Some Factors Influencing Rhizoid Formation in Female Gametophytes of the Marsileaceae

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We have been working with various members of the family Marsileaceae for the past twelve years or more and have become interested in rhizoid formation in the megagametophytes. When first observed the rhizoids were seen to arise from the base of the gametophyte adjacent to the region where the root was forming on the enclosed embryo. Rhizoid formation was usually limited to a small region adjacent to the site where the root emerged and the base of the gametophyte next to the megaspore wall. When this close association of position was noted between the root and rhizoids, it was thought that there might be a hormonal relationship between the megagametophyte and the enclosed embryo sporophyte. Time was not available to explore this relationship when first observed, but during the past year some work was done on this problem and the results are very interesting.

It was suggested to an undergraduate student, Stephen Main, working under a National Science Foundation grant for undergraduate research at Valparaiso University that he spend some time studying this gametophyte-sporophyte relationship. Several approaches to the problem were followed.

Sporocarps of *Marsilea Drummondii* A. Br. collected in October, 1953 by Mr. T. R. N. Lothian of Loxton, South Australia were placed in vials containing tap water. Following dehiscence of the sporocarps, but before the microspores had a chance to germinate and produce free sperm, some of the megaspores were isolated and placed in separate vials. Within several days both the megagametophytes which did not possess embryos and the megagametophytes containing embryos were observed to possess rhizoids. In both cases the rhizoids were concentrated in the same regions on the ventral side of the floating gametophytes. This ruled out a hormonal relationship between gametophyte and sporophyte.

The next effort was directed toward determining whether a limited region was involved in rhizoid formation or whether many different epidermal cells of the megagametophyte were capable of forming rhizoids. To determine this, megagametophytes were again isolated before fertilization could occur, as well as after fertilization, and placed on the surface of mineral nutrient agar in Petri dishes. The dishes were kept covered to retain moisture. Once the megaspores were attached to the surface of the agar they adhered firmly. Some of the dishes were kept upright and others were inverted. Under these conditions rhizoids developed on the gametophytes with embryos as well as those without embryos. In all cases, the roots and rhizoids grew downward, regardless as to whether this was into the agar or into the moist air. This suggested that rhizoid formation was possibly controlled by a hormone that became sufficiently concentrated in the ventral position to initiate cell-changes leading to rhizoid formation.

At this point Mr. Main had to discontinue his work on this problem. The author continued further work as time permitted. Since the evidence obtained thus far suggested a hormone which might be moving due to graviational force to the ventral region of the gametophytes, a method was sought which would prevent such concentration, were it occurring. Several flasks of tap water were prepared. In some, sporocarps were dehisced and the gametophytes were permitted to develop overnight, which was sufficient time (sixteen hours) to permit maturation of the gametophytes and fertilization of the eggs. These were then placed on a shaker. In other cases the sporocarps were dehisced in flasks and immediately placed on shakers. Several control flasks were also prepared and used in making observations to detect the appearance of rhizoids. The work was carried on under constant illumination at thirty-six degrees centigrade.

When rhizoids were observed on the controls, the experimental flasks were removed from the shaker and their contents were examined. The megagametophytes which were not placed on the shaker until sixteen hours after dehiscence of the sporocarps showed fairly normal sporophytes and numerous rhizoids, usually in the expected position. The embryo sporophytes were often curved and twisted. In the flasks in which the sporocarps did not open until the flasks had been mounted on the shaker, several differences were noted. Most of the eggs in the megagametophytes had not been fertilized, hence the megagametophytes did not contain embryos. This was probably due to the disturbance of diffusion patterns of chemotactic substances produced in the archegonia or to the inability of sperm to enter the neck canal due to the constant motion of the liquid. Rhizoids were observed completely around the megagametophyte in an extensive region next to the megaspore wall. Many of the megagametophytes showed some abnormality in shape and some gave evidence of extended gametophyte development beyond that usually noted in plants not containing embryos. The extensive development of rhizoids on plants kept in continual motion to prevent concentration of a postulated hormone subject to gravitational forces lends support to a theoretical hormone control of rhizoid formation, in the megagametophytes of the Marsileaceae. The appearance of rhizoids in the usual position on the megagametophytes in flasks not shaken during the first sixteen hours following dehiscence of the sporocarps indicates that rhizoid formation is initiated during these first sixteen hours of gametophyte development.