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COMPARATIVE VIABILITY OF SPOROCARPS OF *MARSILEA QUADRIFOLIA* L. IN RELATION TO AGE[1]

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The Marsileaceae are a small group of water ferns distinguishable from all other Leptosporangiatae but the Salviniaceae by their heterospory. In contrast to the Salviniaceae, the sporocarps of the Marsileaceae contain both microspores and megaspores. The morphology of the Marsileaceae is discussed rather fully by Bower (3), Eames (6), and Smith (7), and in a number of individual reports dating back to the late nineteenth century.

One of the most unusual characteristics of this group of ferns is their mode of sexual reproduction. Both microspores and megaspores occur in each sorus and are produced in a special organ, the sporocarp. These closed more-or-less globular structures are considered phylogenetically to be leaflets composed of many pinnules. When mature they possess a hard, resistant outer coat which serves to protect the spores against mechanical injury, desiccation, and unfavorable substances to which they might be exposed.

There is considerable interest in the sporocarps of the Marsileaceae because of their apparent resistance to the effect of aging upon the viability of the spores. As early as 1888 Campbell (4) conducted germination tests on *Marsilea aegyptiaca* and found that after five years there was little decline in viability, and sporocarps 11-years old were 50 percent viable. Chamberlain (5) describes germination of poisoned herbarium specimens 50 years old and states that sporocarps in 95 percent alcohol germinate as quickly as those in a dry box. Eames (6) reports that sporocarps taken from herbarium sheets 50 years old have germinated, and Smith (7) reports that spores remain viable 20 or 30 years. Allsopp (1) has recently germinated sporocarps of *Marsilea minuta* 68 years old and *M. fournieri* 61½ years old and found that they showed little loss of viability and produced typical embryo sporophytes. Bloom (2) has shown that in fertile sporocarps of *M. quadrifolia* 30 years old, better than 98 percent of the megaspores produced embryo sporophytes.

Unfortunately, the term *germination* is somewhat ambiguous when used in describing one stage in the reproductive cycle. Expansion causes a rupturing of the sporocarp when water is imbibed. In some species sori contained in the sporocarp are pulled out by means of a greatly expanded gelatinous ring, but in other species the individual spores are eventually freed from the sporocarp or may remain within the sporocarp walls. This phenomenon is commonly referred to as germination although it is purely physical

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and may occur even though none of the spores within the sporocarp are viable. Sporocarps that have been heat-treated to the extent that all spores are killed still "germinate" as have sporocarps stored in higher alcohols, although viable plants were not produced. Apparently some of the earlier reports on germination refer only to this physical phenomenon.

Following germination of the sporocarps in water, or in nutrients of suitable concentration, the gametophytes develop rapidly and the megagametophyte can readily be seen with low magnifications (X10). Slightly higher magnifications (X35) are necessary to see the ruptured microspores which serve as evidence of functional microgametophytes. At magnifications of 100 to 440 diameters, typical motile sperm cells can be seen easily. Under favorable conditions the gametophytes develop within 24 hours, and within 48 hours small sporophytes are distinguishable.

Materials and Methods

Most of the reports referred to previously are based on the germination of one or several sporocarps, without any means of checking the results against younger sporocarps of the same ecotype. Allsopp (1) calls attention to the fact that negative results based on a single or several sporocarps are not conclusive, as it frequently happens that relatively young sporocarps fail to germinate or to produce typical sporophytes. I have been fortunate to obtain from Dr. Paul D. Voth, Department of Botany, University of Chicago, and Dr. Ernest L. Stover, Department of Botany, Eastern Illinois State College, three collections of sporocarps from plants of *Marsilea quadrifolia* growing on the Charleston campus.

The oldest sporocarps were collected in 1922 by the late Miss Ethel Thomas of the Charleston faculty. This material was stored in a cardboard box; for some time it was in the basement of the Hull Botanical Laboratories, University of Chicago. Conditions under which the sporocarps *were* collected are unknown, and the percentage of germination or fertility at the time of collection is not on record. The second collection was made several years ago by a student of Dr. Stover, and I made the most recent collection in the fall of 1953.

To determine whether the spores in the sporocarps were viable, and to what extent they might have deteriorated in storage, the following studies on germination were conducted. Ten sporocarps from the first two collections, and 10 sporocarps from the 1953 collection which had been hastened in maturation by heating at 65°C. in a forced draft oven, were used. Each sporocarp was scarified on emery paper and placed in a separate vial 2 cm. by 8 cm. in 20 ml. of Voth (8) no. 5 solution. Periodic observations were made and data recorded. After 10 days the megagametophytes with attached sporophytes, megagametophytes without sporophytes, and undeveloped megaspores were counted. The results are shown in tables 1, 2, and 3.

None of the sporocarps collected in November 1953 showed typical germination by January 24, 1954,

TABLE 1.—GERMINATION RESULTS OF SPOROCARPS OF *Marsilea quadrifolia*. THOMAS MATERIAL, 32 YEARS OLD.

