GRAVITY PLATES IN THE TEACHING OF MYCOLOGY—AN APPRAISAL

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Abstract

This paper describes a simple teaching tool that requires minimal resources, creates an active learning environment and introduces students to some basic concepts in microbial ecology and fungal taxonomy.

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Introduction

New students of mycology are often presented in weekly practical classes with a set of axenic cultures that show various taxonomic traits. This type of practical class too often presents the picture that mycology is all about classification and taxonomy. Of course, some members of the Mycota are biotrophic pathogens. In such cases infected hosts, processed or otherwise, are usually presented in class rather than axenic cultures. This, of course, is how powdery mildews and rusts are often presented. In both presentation modes, *i.e.* axenic culture or infected host specimen, the new student is presented with a collection of 'instructor chosen' material that limits any random insight into the real world.

This paper presents a synopsis of a study carried out over the past 15 years in which the isolates presented to new students are not derived from an existing culture collection. Rather, they represent those fungi that may normally be found in the aeromycota of a campus. Moreover, the exercise described here introduces the students to several important ecological concepts. This exercise also allows students to construct their own small culture collection, practice aseptic transfers and create an individual dichotomous key utilizing their own microscopic observations of the fungi captured. This exercise can adequately complement a more structured taxonomic approach, if required.

Materials and Methods

Ten days prior to the first laboratory practical class, sterile plates are exposed to the outdoor air for various periods of time. Normally half strength Potato Dextrose Agar (HPDA) is used and this is prepared using half the recommended quantity of PDA L^{-1} plus half the recommended quantity of pure agar L^{-1} to permit gelling. Other media such as Malt Extract Agar or Czapek-Dox Agar may also be used. However, the use of more selective media such as Dichloran Chloramphenicol Agar should be avoided since it limits the range of isolates captured. Sterile plates are placed on a flat surface 50 cm above 'grass level' on campus in an area which is well vegetated and away from any tall structures. If possible, rainy or blustery days should be avoided and half the plates should be exposed mid-morning and the other half in the late afternoon. This has been demonstrated to increase the diversity of taxa captured due to the possible effects of diurnal rhythms in spore liberation. The plates should be prelabelled on their bases according to the medium used and to the exposure period. The lids are then all removed and the media exposed to the air for 1, 2, 4, 8, 16 and 32 minutes. After the desired exposure period the lids are replaced on the bases.

The plates are incubated inverted in an incubator set at 25°C, more or less. They should be examined every two days since rapidly growing fungi such as *Trichoderma* spp. or mucoraceous species may colonise an entire plate within a few days. Such plates could be stored at 4°C till the day of the practical. At the first practical class the plates are distributed around the class. Depending on the class size every student should have 5–10 plates. A laboratory board should be ruled off to allow the number of colonies per plate to be entered, according to the medium used. Students are then instructed as to what constitutes a viable colony and then, using a marker pen to

mark the base of each of their plates, they count the number of colonies. All data are compiled and the average number of colonies per plate per medium calculated. This can be done by the students working in small groups.

Next a discussion is held about the flaws in the sampling protocol. What about variations in wind speed? What about the effects of recent watering or grass mowing? Students are encouraged to speculate about spore size in relation to dispersal and trapping, the suitability of the medium and the potential for trapping fastidious organisms. However, the basic assumption is that if 'n' propagules are caught in 1 minute then the number caught in each successive trapping event can be considered as 2n, 4n ... 32n. Over a short time span of about one half hour the number of trappings (*i.e.* landings) is probably proportional to the exposure period. Now, find the average number of colonies present on the plates exposed for 1 min. Double this number for every successive exposure period to calculate the 'expected number' of colonies for each exposure period.

