Notes on the Physiology of the Sporophyte of Funaria and of Mnium.

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Introductory.

The results presented in these notes are the outcome of experiments performed by the writer at the University of Wisconsin a number of years ago and supplemented by other studies of more recent date.

Although the curvature of the seta of many common mosses and the unsymetric growth of the capsules of not a few others are matters of common observation, students of plant physiology appear to have given but very little attention to the phenomenon. But one author, Wichura (1), seems to have given the subject close study and his conclusions were reached without any considerable amount of experimentation. By observing the conduct of developing sporophytes of *Bryum argenteum* L. with reference to the direction of the strongest illumination when placed in various positions, Wichura came to the conclusion that the position assumed by the capsules of this species is due to the influence of the direction of the incident light rays. After a few experiments with *Bryum argenteum*. he ventured the conclusion that the direction assumed by the shoots and leaves of *Fissidens*, the secund *Hypna*. *Dicrana* and others is likewise determined by their relation to the direction and intensity of illumination. A marked sensitiveness of the sporophyte to light is also asserted. The possible influence of still other factors in shaping the development of the mosses seems not to have occurred to this author.

Goebel (2), however, points out that our knowledge of the influence of factors of the environment on the development of the mosses is in a very unsatisfactory state.

Experimental.

I have given attention especially to the development of the sporophyte of two of our commoner species, *Funaria hygrometrica* Sibth., characteristically found in exposed, sunny spots, and *Mnium cuspidatum* Hedw., one of the shade-loving mosses of the woods. In its mature form, the capsule of *Funaria* is bilaterally symmetrical and decidedly arcuate, being convex in its upper outline, concave beneath. In addition to this curvature of the capsule, the seta shows a very pronounced bend some distance below the capsule. The capsule of *Mnium cuspidatum* is nearly radially symmetrical and takes a pendant position by means of a sharp curvature of the seta formed very the capsule.

Early in April, turfs of both species were transplanted into pots and removed to the laboratory for study. The general course of development is much the same in both species. Following the fertilization of the egg, both the archegonium and the young sporophyte begin growth, the latter as a slender body enclosed in the rapidly enlarging archegonium. At this stage the tip of the calyptra may just be seen among the perichaetical leaves. Soon the growth of the archegonium ceases, and this organ is torn away from its attachment by the elongation of the sporophyte enclosed within it. Goebel (3) suggests that the line at which the archegonium separates from the receptacle is marked by a zone of modified, weakened tissues to facilitate the rupture. In a number of instances, specimens of *Funaria* were seen in which the calyptra failed to separate as usual at the base. and the sporophyte pierced it, growing up through it in a manner recalling the Hepaticas. This would seem to indicate that occasionally the specialized, weakened zone may still fail to tear with the required readiness. The sporophyte continues to grow in length for about a fortnight under laboratory conditions. keeping an erect position. Finally, as the seta reaches nearly the normal length, the curvature begins to appear at the base of the calyptra. Before the curvature of the seta is complete, but usually not until it is well under way, the rudimentary capsule can be detected as a small swelling just below the distal extremity of the sporophyte. This structure now enlarges rapidly while growth in the seta rapidly diminishes and soon altogether ceases. The calyptra is finally ruptured by the expansion of the growing capsule and falls away. The capsule of necessity retains permanently the position assumed at the time the narrow, elongating zone below the capsular rudiment loses the power of growth.

It was first necessary to become oriented in regard to the rate and distribution of growth in the sporophyte at the different stages of its development. For this purpose, *Funaria* was given most study since it is known to bear hard treatment more easily than many other species. India ink dots were placed at intervals along the sporophytes and the intervening distances were measured from time to time. Since, even when carefully carried out, the small size of the objects under study and the interference by the calyptra stood in the way of the highest accuracy, only an approximation to the true relations was obtained. I believe, however, that the chief features of the rate and the distribution of growth were distinctly to be seen. No attempt was made to ascertain the growth rate in the stages prior to the emergence of the tip of the archegonium from among the perichaetical leaves.

