of food,
b. Unrelated groups of fungi have often evolved independently, using different mechanisms, to take advantage of this same 'obscure' food source,
c. A great diversity of fungi are present in many natural substrates, including soil,
d. In such habitats, the filamentous growth form of fungi allows them to connect across time and space, while their nutritional capabilities allow them to connect across many trophic levels of their ecosystem, and
e. Selected fungi can be discovered and isolated from complex habitats by taking advantage of their nutritional specialization.

Here, in a nutshell, is what you will do:

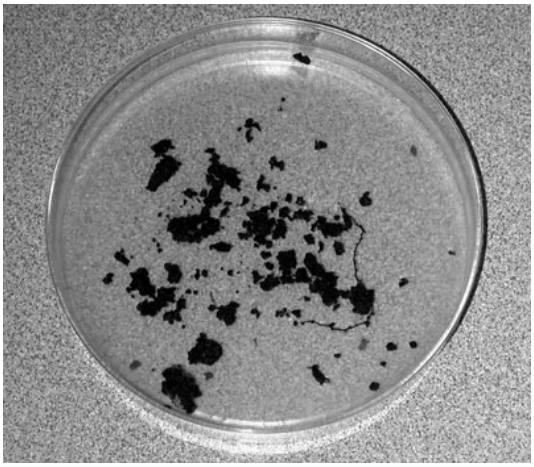
1. Observe, isolate and identify nematode-destroying fungi in soil sprinkle plates
2. Observe the attack of nematodes and bacteria by *Pleurotus ostreatus* on water agar.

Here is an outline of what needs to be done by the instructor [you need to know this in order to understand what you're doing and how it worked]:

1. 25 days before lab: Pour 20 plates PDA or MEA for #3
2. 3 weeks before lab: Instructor will pour 5 plates per student of 1.5% (w/v) distilled water agar (and 40 for the instructor). Allow the plates to dry 2 days before bagging.
3. 3 weeks before lab: Instructor will locate and transfer out cultures of a variety of nematode-destroying fungi onto PDA/MEA plates.
4. 3 weeks before lab: Instructor will transfer out cultures of nematodes (*Caenorhabditis elegans* or *Panagrellus redivivus*) onto ~5 PDA/MEA plates to obtain large numbers.
5. 15 days before lab: Instructor will add 200-400 nematodes as bait to 3 WA plates per pair of students
6. 2-3 weeks before lab: Instructor will inoculate 20 WA plates with *Pleurotus ostreatus* and several plates each of other nematode-destroying fungi (longer for slower-growing strains).
7. 8 days before lab: Instructor will add 200-400 nematodes as bait to 2 WA plates per student
8. One week and again 2 days before lab: Instructor will add a few nematodes to 10 plates of *Pleurotus ostreatus* on WA and to plates of other nematode-destroying fungi.
9. 2 weeks plus 2 days before lab: Instructor will pour 2 plates per pair of students of PDA with 50 mg/L chloramphenicol (as an antibacterial antibiotic). Two days before lab: pour two more plates per student pair.

Here is an outline of what needs to be done by the students:

1. 2 weeks before lab: Each pair of students should collect ± 15 mL (~1 tablespoon) of soil into a small ziploc plastic bag (or used coffee cup). Think about where nematodes might be - most feed on bacteria and all of them require a film of soil water to swim through, so they need a fairly moist (but not waterlogged), rich and undisturbed habitat. For example, don't pick soil from a gravelly parking lot, a construction site, or a sandy beach. Perfectly good soil samples could be obtained behind the Staging Building (with greater difficulty if the ground is covered in deep snow), but it might be more interesting to obtain at least one sample closer to your home - perhaps from beneath or around your compost bin (not the slimy stuff in it), a hollow in a big rotten log, or from an organic greenhouse (remember that both fungi and nematodes may be reduced by pesticide use).



2. 2 weeks before lab: Students will sprinkle a pinch of soil onto 3 WA + nematode plates for each sample (see the illustration at left - be sure to leave lots of open space). This will take about 5-10 minutes. Save the remainder of your soil in a fridge for next week.
3. 1 week before lab: Students will sprinkle a pinch of soil onto 2 WA + nematode plates and 1 PDA + chloramphenicol plate. This will take about 5-10 minutes.

