# Molds As Model Organisms For Undergraduate Structured Inquiry Labs

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## Abstract

Structured inquiry labs allow students who have little prior lab experience to learn the mechanics of experimental design, then immediately apply their new skills to a novel problem. Cultured molds are easily manipulated model organisms that are particularly suited for such investigations. This article outlines a 2–week lab in which students model inter–specific interactions. The first week students grow two species of molds in isolation versus in competition, and use point counting to estimate relative growth rates. Students subsequently design and execute their own experiments. Several commonly encountered student experiments are described, with suggestions for how to accommodate them. Known limitations and concerns about this model system are also addressed.

## Introduction

Biology majors at Wake Forest complete four required "core" courses that include weekly 3–hour laboratories. Students learn more and are better engaged by lab courses when they can conduct their own experiments, but most incoming students have little experience with designing

experiments to test hypotheses, in interpreting complex datasets, or in applying theoretical concepts to novel problems. To address this situation, we have replaced many of our traditional demonstration and confirmation lab exercises with 2– or 3–week long structured inquiries.

In the first week of an inquiry unit students become familiar with a particular model organism or system by completing 1–3 modified confirmation exercises. Students are not told in advance what outcomes to expect, so they must record their observations and use concepts being covered in the lecture portion of the course to explain the observed processes. Before leaving at the end of the first week students design an experiment of their own, and hand in a summary of their experimental design. Students are given a list of reagents and materials that we will have available. They can also request materials, but must provide a reasonable explanation for why they are needed. After their general experimental design is reviewed and approved, the students complete their experiment during the second week. The outcomes of their experiment are summarized in either a formal lab report, an informal directed writing assignment, or an oral presentation to their classmates.

There are several advantages to multi–week structured inquiry labs. They teach students to use higher order critical thinking processes, and provide them with repeated opportunities to test their newly acquired skills. When compared to the traditional mid–term and final lab practicals, we also have many more opportunities to formally and informally assess student comprehension. Our overall experience with structured inquiry has been so positive that we are currently modifying our non-majors lab courses to include more structured inquiry as well.

Our main obstacle to implementing structured inquiry labs has been appropriate model systems. Many of the organisms used routinely for demonstration exercises do not lend themselves to student experiments; the cost or housing requirements are too high, or the number of organisms and ancillary materials needed for experiments require too much preparation time. Fortunately we have found that common molds (the Deuteromycetes, mostly in Phylum Ascomycota) are excellent models for a wide range of structured inquiry labs. Molds are extremely hardy, grow in ambient conditions, and are unlikely to be contaminated in the time frame of a typical student experiment. Students can manipulate molds easily, and since many species have unique morphologies and colors, experiments usually can be scored with the naked eye. Molds are a familiar part of students' life experiences, so are less intimidating. Finally, molds are legitimate long–term experimental models. Basic questions remain unanswered about the natural history, biochemistry, and ecology of many fungi, so students who develop a long–term interest in them can reasonably expect to contribute new knowledge to the field.

Here I describe a structured inquiry lab in which students test interspecies interactions between molds. In the first week, students measure and plot growth rates for two species grown on separate potato dextrose agar (PDA) plates, versus grown together in a single PDA plate. For the second week students can design experiments that test for abiotic and biotic factors that determine which species will dominate and take over the cultures.

## Procedures

### Stock Cultures

Two to four weeks prior to the lab we purchase 1–2 plates of each of the following species (common names, and 2003 catalog numbers from Carolina Biological are in parentheses): *Aspergillus niger* (black bread mold, CE–15–5946), *Aspergillus flavus* (brown bread mold, CE–15–5934), *Penicillium camemberti* (bleu cheese mold, CE–15–6141), and *Rhodotorula rubra* (pink water mold, CE–15–6230). These species were selected because they can be easily distinguished

from one another when grown together. To obtain stocks of "wild" mold species for the second week of the lab, we prepare 100 mm sterile PDA plates using commercial media, and place open plates in various locations around the building. After one hour, plates are covered and incubated in a drawer at room temperature. After 3–7 days, isolated colonies will appear that can be transferred to individual PDA plates and subcultured.

Axenic mold stocks are amplified using the same general sterile techniques as with bacteria. Note however that molds grown on plates require 3–7 days to mature and sporulate, and plates typically are covered with white vegetative mycelium for 1–2 days before the colored fruiting bodies appear.

**Other Materials**

For the first week, a lab section of up to 24 students will need 1–2 stock plates of each of the four named species. Each pair of students will also need four sterile PDA plates, and 5 ml of sterile dissociation medium in a screw–cap tube (2% glucose in distilled water; autoclave to sterilize). Each pair will also need 4–5 sterilized glass Pasteur pipets, 4–5, 1.5–ml microtubes, and 1–2, 1–ml serological pipets. The following items also need to be available: disposable plastic transfer pipets, bent glass or plastic bacterial spreaders, watch glasses with 95% alcohol (for sterilizing the spreaders between plates), three colors of Dry-Erase markers, and clear acetate overhead sheets printed with a 0.5–cm square grid.

