A PROCEDURE FOR PROPAGATING FERNS FROM SPORE USING A NUTRIENT-AGAR SOLUTION

BRUCE C. LANE

Bordier's Nursery, Inc
Irvine, California, 92714

Plantlet crowding and biological contamination are problems experienced by many who propagate ferns from spore. At Bordier's Nursery, our attention has been directed toward finding propagation methods which would work to diminish these problems. The methods we are now using are not uncommon. They require tedious labor with cleanliness being stressed at every step, but they are successful in that we are able to produce the fern liners we need. Briefly, our propagation procedure involves germinating spore and growing the young fern gametophytes on a nutrient-agar solution, transplanting adult gametophytes onto a growing medium where fertilization can lead to fern sporophyte formation and finally, transplanting young sporophytes to give them space to develop into liner-sized plants. Previously, we germinated our spore on fine spagnum peat and had a serious problem with gametophyte crowding, as well as fungal, algal and moss contamination. However, approximately 1½ years ago, we tested and adopted the use of a sterile nutrient-agar solution as a germination and growth medium, a method developed by Tjosvold (1) specifically designed to reduce these problems. The six fern species we produce using our method are: *Cyrtomium falcatum* 'Rochfordianum', *Dicksonia antarctica, Nephropelis exaltata Polystichum setosum* (*P.* × *bicknelii* or *P aculentum,* Bot. ed.) *Rumohra adiantiformis* and *Sphaeropteris cooperi.* Following is the procedure we are successfully using to propagate ferns from spore.

Our spore is collected from stock plants when the sori on the underside of the fronds are plump and ripe, but before these clusters of sporangia have discharged their asexual contents. These fronds are gathered, enveloped in paper plant sleeves and placed in a warm, dry location for two weeks. This desiccating environment dehydrates special cells encircling each sporangium, eventually catapulting the spore away. It is important to separate the released spore, as much as possible, from the crisp fronds and chaff that may carry inocula. A mechanical shaker and a series of soil sieves are used for this separation, the final two screens having a 200 and 270 mesh (75 and 53 micrometer openings, respectively). The powdery spore is stored dry in tightly sealed containers and refrigerated at 4.5°C (40°F).

From this point on, cleanliness becomes very important, especially when working with a sterile growth medium. Any con-

tamination which can be avoided leads to more productive spore germination, more vigorous gametophyte growth and fewer problems in the transplant flats. In our greenhouse, a laminar-flow hood provides us with an inocula-free work space in which to conduct our sporing. Precautions taken to insure cleanliness within the transfer hood include spraying the working surface, walls and filter with aerosol Amphyl and operating the filter 30 minutes prior to use. Cleanliness is enhanced when sporing is conducted atop disinfectant saturated paper towel. In addition, all sowing instruments are cleaned in a 5% Clorox solution.

Our spore is sterilized immediately prior to sowing to insure that it is free of biological contamination that may have passed along with it through the soil sieves. One part spore is shaken vigorously in 5 parts of a 5% Clorox solution. After 1½ minutes this mixture is quickly poured into a Buchner funnel-filter setup and with the aid of a vacuum, rinsed several times with water. This dilutes the biocide so harm to the spore is avoided. After rinsing, we allow the vacuum to pull air through the spore for an additional 5 minutes before letting the spore air-dry under the hood. If this vacuum airing is not done before drying, caking of spore results.

Tjosvold was trying to accomplish two things when he developed his method of sowing fern spore on a warm nutrient-agar solution. He wanted to improve the distribution of spores on the sowing medium to reduce gametophyte competition and provide a nutrient-rich environment free of contamination from fungi, algae and mosses. He found that by using an agar concentration of 2 grams per liter of double strength Hoagland solution #2 in combination with a solution temperature of 120°F at the time of sowing, a favorable surface tension occurred which distributed the spore uniformily on the medium. Gametophyte growth was enhanced when the strength of the Hoagland solution was doubled. Heating to the boiling point involved in preparing the solution produced a sterile medium on which the fern gametophytes could grow without competition. Our nutrient-agar solution is prepared by Soil and Plant Laboratory, Santa Ana, California, according to Tjosvold's formula. We receive this solution after it has been autoclaved and sealed in Mason jars.

