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# DEMONSTRATING MINERAL-NUTRIENT DEFICIENCIES WITH *REGNELLIDIUM DIPHYLLUM*

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## Introduction

The Brazilian waterfern, *Regnellidium diphyllum* Lindm., resembles the more familiar species of *Marsilia*. However, the large paired leaflets, the vigorous rhizome which yields latex, and the rotund sporocarp which approximates in size a single pea seed are striking features of a plant which has been propagated successfully in greenhouses in the United States. The ease with which *Regnellidium* is grown under several different cultural conditions indicates its versatility in controlled experiments or in classroom demonstrations.

Bloom (1954) placed quartz sand in a 2-gallon glazed crock and added solution to a depth of 5.5 inches. The kind of solution used was determined by the familiar experimental design of the nutrient triangle (Voth and Hamner, 1940). The osmotic concentration of each solution approximated 0.5 atmosphere. One transplant was placed in each container. Equally successful was soil with a similar depth of supernatant. Under certain cultural conditions the rhizome of a well-established plant of *Regnellidium* grew forward at a rate of two inches per day. Responses of this plant to excesses, deficiencies, and imbalances of the common nutrient anions and cations were striking and measurable. Plants placed in solutions relatively high in magnesium salts possessed wilted leaflets within an hour. Responses to other nutrient variations often were not as readily observable and required careful measurements of dimension and of weight to demonstrate differences.

In the autumn of 1954 it became desirable to demonstrate the effects of some nutrient deficiencies in plants to a biology class at a level comparable to the junior year in college. In Chicago, growing conditions became progressively less favorable in October and November, even in the greenhouse. In former years tomato or other plants growing in quartz sand had been available as surplus from investigative work. Lacking the usual plants, it was necessary to rely on expedience and innovation. *Regnellidium* offered the best possibility.

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### Observations

Early in October a Mason fruit jar of two-quart capacity was filled with the desired nutrient solution to within an inch of the neck. No solid substrate was provided. Instead, a transplant consisting of a rhizome-node with a well-developed leaf, an unopened but healthy axillary bud, and the internode fragments was weighted with a tightly bent U-shaped length of four-millimeter glass rod hung around the petiole and over the creeping stem. The entire assembly was lowered into the liquid (Fig. 1). When the petiole was too short to permit flotation of leaflets, liquid was drained off until the level was suitable. Greatly elongated petioles may bring the leaflets some distance above the jar, but Bloom (1954) found that extended leaves remained functional except in a few treatments. His trials were made in April and May. No observations have been made upon extended petioles placed in shallow solutions late in the year.

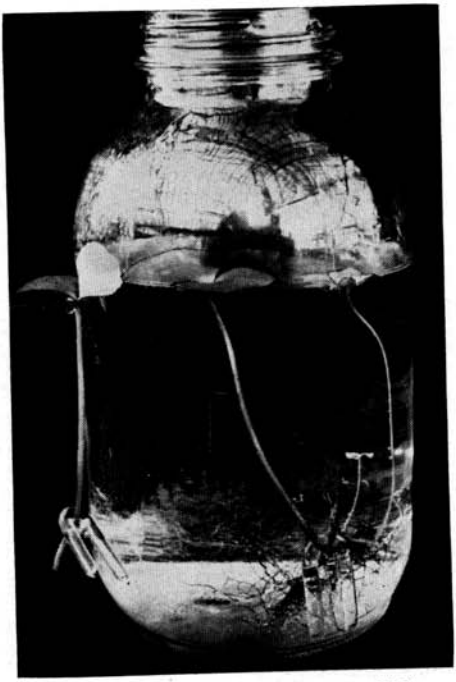

FIG. 1.—Transplant of *Regnellidium diphyllum* with several leaves on lateral rhizome. Tap water. Age of transplant is nearly two weeks. To left of jar is newly-cut transplant. Marks on middle of jar indicate vertical inch.

Treatments were prepared in duplicate, and the fruit jars containing the plants were placed on inside window ledges of a large laboratory. Exposure was to the south but shadows from a building lessened direct sunlight in the morning hours. Solar radiation was weak and passage through the thick window panes decreased the intensity still more. At no time did the plants appear to be overheated in the jars.

