

Growth Responses of *Regnellidium diphyllum* Sporelings to Variations in the Concentration of the Nutrient Solution¹

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Ferns have not been used extensively in studies of plant nutrition. Culturing the gametophytes to maturity is often difficult and slow and growth of the sporeling is relatively slow. Some experimental studies have been conducted using suitable solutions for germinating spores and for growing gametophytes, but sporophytes of ferns seldom have been grown in nutriculture. *Azolla* has been used for limited nutritional studies. Allsopp (1, 2, 3) has employed species of *Marsilea*, using aseptic methods, to study carbohydrate utilization and the effects of starvation upon the morphological and growth responses of the plants.

In some studies on the nutritional requirements of the tropical water fern, *Regnellidium diphyllum* Lindm., it was first desirable to know more about the essential range of concentrations of the nutrient solution suitable for growing this plant. The work reported here was one of several avenues of investigation pursued to find out more about its optimal growth requirements.

General Methods

The experimental plant, *Regnellidium diphyllum* Lindm., is a monotypic genus, one of the largest members of the Marsileaceae. Its anatomy is discussed fully by Smith (6) and Eames (4). As with other members of the Marsileaceae, the spores are produced in sporocarps, which contain both microspores and megaspores. A single sporocarp may contain over 240 megaspores.

Culture solutions. To secure a suitable range of nutrient solutions for experimental purposes, the dilution method used by Voth (7) for *Marchantia polymorpha* was used. Solution number 1 was approximately twice the usual strength of the nutrient solutions in common use. Solution number 2 was the same strength as the usual solutions. Each successive dilution was one-half of the previous solution so that solution number ten was one-two hundred fifty-sixth (1/256) of the usual concentration used in nutriculture of higher plants.

Individual sporophytes were cultured separately in glass vials 20 mm. in diameter and 85 mm. deep. Twenty milliliters of nutrient solution were used in each vial. Ten replicates of each of the 10 Voth solutions and of de-ionized distilled water were employed. The vials were placed in a covered aquarium to maintain a high humidity over the plants and a temperature of 26° ($\pm 1^\circ$) was maintained. A 13 hour photoperiod was provided by means of two 20 watt fluorescent tubes which provided about 300 foot-candles at the level of the plants.

1. This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

One sporocarp, harvested in 1943 by Dr. Paul D. Voth of the Department of Botany of the University of Chicago from material grown in the university greenhouses, was germinated on Dec. 11, 1953. Development of the gametophytes and of the embryo sporophytes proceeded so rapidly that individual plants could be transferred with forceps to the nutrient solutions on December 18. Thus, in one week, spores germinated, both kinds of gametophytes matured, fertilization ensued and the young sporophyte (sporeling) was well established. Observations were made from time to time and recorded. Plants were harvested on February 4, 1954, and dried in an 80°C gravity oven, and dry weights determined.

Results

Some plants were injured during the transfer operations and showed early signs of necrosis. Uninjured plants showed steady and uniform growth in each solution. The flask used to prepare solution number 5 apparently retained some toxic substance in spite of careful washing and repeated rinsing with distilled water. All the plants transferred to this solution soon showed signs of necrosis. This solution will be omitted in the treatment of the results.

Regardless of the concentration of the nutrient supply and size of the plants, the sequence of appearance of the leaflets was fairly uniform in all but the last two solutions. Plants in solutions 1 to 7 had all produced 8 leaflets by January 20, 1954. Those in solutions 8 and 9 had produced 7 leaflets. Plants in solution 10 had produced 6 leaflets and in de-ionized distilled water 5 leaflets.

The differences in gross morphology were quite apparent. Plants in solution 1 had the coarsest leaves and roots. The roots were the shortest of any of the solutions, including de-ionized, distilled water. As solutions became more dilute the roots became longer and finer and reached the maximum length in solution 6. From solutions 7 to 10 the roots became progressively shorter and finer. The roots in de-ionized distilled water were fewer in number, fine and short.

Figure 1a illustrates the general characteristics of a plant grown in solution 1. Figure 1b illustrates the longer roots produced by plants in solution 3. Figure 1c demonstrates the long roots produced in solution 6. Figure 1d illustrates the overall reduction in plant size in the highest dilution of the nutrient, solution 10.

The dry weights of the plants show a steady decline after solution 3 as the nutrient solutions in which they were grown became more dilute. The dry weights ranged from 2.6 mg. per plant in solution 1 and 3 to 0.7 mg. in solution 10. The only solution which did not fit this pattern was solution 2. Plants grown in solution 2 averaged 0.6 mg. less per plant than those in solutions 1 and 3. This could possibly be an experimental error rather than a pronounced effect of the nutrient solution itself.