Prior to sowing, we reheat the prepared solution in a hot water bath shaking the quart jars several times to insure a homogenous medium. After it has cooled to the desired sowing temperature of 120°F, each sterile, plastic, 100 × 200 mm petri dish is filled with ¼ inch of the solution. Sowing is done by lightly tapping a 270 mesh screen containing the spore over the medium, quickly replacing the petri lid when finished. A key advantage to sowing on this medium as opposed to peat moss is that one can see the individual spores sown due to the color contrast and

clarity of the solution. If density appears to be low, more spore can be added.

After sowing is completed, a flame is used to sterilize the exposed edges of the petri dishes. This extra precaution is taken to eliminate inoculum that may have been introduced with handling and could find its way into the plate. These petri dishes are then placed and sealed in polyethylene bags to minimize dehydration of the solution and keep out fungus gnats and other insects.

Germination of spore usually occurs within 10 to 14 days, the gametophytes appearing as little green dots on the solution. For germination and growth, our fern house daytime temperature is kept between 21° and 26.5°C (70° and 80°F), and cooled by means of an evaporative cooler. Night time temperature is maintained at 18.5°C (65°F). Light intensity is kept in the range of 200 to 400 foot-candles.

Two or three months after germination, gametophytes are usually ready to be transplanted. Transplanting is done because the small plants have grown to a desired size and conditions more conducive to fertilization for sporophyte formation are needed. On the underside of the adult gametophyte, motile sperm from male antheridia are attracted to eggs within female archegonia by a chemical substance. Watering and misting the transplant flats provide free water in which the multiflagelate sperm can swim to their destination. We have noticed reduced numbers of sporophytes when adult gametophytes are kept on the more viscous nutrient-agar solution for a long period of time. Transplanting is also needed when gametophyte crowding starts to retard growth. This occurs after the young gametophytes have spread over the solution to the walls of the petri dish.

At the time of transplanting, gametophytes in petri dishes are floated on water, broken apart and spooned with water onto a prepared flat. Usually 10 to 20 gametophytes are spooned together in a group with groupings spaced about an inch apart. We also transplant by combining the contents of several petri dishes, diluting and agitating with water, and pouring this mixture over the prepared flat until the desired gametophyte density is obtained. The water used in both of these transfers permits a close contact between gametophyte rhizoids and the growing medium. Our transplant mix consists of equal parts of fine peat moss, fine vermiculite and graded, sand-sized perlite. All flats are kept moist with a ⅓ strength Peter's solution (20-20-20) made from chlorinated water low in salts. Flats are placed on a bench beneath a polyethylene tent to maintain high humidity.

Sporophytes start emerging from the adult gametophytes 3 to 7 months after transplanting, depending on the species. These

sporophytes are transplanted as single plants to another prepared flat while the second leaf is still developing. The transfer is made at this young stage to avoid the root intertwining that is characteristic of older sporophytes that have grown closely together. There is reduced root tearing when intertwining is minimized. Plastic tweezers are used for the delicate separation and placement into flats and care is always taken to keep the fern crown above the soil level. As sporophytes become large enough, they are finally potted into liner containers, again keeping the crown above soil.

While our transplanted ferns are vigorous plants seemingly tolerant of fungi, algae and mosses, inoculum are ever present and cause serious problems if left unchecked. Spacing for adequate air circulation, proper irrigation using water free of inoculum and transferring only clean plants to transplant flats reduces or prevents some of these problems. Removal of diseased and infested areas is done by hand. Problems that are present in petri dishes are easily elimated by throwing away contaminated plates. We are quite inexperienced when it comes to chemical control, but have found that tender young gametophytes and sporophytes are easily burned by particular chemicals in combination with our greenhouse temperatures and humidity.

Germinating spore and growing gametophytes on nutrient-agar solution has improved our fern propagating procedure. The transplanted gametophytes are vigorous plants having grown in a medium free of contaminants, uncrowded, and well supplied with nutrients. We rely on the vigor of these transplanted gametophytes along with cleanliness and proper cultural care to provide and maintain the sporophytes required for our canning production.

LITERATURE CITED

1 Tjosvold, Steven 1978 Uniform fern spore dispersal on warm nutrient agar solution University of Califoria Nursery and Flower Report Summer, 1978 p 7

MAHONIA PLANT CONDITIONING AND PROPAGATION

CHET BODDY
Boddy Nursery
Fort Bragg, California 95437

Of the four mahonia cultivars we grow, *Mahonia aquifolium* 'Compacta' is the most popular — and also the most difficult to propagate from cuttings. This paper deals mainly with 'Com-