The most successful nutrient solutions in the demonstration were the complete one and those lacking calcium, nitrates, or phosphates. In the following formulations, which are based on Hoagland's solution 1, not only the solutions used in the demonstration are given, but also others which may be added to a classroom experiment (Hoagland and Arnon, 1950). For the present purpose no micronutrients except iron need be added. Chelated iron is the most effective form of this mineral nutrient (Weinstein, Robbins and Perkins, 1954). In each instance add distilled water to make one liter of solution.

#### Complete Solution

| | |
|---|---|
| $KH_2PO_4$ | 136.0 mg. |
| $KNO_3$ | 505.5 mg. |
| $MgSO_4 \cdot 7H_2O$ (Epsom salt) | 493.0 mg. |
| $Ca(NO_3)_2 \cdot 4H_2O$ | 1180.8 mg. |
| Chelated iron | 1.0 mg. |

SOLUTION LACKING POTASSIUM

| | |
|---|---|
| $Ca(H_2PO_4)_2 \cdot H_2O$ | 116.0 mg. |

$MgSO_4$, $Ca(NO_3)_2$, and chelated iron as in the complete solution.

SOLUTION LACKING NITROGEN

| | |
|---|---|
| $MgSO_4 \cdot 7H_2O$ | 493.0 mg. |
| $K_2SO_4$ | 435.6 mg. |
| $Ca(H_2PO_4)_2 \cdot H_2O$ | 116.0 mg. |
| $CaSO_4 \cdot 2H_2O$ | 344.0 mg. |
| Chelated iron | 1.0 mg. |

SOLUTION LACKING PHOSPHORUS

| | |
|---|---|
| $KNO_3$ | 606.6 mg. |
| $MgSO_4 \cdot 7H_2O$ | 493.0 mg. |
| $Ca(NO_3)_2 \cdot 4H_2O$ | 944.6 mg. |
| Chelated iron | 1.0 mg. |

SOLUTION LACKING MAGNESIUM

| | |
|---|---|
| $KH_2PO_4$ | 136.0 mg. |
| $KNO_3$ | 606.6 mg. |
| $Ca(NO_3)_2 \cdot 4H_2O$ | 944.6 mg. |
| $K_2SO_4$ | 261.4 mg. |
| Chelated iron | 1.0 mg. |

SOLUTION LACKING CALCIUM

Omit only $Ca(NO_3)_2$ from the complete solution.

SOLUTION LACKING SULPHUR

| | |
|---|---|
| $Mg(NO_3)_2 \cdot 6H_2O$ | 256.4 mg. |

$KH_2PO_4$, $KNO_3$ $Ca(NO_3)_2$ and chelated iron as in the solution lacking magnesium.

SOLUTION LACKING IRON

Complete solution without the chelated iron.

In the spring of the year Bloom found that differences in growth rates became evident within a few days. Striking differences in the transplant leaf and in the development and linear growth of the axillary buds were measurable within a week. However, when *Regnellidium* was grown in the laboratory in glass jars under autumn conditions, more than a week passed before the earliest symptoms were apparent. The desired condition of the plants was attained after five weeks under the described conditions.

The leaflets on the original portion of the plant remained green in these solutions: complete, lacking calcium, and lacking phosphates. Yellowing was apparent in original leaflets found growing in solutions deficient in nitrates.

The axillary bud developed the largest and longest rhizome, the greatest number of leaves, and most vigorous secondary rhizomes when placed in the complete solution. Other solutions supported plants of smaller size and with different growth characteristics.

Calcium deficiency was apparent in the newly formed leaflets which are wedge-shaped, stunted, and submerged. The rhizome producing such leaves remained very short.

Symptoms of phosphate deficiency included a very dark green transplant leaf and a number of nearly full-sized leaves. The total growth of tissue, however, was small so that fresh and dry weights were low.

When nitrates were omitted the original leaflets became yellow, and subsequent leaflets were decreasingly smaller in size and lost their green color in turn.

Lack of iron also resulted in the production of new leaflets that were yellow, but the transplant leaf generally remained green. Stunting was not severe during the demonstration. If iron chlorosis develops, chelated iron may be added at the rate of 0.7 p.p.m. (Bloom, 1954; Weinstein, Robbins and Perkins, 1954) or at 1 part per million (1 mg. per liter).