**Demonstration Phase of the Lab**

Each pair of students selects two mold species they can distinguish by eye. Using the **larger** end of a sterile Pasteur pipet as a punch, they remove a ~4 mm plug of agar with spore-bearing mold from each of the appropriate stock plates, transfer the plugs to separate microtubes, and add 1 ml of dissociation media to each plug. The plugs and dissociation media are shaken vigorously, then exactly 0.5 ml of slurry from each species are transferred to a third microtube and mixed. Next students inoculate each of their three PDA plates with one drop of mold slurry. Two plates are inoculated with slurry from the individual species, and the third plate with slurry containing a 1:1 mix of spores from both species.

Next students remove a glass or plastic spreader rod from its alcohol bath, allow the excess alcohol to evaporate, then spread the drop of mold slurry evenly across the first PDA plate. The spreader is sterilized in alcohol again for 30 seconds, and the procedure repeated with the other plates. Finally, students use two pieces of clear tape to attach each lid to its corresponding plate, label them, and place the plates face up in a dark storage drawer in the lab.

Each day for the next six days, someone from each pair must return and measure how much of each PDA plate is covered with spore–bearing (*i.e.*, reproductively mature) mycelium. Point counting is used to estimate relative coverage of each plate. With practice, students can estimate coverage on all three plates in as little as 10 minutes.

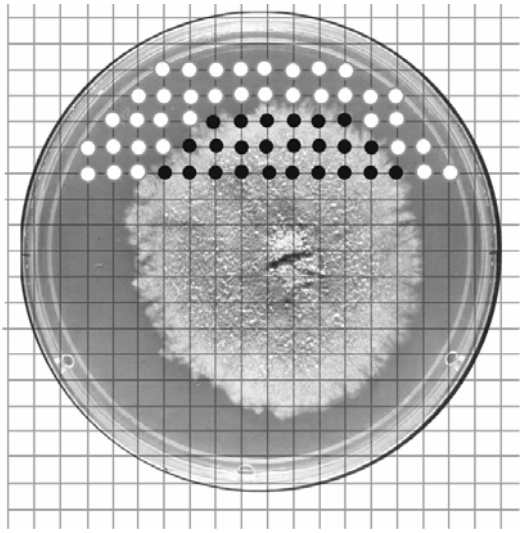
**Point Counting Procedure**

Students should practice this procedure in lab with stock plates before they begin collecting their own data. To estimate relative coverage of mold on a plate, they attach an acetate grid sheet to the lid of the first single–species plate to be counted. Looking down through the clear sheet onto the agar surface, they should be able to see where colored, spore–bearing mold is growing. If there is too much condensation to see clearly, students should open the plate and wipe it away with a Kimwipe.

Starting at the top of the plate, students must score every point where two lines intersect over the surface of the PDA medium in the plate. Using one color of Dry–Erase marker they place a dot at every intersection overlying mature mold. Using a second color, they place dots at the remaining intersection points that lie over bare agar, or over white, immature mycelium. Finally, they count and record the number of dots of each color. The same procedure is used to score the plate containing the second species of mold. To score the plate containing both species, students must use three markers: one to indicate where Species #1 is located, a second color to indicate Species #2, and a third to indicate areas that have no mold or immature mycelium only. Once the numbers of dots are recorded, the acetate grids can be removed, wiped clean, and reused. Plates are returned to their storage drawer until the next day.

To calculate relative coverage of the plate by a species, divide the number of dots for that species by the total number of dots, and multiply by 100%. When the students have an estimate of percent coverage for 6 consecutive days, they summarize their results graphically by plotting the percent coverage versus time. Plots are generated for each mold species alone, and for each species when they are grown together,

**A.**



**B.**

| | |
|---|---|
| # points over mature mold | **24** |
| # points over empty plate | **36** |
| Total # points | 24+36 = **60** |
| % coverage by mold | (24/60)*100% = **40%** |

**C.**

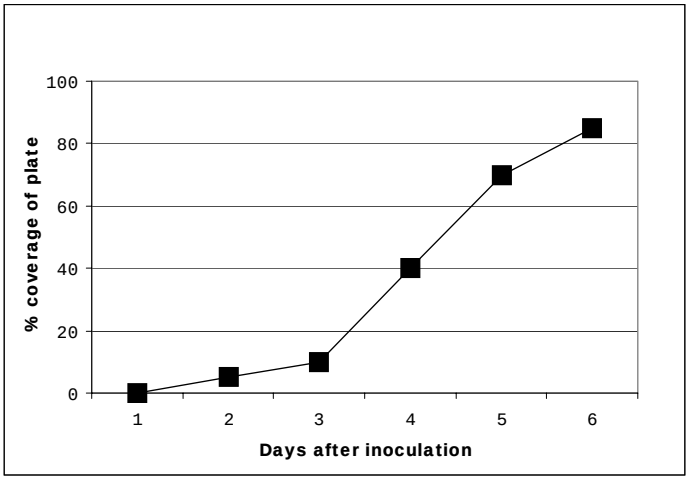



**Figure 1. A.** Estimating the growth rate of a single mold species in culture. In this experiment students only inoculated the center of the plate with spores. Black dots indicate where the grid intersections overlie sporulated mold; white dots indicate intersections where the plate has not been colonized yet. For clarity, only the top third of the plate has been marked; normally students would score the entire plate. **B.** Estimating coverage. The dots shown in Part A were counted and tabulated. Relative coverage was determined by dividing the number of white points by the total number of points (both black and white). Using this method, 40% of the area

in the top third of the plate shown in Part A is covered with mature mold. **C.** Typical growth curve for a single species of mold in culture. A lag phase of 1–3 days before mature spores appear is normal.

