A Study of Sleep Movements in the Genus *Marsilea*

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Abstract

Studies of young sporophyte plants of *Marslea mucronata* indicate that the first bifid leaves are capable of sleep movements. A mechanism for the sleep movements in the Marsileacease is proposed.

Many plants, notably *Mimosa pudica* and *Oxalis* (4), exhibit the so-called "sleep movements" which are thought to be the result of reversible changes of turgor pressure in certain of the plant cells. The movements may be initiated in several ways, although the mechanism for causing the turgor changes is not yet clearly understood. There are a few references to the "sleep movements" in the *Marsilea* in the literature (1, 2). In *Marsilea* the mature leaves are extended in a plane at right angles to the sun's rays during the day, but they assume a folded position with the four leaflets together, the basal pair on either side of the distal pair, at night.

To determine any difference in the time required for opening and closing at different times of the day, three pots of *Marsilea mucronata* were kept in the greenhouse and six healthy leaves at different stages of maturity were labeled in each as references. At each hour between 8 AM and 5 PM a pot was placed in the dark for one hour and then brought back into the light and the time required for the labeled leaves to open completely was noted and recorded. An hour of darkness was sufficient to cause the leaves to close completely during the morning and late afternoon, and they opened again in an average of 20 to 25 minutes in the morning and 15 to 20 minutes in the afternoon. Timing was complicated by the fact that in full sun and high afternoon temperatures the leaves often assumed a partially folded position for several hours. In this case one hour in the dark caused little additional closure and upon being readmitted to light the leaves opened rapidly, often in less than ten minutes; or they did not open at all until later in the afternoon.

Marsilea quadrifolia sporelings were used to determine the stage in development at which the sleep movements first occur. To secure sporelings a sporocarp was scarified and placed in a vial with 30 ml. of tap water. Development of the gametophytes occurred rapidly and numerous sporelings were available within a week. These were then transferred to soil kept moist with a constant watering device. In the development of the young plant there are first two to four spatulate leaves followed by two to four bifid leaves, in turn followed by the first quadrifid leaf. There are many variations in the amount of indentation of the spatulate leaves and of the completeness of division of the bifid leaves. There is also an occasional trifid leaf. It is clearly seen by observing the young

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sporelings that the bifid leaves do close in a manner similar to the quadrifid leaves of the mature plant. Some variation exists, however. In some the second bifid leaf, or the first to be completely divided, is the first to react to a change in light; while in others even an incompletely divided leaf showed some degree of closure. Documentation of the movement in the young leaves was done photographically showing the entire young plant with its bifid leaves closed, and comparing open and closed leaves removed from the plant (Fig. 1).

Both quadrifid leaves and bifid leaves were sectioned and stained in an attempt to determine the tissue responsible for the leaf movements. Those portions of the adult leaflets that are attached to the petiole and the entire bifid leaves were fixed in either Bouin's or Formalin and embedded in paraffin according to general fixing and embedding schedules and procedures. Both longitudinal and cross sections were made at ten microns, and stained with a safranin and fast green combination (5).

In some plants the turgor movements are reported to result from changes in certain thin-walled cells of the pulvini. When these cells are evenly distended the leaf is supported, but unequal turgor pressure on one side of the pulvinus causes the petiole to move toward the side with the reduced pressure (4). *Marsilea* does not seem to possess this pulvinus-type arrangement. Examination of the sections revealed an area of specialized cells in and sometimes adjacent to the upper epidermis, near the junction of lamina and petiole (Figs. 2 and 3). These specialized cells stain more densely with safranin and contain many safranin-stained granules which also occur in large numbers near the vascular bundles. Similar specialized cells occur in the petiole near the point of attachment of the leaflets.

This finding suggests a possible explanation for the "sleep movements" in *Marsilea* similar to that cited by Steward (6) for the behavior of the guard cells of stomata. Guard cells are thought to respond to light with a starch to sugar conversion and an increase in turgor pressure resulting in a change in shape and volume. Steward suggests that the starch is converted to glucose-1-phosphate and finally to glucose and free phosphate. These reactions would occur in light as carbon dioxide is used in photosynthesis and the pH of the cell rises favoring the catalytic action of phosphorylase in converting starch to sugar. In this scheme the conversion back to glucose-1-phosphate would require energy and the hexokinase enzyme system, and would require oxidation. Stomatal closure would depend on energy and respiration but opening in light would occur as a result of photosynthesis. If the specialized upper epidermal cells in *Marsilea* decrease in size and lose turgor pressure due to loss of water as sugar is converted to starch in the dark, the greater turgor pressure of cells opposite these specialized cells would cause the leaflets to close. In light a conversion of starch to sugar would increase the sugar concentration and raise the turgor pressure in the specialized cells to a higher level than that in adjacent cells, and the leaflets would open. Further studies to test this hypothesis as the cause of the "sleep movements" in *Marsilea* are in progress.

