CULTURE METHODS IN THE PRODUCTION OF POLYEMBRYONY IN CERTAIN FERNS (POLYPODIACEAE).

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To Secure the Stock Culture.—In both stock and transplanted cultures the prothallia are grown upon rich soil, preferably woods earth. Unglazed earthen ware vessels, flower pot saucers, are used as containers.

The thoroughly moistened (not wet) soil is passed through a sieve with one-fourth inch mesh. First a thin layer of coarser earth is placed in the saucer, which is then filled three-fourths full with the sifted earth. Upon this is evenly spread a thin layer of earth which has been passed through a finer sieve such as may be made from fly screen wire. The surface is then gently firmed with the hand, so that an even and sufficiently compact surface is provided. A moat is now made around the edge by pressing the soil back from the side of the saucer with a stick or finger.

The vessel with the soil thus prepared is sterilized in a steam sterilizer two hours on each of two successive or alternate days. If the soil be too wet before sterilization, the result will be a mud pie instead of a suitable substratum.

The soil is now evenly coated with the spores, and the culture covered with a belljar. It is well to prop up the belljar on one side to a height of about one-fourth inch to insure ventilation and to facilitate evaporation should the culture be made too wet in watering. The soil is kept moist by sub-irrigation, the water being gently poured into the moat. This method is preferred to that of allowing the saucer to stand temporarily in shallow water. Cultures should never be watered by spraying, and care should be taken not to keep the soil wet.

Good diffused light provides the best illumination. However, direct sunlight for an hour in early morning is beneficial.

Transplanting and Cultivation.—The soil is prepared as for the stock culture, and the conditions are to remain the same.

When the prothallia are from one to three millimeters in breadth, or larger, they are lifted from the stock culture with needle or forceps, along with sufficient earth to preserve the majority of the rhizoids, and set out in the new dish about an inch apart. Care should be taken to remove all smaller adhering prothallia. The soil should be quite moist at the time of transplanting so that the earth about the rhizoids may be pushed easily into it. The under free surface of the prothallium should not come into direct contact with the soil. If after a time weed-prothallia, mosses or other plants appear in the cultures, these should be removed. If the surface of the soil should become caked or grown over with blue-green or other algae, as may happen in long-standing cultures, the earth should be allowed to dry somewhat and the caked or matted surface removed and fresh sterilized soil added. Sometimes it is desirable to transplant the prothallia to newly prepared culture dishes. They should be lifted with enough earth to preserve the

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rhizoids. Growing prothallia for longer periods of time than obtains under natural conditions out of doors, however, requires experience to insure success.

Fertilization or Fecundation.—When the plants have attained the desired size—eight to ten millimeters in width—fecundation should be begun. If the prothallia be monoecious, three or four drops of water are allowed to fall upon the growing notch. It is better to do this near mid-day when the cultures are a little drier, as the mature archegonia will then open readily, and at the same time sperms will escape from the antheridia. This procedure should be repeated every other day for a few times, so that several egg cells may be fecundated upon a given prothallium.

If the prothallia tend to be dioecious, as in *Onoclea sensibilis* and *Matteuccia Struthiopteris* (*Onoclea Struthiopteris*), it is well to place a small tuft of male plants close to the apical sinus before dropping on the water, in order to insure fecundation.