Growing Fern Prothallia

HERBERT M. CLARKE
Botany Department
University of Wisconsin

The following method has been successfully employed in growing fern prothallia for classroom and research use. Spores from a number of genera have been utilized; however, spores from various species of *Osmunda* have been the ones ordinarily used.

Fertile fern leaves or leaflets bearing almost mature sporangia are placed on clean pieces of smooth paper. As the sporangia dry the spores are discharged onto the paper. After a quantity of spores has accumulated on the paper, they are put into number 1 gelatin capsules for storage until the spores are to be planted.

The gelatin capsules containing the spores are placed in small bottles which are then tightly stoppered. At average summer room temperatures the stored spores of *Osmunda* retain their viability for about three months, in a 40° F. refrigerator they retain their viability for about a year and a half, and in a 5° F. refrigerator viability has been retained at a rather high level for more than thirty months.

A mineral nutrient agar made with a modified Beijerinck's solution is used for the germination of the spores and for growing the prothallia. The formula for the stock solution of this mineral nutrient is as follows:

Ammonium nitrate (NH_4NO_3)	5 grams
Magnesium sulfate ($MgSO_4$)	2 grams
Monobasic potassium phosphate (KH_2PO_4)	2 grams
Calcium chloride ($CaCl_2$)	1 gram
Distilled water	1000 cc.

This stock solution is diluted in the proportion of 1 cc of stock solution to 9 cc of distilled water. One cc of a saturated solution of ferric chloride is added to a liter of the diluted stock solution just before it is used. The same nutrient solution is used for the germination of moss spores and the growth of moss protonema, but for mosses it has been found that a 1:99 dilution of the stock solution is more satisfactory than the 1:99 dilution used for ferns.

Agar is added to the diluted stock solution in the amount of 16 grams per liter. This mineral nutrient agar is sterilized in an autoclave at 15 pounds pressure for thirty minutes. A kitchen pressure cooker may be used if an autoclave is not available. After sterilization the agar is allowed to cool slightly and then is poured into sterile petri dishes to a depth of approximately one quarter of an inch. When the agar has cooled and jelled the spores are planted.

When the spores are to be planted, the contents of one of the gelatin capsules is emptied into a prescription bottle or flask containing 100 cc of sterile distilled water. After thoroughly agitating the spore suspension by blowing into it through a pipette, 1 cc of the suspension is taken out and spread over the surface of the agar in a petri dish. The lid is replaced and the dish is tilted back and forth a few times in order to obtain an even distribution of the spores. If the spores are fresh and viable this concentration will give a large number of germinating spores—far too many for good growth of the prothallia. Therefore, it is advisable to dilute this spore suspension. This dilution is made by taking 1 cc of the spore suspension and adding it to another 100 cc of sterile distilled water. This new suspension is agitated thoroughly and liquid is drawn up and blown out of the pipette several times in order to wash all of the spores out of the pipette into the water. One-cc portions of this second suspension are placed in the petri dishes containing the agar, and the dishes are tilted back and forth to spread the suspension evenly over the agar. To get still fewer prothallia per petri dish, another dilution may be made. A 1:25 dilution of the second suspension is suggested.

After planting of the spores, the petri dishes are illuminated by either natural light from windows with a north exposure or artificial light from fluorescent lights. The fluorescent

lights used by the author were regulated by a clock and the period of illumination extended from 5 a. m. to 10 p. m. This period of illumination was decided on in an arbitrary manner and not from any particular evidence except that it had been observed that cultures of *Euglena* sharing the illumination grew better when there was less than 24 hours of continuous illumination.

After about two weeks, petri dishes containing the 1-cc portions of the original spore suspension will become green and various stages in development of young prothallia will be evident. After about six weeks, prothallia producing sex organs will be found in the petri dishes which were planted with 1-cc portions of the diluted spore suspensions. If not crowded the prothallia will show the form characteristics of the species planted. Ordinarily sporophytes will not develop unless the cultures are flooded with water to provide a medium for the movement of the antherozoids to the egg. If the fern species utilized as a spore source is one in which apogamy occurs, sporophytes develop without flooding.

Occasionally contamination of the cultures by fungi may occur. Most fungi grow poorly on the mineral nutrient agar and thus do not interfere with the growth of prothallia. Mites and small insects have at times tracked in the spores of fungi which have contaminated the cultures.

Prothallia may be left in the petri dishes for several weeks. However, after two to three months the agar will dry down somewhat and crack. Prothallia may be transplanted to fresh agar if it is desired to maintain them for a longer time. Some old prothallia proliferate freely, and new cultures of prothallia may be started by picking off the adventive prothallia and placing them on fresh agar plates.

The Mid-west Conservation Education Conference was held at Higgins Lake Conservation Training Camp at Roscommon, Michigan, October 10-13, 1954. Responsibility for the program was shared by Dr. G. W. Mouser, Michigan State College, and Dr. R. L. Weaver, the University of Michigan.

Biology in the News

BROTHER H. CHARLES, F.S.C.
Saint Mary's College
Winona, Minnesota

SHOULD YOUR BOY PLAY FOOTBALL?, Al Stump, *American Mag.*, October 1954, pp. 26, 109-113.

A sports writer gives some shocking reasons for the mounting toll of accidents on high school gridirons. The safety code which is included is worth considering by students and administrators alike.

THE RAIN FOREST, Lincoln Barnett, *Life*, Sept. 20, 1954, pp. 76-106.

The ninth in the series "The World We Live In." The color pictures and the descriptions of life in a tropical rain forest are equal to those which have preceded it in the series. Several copies of this article should find a place in your file of really worthwhile bulletin board materials.

THIS SCHOOL IS READY FOR THE H-BOMB, Herbert and Dixie Yahraes, *Sat. Ev. Post*, Sept. 25, 1954, pp. 45, 111-114.

Do you have disaster training in your school? Would you be prepared to meet emergencies if your city were bombed? The plan described in this article might be used, with a few modifications, in case of other types of disasters. This article might excite lively discussion about what protective training your school should have.

BEFORE YOU DIET, Ethel Strattan, *Cosmopolitan*, October 1954, pp. 46-49.

Thumb nail discussions of twenty key theories on dieting. A thoughtful reading will provoke many reactions from students.

12 DIETS FOR YOU AND YOUR FAMILY, Herbert Pollack, M.D., *Woman's Home Companion*, October 1954, pp. 109-112.

This article, based on recent research, contains good news for those who would diet successfully to gain weight, to lose weight or to maintain normal health.

THE GREAT CARNATION LOTTERY, Frank J. Taylor, *Sat. Ev. Post*, Oct. 9, 1954, pp. 28, 112-115.

Denver's nearly 300 days of sunshine each year has enabled its carnation growers to develop new varieties and to take top honors in kinds and carnation production in the United States.