If responses are consistent and growth fairly extensive, a number of measurements may be made on lengths of rhizomes and petioles, on numbers of fully expanded leaves, nodes, and secondary branches, and on the areas of the leaflets. Areas may be computed easily. Length of a leaflet multiplied by its width, multiplied by 1.6 will closely approximate the upper surface area of the entire blade as reported by Bloom.

Observation of qualitative differences and the gathering of quantitative data can be valuable if planned as a class exercise and correlated with well-known symptoms of higher plants as reported by Hambidge, *et al.* 1941. Determination of fresh weights is easily made. Root growth as well as top growth may be estimated.

The greatest deterrent to the extensive use of *Regnellidium* in nutrient demonstrations will be the propagation of plants, if greenhouse facilities are limited. Bloom used deep metal tanks into which a wooden platform was built. Soil was placed on the boards to a depth of two inches and the tank filled with tap water so that the soil was submerged about three inches. Transplants grew rapidly in this type of tank and survived for more than a year (Fig. 2). This culture which was started by Bloom has been perpetuated with little attention by greenhouse personnel. The water level has often dropped several inches below the level of the soil. No supplementary heat was provided in the winter of 1954-55. There is no doubt, however, that a submerged heating cable or a radiant heater placed near the tank would have promoted growth in the winter months. Experiments with vermiculite may solve the problem of propagation, once a clone has been started.

On plants with leaves floating or emergent, no sporocarps have grown on any of the substrates in our greenhouses. In soil culture a few sporo-

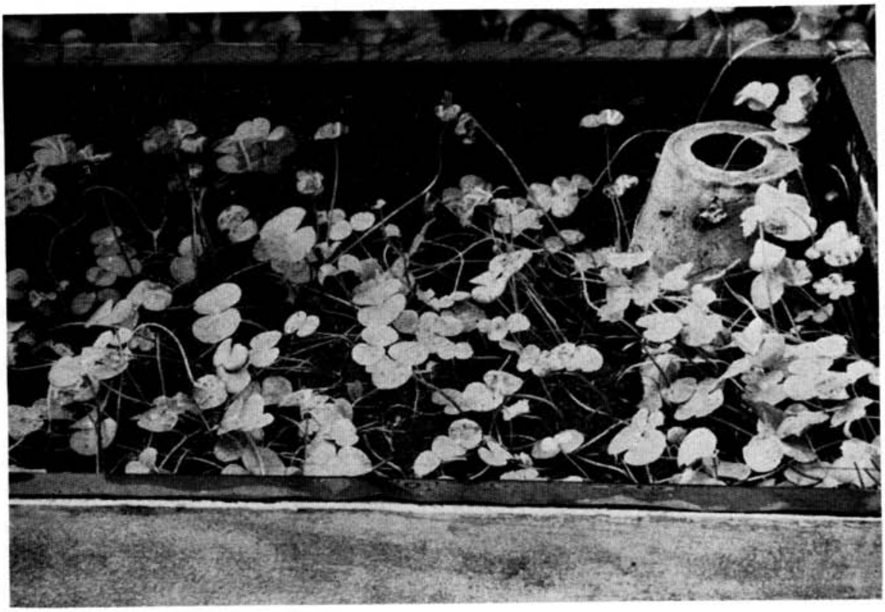

FIG. 2.—*Regnellidium diphyllum* propagated on soil in metal tank, January 30, 1955.

carps formed when the water level was permitted to drop gradually. The factors which control spore production are not known, and this aspect of *Regnellidium* culture will bear much more investigation.

### Summary

Teachers and students are encouraged to utilize a window ledge in the classroom as a miniature laboratory. Responses of plants to mineral nutrients can be demonstrated with simple glassware and easily compounded laboratory chemicals. Stock plants of *Regnellidium*, once procured, should be propagated by a variety of methods to ascertain the most successful ones for each locality and climate. If greenhouse space is limited or unavailable, the perpetuation of the clone during summer months requires additional resourcefulness, but it may be possible to use aquaria or small outdoor ponds to accomplish this.

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