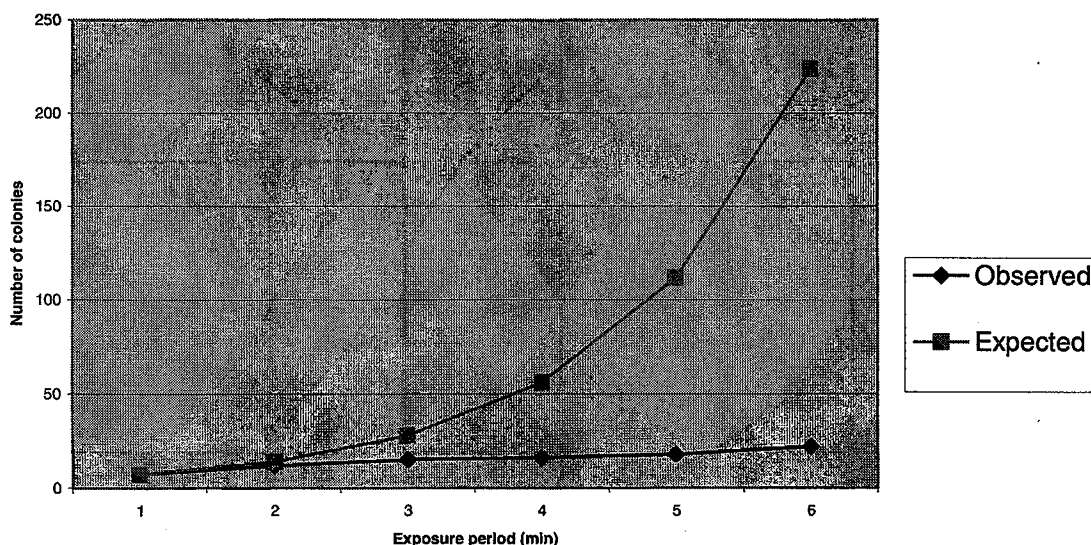
Now plot these expected numbers against the exposure period and then, on the same graph the 'observed number' of colonies. An example produced by students in a recent class is shown below.

Table 1. Typical class data from a gravity plate experiment.

Exposure period (min.)	Colonies observed	Expected number of colonies
1	7	7
2	12	14
4	15	28
8	16	56
16	18	112
32	22	224

This can be shown graphically as:

Figure 1. Typical gravity plate data.



The clear increasing discrepancy between the observed colony count and the expected colony count creates much speculation in the class. Usually, after some discussion the students erect the hypothesis that this difference is due to competition. They readily accept the ecological concepts that faster growing and perhaps more aggressive species may inhibit the growth, or even germination, of slower growing and less aggressive species. They accept that there is finite space and nutrient resources in the plates and that competition between species is a key determinant in microbial ecology. Often, by examination of the periphery of adjacent colonies, the effects of amensalism can be seen when one colony has a clearly obvious concave indentation.

Individual students are then asked to choose 10 colonies that look different from each other. Some colonies are effuse or floccose. Others may have immature fruiting structures apparent in the medium. Others may reveal

markedly different pigmentation. The students are demonstrated aseptic transfer technique when working close to a Bunsen flame. Each student is provided with 10 plates and 10 slopes of HPDA. The rest of the practical is taken up by carefully aseptically transferring 10 different isolates to a sterile plate and slope. These are labeled with the student's initials, date and culture number and form the basis for a subsequent exercise in which a dichotomous key to the set of 10 individual cultures is prepared.

Some discussion must be held about 'What constitutes a good dichotomous key?' A simple but workable answer to this question is:

- If there are 'n' individuals the number of dichotomies should be n-1.
- Characters used should have no 'plasticity'—they should be clear.
- If possible, only one character pair should be used at each dichotomy. If this is not possible then a re-examination of the appropriate specimens is required.
- Avoid characters that use, for example, ranges of spore size unless the ranges are quite discrete, *e.g.* 2.5–5.0 µm and 10.0–16.5 µm.
- Use the minimum number of words at each dichotomy.
- The key should work—testing can be done by choosing a culture and working 'backwards' from the endpoint of the key to the first dichotomy.
- Culture collections and keys can be exchanged and a random group of cultures keyed out by a fellow student. Amongst better students this is seen as a chance to outdo a classmate.

Discussion

Surveys done at the end of each course have shown that the students like to 'find their own' material. Students who have labored over keys in botanical courses or in entomology are 'refreshed' by the chance to construct their own key. It also creates awareness in students about the amount of effort that has gone into producing keys such as those found in Ellis (1971) or Pitt & Hocking (1997).

In summary, this is a useful teaching tool that requires modest resources, teaches a range of mycological skills including that of maintaining a small culture collection.

References

Ellis, M.B. (1971). *Dematiaceous Hyphomycetes*. CAB, Kew, UK.
Pitt J.I. & Hocking A.D. (1997). *Fungi and Food Spoilage*, 2nd edn. Blackie, London.