Discussion

As with higher plants, it is difficult to determine the individual factors responsible for the reduced growth in higher dilutions of the nutrient. As the amount of a particular nutrient diminished with dilu-

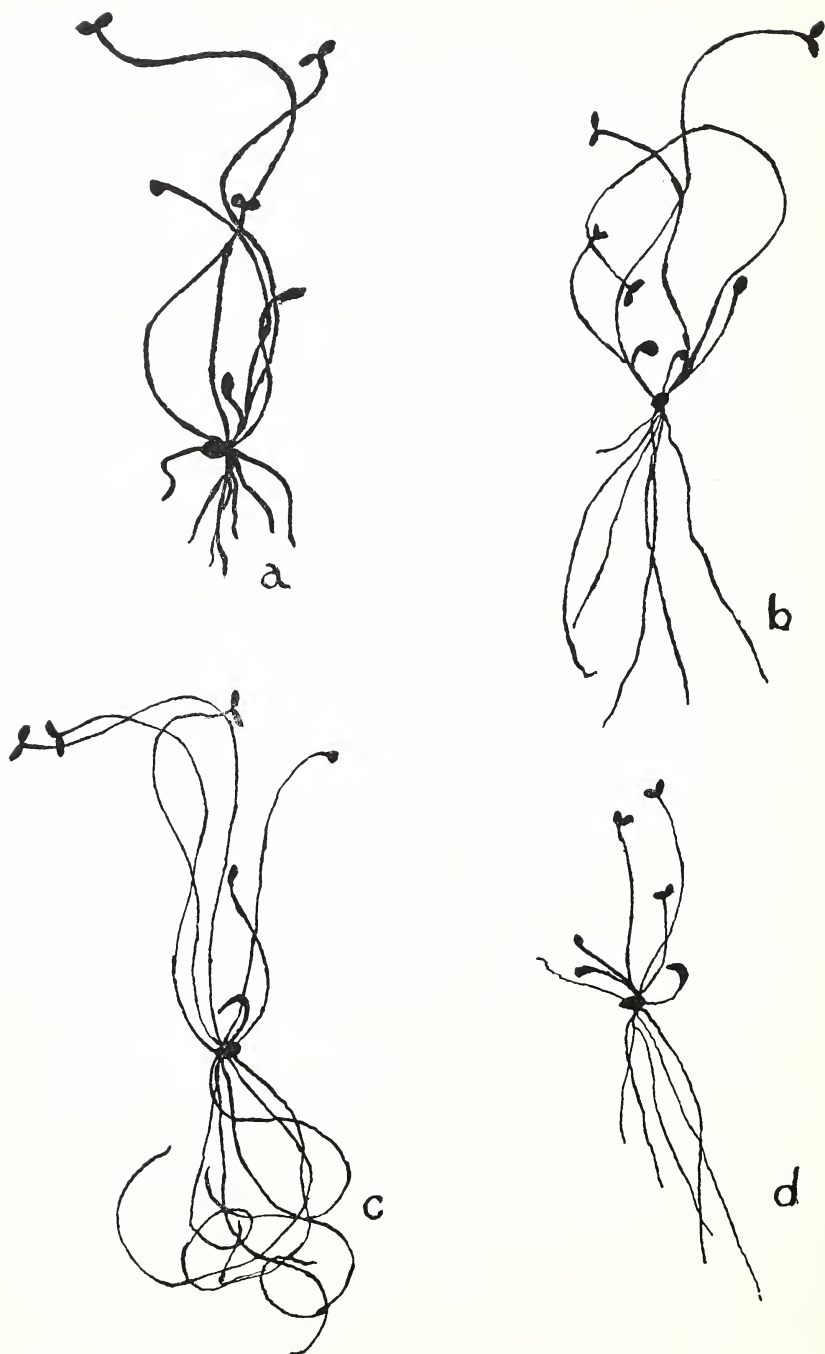


Figure 1. a. Plant grown in solution 1. b. Plant grown in solution 3. c. Plant grown in solution 6. d. Plant grown in solution 10.

tion, the growth responses may even be the result of a deficiency of a single element, such as nitrogen.

The results, while based on a limited sample, suggest that a range of concentration of nutrients between double that of the usual nutrient solution and one-half the usual nutrient would support vigorous growth of this fern. This assumption was further tested by transplanting cuttings to larger 2 gallon glazed containers, containing loam soil or washed quartz sand. Water was maintained over the soil at a depth of 140 mm. Full strength, half-strength, or one-quarter strength Hoaglands' number 1 (5) solution was maintained to a depth of 140 mm. over the quartz sand. The full and half-strength solutions produced growth very similar to that produced on loam soil. In view of these results, experiments that followed were conducted in nutrient solutions similar in total concentration to Hoagland's number 1 solution.

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