On Lab Day [the instructor and TA will assist you with these steps]

1. [Consider doing step #5 first, so you know what you are looking for in step #1.] Using the 4x or 10x objective of your compound scope (and being careful not to run into clumps of soil), scan the soil sprinkle plates for dead nematodes trapped in nets, loops or rings, or growing 'fur coats' of fungal hyphae (see illustrations in Chapter 15 of The Fifth Kingdom). Look for conidiophores and conidia of the *Arthrobotrys* group (anamorphic *Orbiliaceae*; Figure 15.5). Don't rush this step - expect to spend 10-20 minutes per plate in order to really find the nematode-destroying fungi that are there. For each soil sample, record in your lab notebook the identities of any nematode-destroying fungi that you encounter, as well as any other interesting fungi, microbes, and micro- and mesofauna.
2. Try to identify your *Orbiliaceae* to genus and species using the drawings provided in lab. You will need to make microscope slides and use a compound microscope at 40-200x and look carefully at the trapping devices and conidia; you may also need to measure the conidia and possibly other structures to reach an identification. **DRAW** this fungus, highlighting its identification features. If possible, isolate your nematode trapper into pure culture by aseptically transferring one or two conidia from a conidiophore onto a plate of PDA + chloramphenicol. [Instructions provided in the lab. A Pasteur pipette drawn out in a flame to a fine tip makes a great transfer tool for this, but a dissecting needle, sterilized in ethanol then wiped through sterile agar, works too. This needs to be done under a dissecting microscope, with the soil plate placed on a black background and with low-angle epi-illumination to be able to see the *Arthrobotrys* conidiophores.]
3. What other fungi, and other organisms, do you see on your WA + nematode plates? How do they look? Record these observations in your lab book.

4. Compare the fungi growing from soil sprinkles on your WA + nematode plates with those growing from soil dilutions on your PDA + chloramphenicol plates. Do you see any *Orbiliaceae* on your PDA plates?
5. Using your compound microscope at 40-400x, observe the demonstration plates of other genera of nematode-destroying fungi provided by the instructor, and **DRAW** what you see in your lab notebook. (You might just want to do this step before #1) Be sure to observe and draw at least one predator and one parasitoid. [These demo plates are a back-up for those students whose soil plates yield no n-d fungi. We provide *Harposporium, Arthrobotrys, Hohenbuehelia* (anamorphic), and *Pleurotus*.]
6. Using your compound microscope at 40-400x, observe the demonstration plates provided of *Pleurotus ostreatus* consuming nematodes and bacteria, and answer question #6 (below) in your lab notebook. Find and **DRAW** a nematode that has been attacked, and any other pertinent details you see. You'll have to look carefully (and ask your instructor the right questions) to determine what is going on.

Questions to answer in your lab notes:

1. What is the fundamental difference between the parasitoid ('endoparasitic') and predatory nematode-destroying fungi?
2. What are some genera of parasitoid nematode-destroying fungi? What is their classification (phylum, class, order, family)? What mechanism(s) do they use to capture nematodes?
3. What are some genera of predatory nematode-destroying fungi? What is their classification (phylum, class, order, family)? What mechanism(s) do they use to capture nematodes?
4. What genera and species of nematode-destroying fungi did you find in your plates? Draw what you saw that enabled you to identify them. Are they reproducing sexually or asexually?
5. What is a conidiophore? What is a conidium (plural conidia)?
6. What is the classification (phylum, class, order, family) of *Pleurotus ostreatus*? What mechanism does it use to capture nematodes? How does it attack and consume bacteria? What is going on during the attack of bacteria (and nematodes) that is not visible even with the highest magnification of a microscope? How do you think the oyster mushroom finds bacterial micro-colonies and immobilized nematodes?
7. Some nematode-destroying fungi eat only nematodes (which ones that you saw?). What about *Pleurotus*? Why is it advantageous to the oyster mushroom to consume nematodes and bacteria in nature? Think about this question and how it relates to question #1 and the last part of question #4. That's what ecology is like -many things are interrelated.
8. Why did you find different fungi growing from the same soil samples when plated on PDA + chloramphenicol versus WA + nematodes?
9. How else could you detect nematode-destroying fungi in soil?

[We provide illustrations from George Barron's "The nematode-destroying fungi" (1977) and from Charles Drechsler's original papers (1930s to 1975!)]