PROJECT 3-2 GERMINATION AND GROWTH OF MOSS SPORES

PROBLEM

It is fairly simple to grow moss spores under sterile conditions. In time, leafy gametophyte may grow from the protonoma. You might like to try to grow spores of various Australian mosses about which little is known. Alternatively, you could pick one that is easy to grow like *Funaria hygrometrica* and conduct experiments to determine what controls germination, growth, differentiation of leafy gametophytes etc.

INFORMATION

1. Ripe unopened capsules can be stored dry in clean containers in cool conditions for at least some species.

2. Read Section G on sterile techniques.

3. A suitable medium for spore growth is Knops.

Solution A	Magnesium sulphate	1 g
	Potassium nitrate	1 g
	Potassium phosphate	1 g
	Water (distilled	1 litre
Solution B	Calcium nitrate	4 g
	Water (distilled)	1 litre

Combine solutions A and B, bring to boil, add 0.8% agar (or 1% if this proves too sloppy) and stir until clear. Cool slightly then pour into jars and sterilize in the pressure cooker. Pour into sterile Petri dishes, about 25 ml per dish. Solutions A and B need not be all used at once and can be stored in the refrigerator.

4. Ripe capsules with a short stalk to use as a handle are treated as follows sterilize a small tube or jar with a lid and a small volume of sterile water in a separate bottle.

Shake capsule in a non-sterile container of water with 1 drop of detergent per litre.

Remove to second container and fill this with freshly made 2% sodium hypochlorite or undiluted Milton solution. Shake for 2-4 mins. From here on use sterile forceps. Transfer capsules to the sterile container add water shake for 1 minute then pour off water repeat the wash remove capsules to a sterile Petri dish crush capsule in a small drop of water and then smear the spore suspension onto the surface of the agar. A couple of brown streaks per dish is plenty seal edges of dish with gladwrap and place culture in dim light. For *Funaria hygrometrica*, germination might occur within 24 hours and leafy gametophytes can be seen after about 3 weeks. For some mosses it is not known how to induce formation of leafy gametophytes in culture so you might well discover something new.

5. When you want to look at the cultures place the dishes upside down on the microscope stage and use low power. You can see through the bottom of the Petri dish and the agar to the spores but the lid of the dish is usually too fogged with condensation to see through.

6. Factors that might affect spore germination and growth and leafy gametophyte differentiation are light and dark, temperature, light of different wavelengths (wrap plates in coloured cellophane), the age of the spores etc. To create different light conditions you can use cellophane or coloured filters from a theatrical lighting supplier. Check absorbance using a spectrophotometer if possible.

7. See Project 3-1 for further information on mosses.

DESIGN OF PROJECT

1. Mature spore filled capsules are mostly available in the latter half of the year and ones collected

2. What will you look for as an early sign of leafy gametophyte development?

3. How might you count the numbers of spores per capsule, per culture, or the number of leafy gametophytes that form?

REFERENCES

Conrad, H.S. (1956). How to know mosses and liverworts (Brown : Dubuque Iowa). (useful for identification but deals mainly with N. American and European material.)

Doyle, W.T. (1970). The Biology of Higher Cryptogams (Macmillan : London

Doyle, W.T. (1973) Non-Seed Plants Form and Function (Macmillan : London)

Ingold, C.T. (1974). Spore liberation in Cryptogams (Oxford Biology Reader No. 49) (Oxford University Press).

Scott, G.A.M. and Stone, I.G. (1976). The Mosses of Southern Australia. (Academic Press : London) (expensive and advanced reading).

Watson, E.V. (1971). The structure and Life of the Bryophytes (3rd Ed.) (Hutchinson : London) (advanced reading).

Watson, E.V. (1972). Mosses (Oxford Biology Readers No. 29) (Oxford University Press)