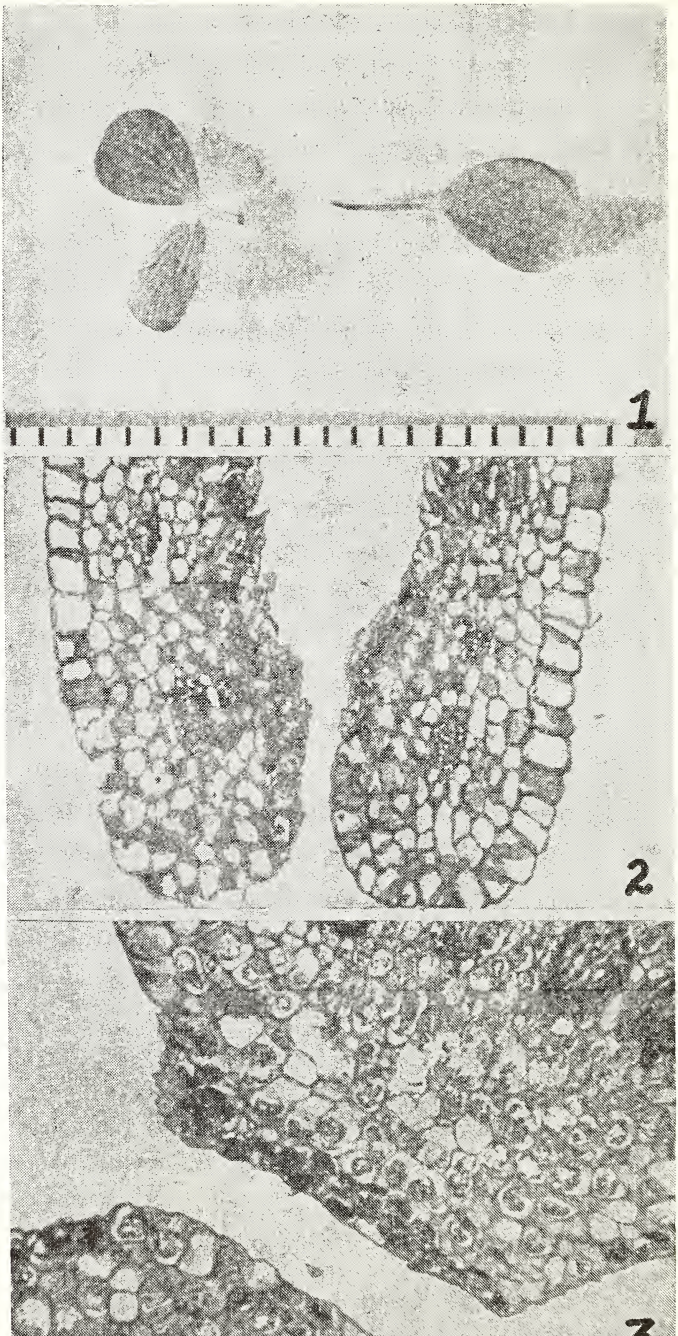
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Figure 1. Bifid leaves from young plants of *Marsilea quadrifolia* in the open (left) and closed (right) positions.

Figure 2. Longitudinal section of a bifid leaf of *Marsilea quadrifolia* in the closed position. Note the dense protoplasts of the cells of the upper epidermis on the facing surfaces in the center of the figure in contrast to the rest of the cells, especially the lower epidermis at the exereme left and right of the section.

Figure 3. Cross section of a mature quadrifid leaf of *Marsilea mucronta* in the open position. Only a portion of one leaflet (upper right) and the petiole (lower left) just above the point of attachment of the leaflet is shown. Note the dense protoplasts of the upper epidermis and adjacent cells (area nearest the petiole) in contrast to the rest of the cells.

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