**Investigative Phase: Typical Student Experiments**

In our labs students begin their own experiment during the next weekly meeting. Depending on the group though, other instructors may want to give their students an intervening week to do more background research and planning. Regardless of the interval, students always should be required to provide the instructor with a 1–page outline of their rationale and experimental plan, including a list of materials they will need, before beginning their experiment.

It is not possible to provide a comprehensive list of materials needed for students' experiments, but based on our experience, certain materials are used frequently. Our students routinely test the effects of abiotic conditions such as light, substrate pH, micronutrient requirements, or preferred substrates. Fortunately, both the composition of PDA plates and local environmental conditions can be modified easily. Normal pH of solid PDA media is around 5.5, but students can adjust it by overlaying pre–made plates with 10 ml of 100 mM acetate, phosphate, or other physiological buffer of the desired pH. After soaking for 30 minutes, the plates are drained then inoculated as usual. PDA plates can be amended with most other soluble components (additional carbon sources, micronutrients, toxins, etc.) in essentially the same way. Rather than maintain multiple light chambers for each wavelength or light intensity desired, we have students cover plates with colored acetate film purchased from a craft supply store, or with shade cloth obtained from our greenhouse. Relative humidity can be varied by placing plates in a container filled with desiccant for dry conditions, or distilled water for high humidity.

Frequently students compare growth rates between 2 molds that have been inoculated onto one species' preferred substrate. For example, they might compare growth rates between *Aspergillus niger* (a bread mold) and *P. camemberti* (a cheese mold) on stale bread. To make these assay plates, students use an opened, pre–poured PDA plate to punch out circles from thin slices of bread or cheese. The bread or cheese is pushed down onto the PDA media (which provides the moisture needed for spore germination) using a sterile glass spreader, then inoculated as described before. Students have also compared growth rates between wild and laboratory strains of molds. A few more advanced students have even asked whether an established mycelium of a "poor competitor"(which they identified in the initial demonstration exercise) can prevent colonization by the more aggressive species.

## Discussion

Although originally developed for a majors course, the methods described for culturing molds and measuring growth rates are not a major technical challenge for students with limited laboratory experience. Immaculate sterile technique is not essential either; students inoculate their plates with so many spores from their test species that unwanted molds rarely gain a foothold. Bacterial contamination also is rare, possibly because PDA has a lower pH than traditional bacteriological media (making it mildly bacteriostatic), or as a result of secreted products from the mycelium. The culture system has proven so robust that we now use essentially the same exercise into two non-majors lab courses as well. The exercise described also is extremely cost–effective. Our estimated cost for the entire 2–week lab is about $6.00 US per student, with the only additional expenses being for experimental materials not already on hand.

Depending upon the introductory material used, other labs can be developed that exploit this culture model. Currently we are considering exercises in which students can explore:

**Majors**

- Microevolution (Can molds develop drug resistance?)
- Mathematical modeling (logistic growth curves, survival curves)
- Allelopathy and other complex inter–specific interactions (How do molds compete with bacteria in the soil? Do molds modify their local environment to improve fitness?)

**Non–Majors**

- Food safety (How good are our preservatives?)

There are some issues that lab instructors need to consider before using molds as a model system. First, students need to have access to their plates outside of the normal lab meeting. One solution is to have students take their plates home, but that increases both safety and contamination risks. The best solution for us has been storing experimental plates in a central lab room that is left unlocked during the day. Students must come to collect their data when the building is open, or lose that day's data. A more serious concern was voiced at the 2003 ABLE meeting: can *A. flavus* be used safely in a classroom setting? This mold species is the primary source of aflatoxin, which is both an acute toxin and potent carcinogen. Fortunately, the particular strain we purchase does not produce aflatoxin, even when grown on powdered rice or peanuts (Wei *et al.*, 1986). There also is the potential for a severe allergic reaction by a student. We routinely warn our students in advance that they will be using molds, and we keep disposable dust masks on hand for individuals who know they are particularly sensitive.

In summary, structured inquiry labs have been a good compromise for our students between traditional confirmation labs, and true open inquiry projects. The author welcomes any suggestions of other uses for molds in the lab setting, or ideas for new labs. Electronic versions of the formal exercise we provide students, as well as a complete set of instructor and preparatory notes are available from the author as well.

## References

Wei, D.L., and Jong, S.C. 1986. Production of aflatoxins by strains of the Aspergillus flavus group maintained in ATCC. Mycopathologia. 93:19-24.