Effect of Aging on the Viability of Sporocarps of *Marsilea quadrifolia*[1]

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The Marsileaceae is 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 (1), Eames (2), and Smith (3) and in a number of individual reports dating back to the late nineteenth century.

One of the more unusual characteristics of this group of ferns is their mode of sexual reproduction. Both megaspores and microspores occur in each sorus and are produced in a special organ, the sporocarp. These closed, more or less globular structures are developed from leaves or leaf segments. When mature they possess a hard, resistent outer coat which serves to protect the spores against mechanical injury, desiccation and some 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. Eames (2) reports that sporocarps taken from herbarium sheets 50 years old have "germinated" and Smith (3) reports that spores remain viable for 20 or 30 years.

The successful sexual reproduction of the Marsileaceae depends on several successive steps. When placed in water, especially if the hard outer coat is ground or filed, water is imbibed, resulting in expansion and rupturing of the sporocarp. In some species the sori contained in the sporocarp are pulled out into the water 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 and may occur even though none of the spores contained in the sporocarp are viable. It is evident that some of the reports on germination represent an observation of this first stage only.

Following germination the spores develop into gametophytes rapidly, the rate being partially controlled by temperature, and varies considerably for different species. Generally 24 to 48 hours are sufficient for the complete initiation and maturation of the gametophytes. This development can be readily detected under low magnifications of the microscope. In mature microgametophytes the motile sperms are readily detected and the ruptured microspore walls are in evidence. The megagametophyte can be recognized as the cushion of green cells at the site of what was a small

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TABLE I. Sporocarp germination, gametophyte and functional embryo sporophyte formation.

No. of sporocarp	Germination	Microgametophyte development	Megagametophyte development	Functional embryo formation
1	+	+	+	+
2	+	+	+	+
3	+	0	0	0
4	+	+	+	+
5	+	+	+	+
6	+	0	0	0
7	+	0	0	0
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+

TABLE II. Viability of megaspores as shown by the development of embryo sporophytes.

No. of sporocarp	No. of viable megaspores	No. of non-viable megaspores	Percentage of viability
1	105	0	100.00
2	98	3	97.03
3	0	87	0.00
4	75	0	100.00
5	93	1	98.94
6	0	91	0.00
7	0	69	0.00
8	57	1	98.27
9	73	3	96.05
10	78	2	97.50

protuberance at the anterior pole of the megaspore. The neck-cells of the single archegonium are usually discernable.

A collection of sporocarps of *Marsilea quadrifolia L.* made by the late Miss Ethel Thomas of the Department of Botany of Eastern Illinois State College at Charleston, Illinois, was furnished the writer by Dr. Paul D. Voth of the Department of Botany of The University of Chicago. These sporocarps had been collected in 1922 and preserved dry in a small cardboard box. For at least a part of the 30 years since they were collected they were stored in the humid basement of the Hull Botanical Laboratories at The University of Chicago. Conditions under which they were collected are unknown and the percentage of germination or fertility at the time of collection was not determined.

In order to determine whether the spores in the sporocarps were still viable and to what extent they might have deteriorated in storage, 10 selected sporocarps were rubbed on fine emery paper to hasten germination and placed in individual glass vials, each 2 cm. by 8 cm. and containing 22 ml. of tap water. Observations were made daily and the results recorded. The results are shown in Tables I and II. The opening of the sporocarps with the development of the gelatinous ring was taken as a criterion of germination. The presence of opened microspores and of motile sperms was taken as evidence of viable microspores. The development of the megagametophyte and subsequent formation of an embryo was taken as evidence of a viable megaspore.

Discussion

Several facts are noted from an examination of the results shown in Tables I and II. In numbers 3, 6 and 7 all the megaspores and apparently the microspores are 100% non-viable. It is impossible to attribute this to conditions peculiar to them at the time of harvesting or to an effect of aging. Judging from the completeness of non-viability, the former would seem to be most likely, especially when compared with the high percentages of viability in the other sporocarps. The behavior and appearance of the megaspores also suggests this. In no. 3 most of the megaspores remained within the sori and had a deflated appearance. The megaspores in no. 7 had a peculiar structure extending out from the anterior portion of the megaspore and the spores hung down rather than assuming a horizontal position. The megaspores in no. 7 remained within the sori.

Of the seven sporocarps showing viability, 579 megaspores were viable and developed functional gametophytes resulting in the formation of embryo sporophytes as compared to 10 non-viable megaspores. For these seven sporocarps a decline in fertility at most was 1.7% in 30 years. It will be noted that this is a much smaller decline than is shown by the seeds of most spermatophytes and probably exceeds that of even the more resistant bacterial endospores.

It is interesting to note the wide variation in the number of megaspores that developed in various sporocarps. Number 1 had a total of 105 megaspores while number 8 had only 58.

Summary

1. Germination of sporocarps of *Marsilea quadrifolia L.* is a physical phenomenon and is independent of viability of the spores.
2. A total of 589 megaspores of *Marsilea quadrifolia L.* 30 years old showed a loss in viability of less than 2%.

Literature Cited

1. BOWER, F. O. 1926. The Ferns, Vol. II. Cambridge University Press. London.
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3. SMITH, G. M. 1938. Cryptogamic Botany, Vol. II. McGraw-Hill Book Co., Inc. New York.