No. of sporocarps	Germination	Megaspore failure	Megagametophytes without sporophytes	Megagametophytes with attached sporophytes	Percentage of viable megaspores
1	+	all*	0	0	0.0
2	+	0	0	74	100.0
3	+	0	3	81	100.0
4	+	0	5	81	100.0
5	+	0	77***	0	100.0
6	+	0	1	72	100.0
7	+	all*	0	0	0.0
8	+	0	0	96	100.0
9	+	0	4	83	100.0
10	+	1	30	60	98.9
Totals	10+	1**	120	547	99.8**

* Spores remained in sori; no development was apparent; spores were not counted.
** Exclusive of nos. 1 and 7.
*** No sperms were detected.

TABLE 2.—GERMINATION RESULTS OF SPOROCARPS OF *Marsilea quadrifolia*. STOVER MATERIAL; RECENT COLLECTION; NATURAL MATURATION.

No. of sporocarps	Germination	Megaspore failure	Megagametophytes without sporophytes	Megagametophytes with attached sporophytes	Percentage of viable megaspores
1	+	2	7	52	96.7
2	+	2	1	84	97.7
3	+	0	2	83	100.0
4	+	0	3	54	100.0
5	+	all*	0	0	0.0
6	+	8	6	70	90.5
7	+	0	0	79	100.0
8	+	0	0	85	100.0
9	+	0	0	88	100.0
10	+	0	5	84	100.0
Totals	10+	12**	24	679	98.3**

* Spores remained in sori; no development was apparent; spores were not counted.
** Exclusive of no. 5.

unless treated to hasten maturation. Of the 10 untreated sporocarps of the 1953 collection, 4 remained closed, 3 developed partially extruded gelatinous rings and freed some spores, and the remaining 3 showed slight extrusions at the sites of the openings made in the sporocarp walls, and eventually some spores were freed. Some megaspores from 5 of the sporocarps developed megagametophytes and typical sporophytes.

Discussion

Allsopp (1) has pointed out that sporocarps of all ages may show complete failure to germinate or produce typical sporophytes. His observations are confirmed in this and other studies. Since all three collections showed such failures, it is unlikely that failure of the Thomas material was attributable to any effect of aging; it was probably the result of some developmental failure during the development of the sporocarps prior to harvesting.

Preliminary experiments with various concentrations of nutrient solutions used by Voth (8) for *Marchantia polymorpha,* tap water and deionized distilled water have also resulted in some failure of spores from the sporocarps to develop into normal gametophytes with the production of sporophytes. Germination in deionized distilled water resulted in a number of ruptured megaspores owing to increased osmotic pressure. Very low concentrations of nutrient solutions at higher temperatures produced similar rupturing of the megaspores. Residual chlorine or other toxic substances in tap water may be harmful, especially to the sperms.

Not infrequently, as in no. 5 of the Thomas material and no. 6 of the heat-treated fresh material, megaspores develop normal gametophytes but will not produce sporophytes.

TABLE 3.—GERMINATION RESULTS OF SPOROCARPS OF *Marsilea quadrifolia.* 1953 COLLECTION; HEAT TREATED TO HASTEN MATURATION.

No. of sporocarps	Germination	Megaspore failure	Megagametophytes without sporophytes	Megagametophytes with attached sporophytes	Percentage of viable megaspores
1	+	3	41	43	96.6
2	+	5	27	43	93.3
3	0	all*	0	0	0.0
4	0	all*	0	0	0.0
5	+	9	33	43	89.4
6	+	2	62***	0	96.9
7	+	29	9	20	50.0
8	+	3	7	56	95.5
9	+	21	38	18	72.7
10	0	all*	0	0	0.0
Totals	7+	72**	217	223	85.9**

* Spores remained in sori in sporocarp; no development was apparent; spores were not counted.
** Exclusive of nos. 3, 4, and 10.
*** No sperms were detected.

Acetocarmine preparations of such materials failed to reveal the presence of typical sperm cells always associated with material of the same age which produced sporophytes. This suggests that the microspores are more susceptible to aging or physical agents used to hasten maturation. The failure of seemingly normal gametophytes to produce embryos in this and other experiments in progress raises some question as to the reports of frequent parthenogenetic development in this group of plants and suggests the need for further study.

The exceedingly high percentage of viable megaspores in the 32-year-old material, which even exceeded that of the more recent mature material (the difference is not significant however, since it lies within the range of chance variation) indicates that the spore longevity in this group of plants is unique. There are undoubtedly sporocarps available on herbarium sheets that are more than 100 years old, and it would be of interest to see how such material would respond in germination tests.

Counts were made of the number of sori in the Thomas and Stover material. The number of sori varied from 12 to 17, with 9 showing a count of 16. The number of megaspores in a sporocarp varied from 57 to 96, with the average for 24 sporocarps being 79. These variations are attributable, at least in part, to aborted sori and spores.

Summary

1. Sporocarps of all ages show some loss of viability, probably due to failures in development and maturation.
2. Sporocarps that contain viable spores show a high percentage of megaspore viability, usually 100 percent after 32 years.
3. Microspores appear to be more susceptible to aging effects than megaspores.
4. The number of sori in a sporocarp varied from 12 to 17, and the number of megaspores in a sporocarp varied from 57 to 96.

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