Measurements initiated as soon as the appearance of the growing archegonium above the perichaetium would permit indicated that the growth rate was very slow, about 0,13 mm. in twenty-four hours in a temperature ranging between 20 ⁰ C. and 25 ⁰ C. Perhaps this slowness was in part due to the restraining action of the archegonium which was still attached to the gametophyte and probably not keeping pace in growth rate with the included sporophyte. At all events, as soon as the archegonium was torn loose at its base, a rapid increase in the growth rate of the sporphyte was observed. When the edge of the calyptra presented itself above the perichaetium, the growth rate seen to have risen from 0,2 to to 2,0 2,5 mm in twenty-four hours. Subsequent measuremeuts showed that this rate of elongation was slowly and steadily increased until the sporophytes reached a total length of from 10 to 12 mm, the stage preceding the first indication of the curvature. As the curvature became more pronounced, the growth rate fell off rapidly, and when, in about forty-eight hours, the rudimentary capsule became distinguishable, growth of the seta sank to nearly zero and, as the growing capsule became increasingly prominent, soon ceased altogether. The growth made during this period of declining elongation in the seta was unequally distributed and, being more rapid on the convex side of the seta, served to bring about the curvature noted. That this curvature was a permanent feature connected with growth, rather than a result of unequal turgor pressure, appeared on placing the young sporophytes in plasmolizing solutions. No changes in form was seen to accompany the loss of osmotic pressure.

Thus the growth curve in its chief features is plainly not widely different from that characteristic of many other plant structures that have been studied. The regularity of the ascent during the earlier stages it somewhat interefered with by the mechanical restraint prior to the breaking away of the archegonium from the gametophyte. The elongation of the seta is completed in about ten days from the appearance of the calyptra. Experiments were made for the purpose of ascertaining the location and extent of the growing zone. By observing the movement of the edge of the calyptra from dots placed near it on the seta, it soon appeared that the entire zone of elongation was situated inside the calyptra. Further experiments showed that in all stages of growth open to study this was true. Accordingly, to more accurately define this zone, it was necessary te remove the calyptra. After a number of failures due to the great delicacy of the tissues in the end of the young sporophyte, it was found possible to remove this structure and fix dots of India ink at close intervals along the apical region. Measurements made at intervals of twenty-four hours thereafter showed that the zone of elongation was limited to a space within 2 mm from the apex, most rapid growth being found about 0,8 mm from the tip. The young seta was found to have reached its complete diameter at any given point as soon as its growth in length was completed. The diameter near the base averages about 0,15 mm and about 0,14 mm near the base of the capsule.

In a number of young sporophytes from which the calyptrae had been removed, sharp curvatures appeared, resembling very strongly the traumatropic curves seen in roots (4). These curvatures were probably due to slight unavoidable injuries inflicted in removing the calyptrae. To slightly inflict a wound near the apex was found to produce similar results in erect sporophytes from which the calyptrae had been successfully removed. Decapitation experiments seemed to show that the perceptive region lies in the immediate apex of the sporophyte.

Having traced roughly the course of the elongation of the sporophyte and its distribution as seen in *Funaria*, attention was directed toward determining the influences bringing about the well-known curvature of the seta seen in *Funaria* and of *Mnium*.

Mnium showed itself very sensitive to conditions of light in its natural habitat as indicated by the uniformity with which the individual sporophytes of a turf assume their positions with regard to their environment. Since *Funaria* shows itself to be less acurately directed, *Mnium* was selected for these experiments. Pot cultures were exposed in various positions to the action of gravity and light in the hope of ascertaining whether light is the sole directive influence, as indicated by Wichura.

On April 11 at 11 : 45 a. m. a pot of *Mnium* having 30 young, erect sporophytes was placed in a dark box in such a manner that the perpendicular sporophytes were subjected to lateral illumination through a hole two inches wide by four inches long at the level of the culture. The pox was placed near a south window in strong diffused light.

Twenty-four hours later, eight of the sporophytes were inclined about 20 ° from the perdendicular toward the source of light, by means of a bend within or at the base of the calyptra.

After another twenty-four hours, fifteen individuals had made similar curves toward the light. On April 16, about 120 hours from the beginning of the experiment, all but three showed plain inclination. It was noted that in every case, the more advanced sporophytes performed this reaction, indicating that for a time, the young sporophytes are not very sensitive to light, becoming increasingly so until they react through a distinct change in the distribution of growth, resulting in the inclination seen.

A *Mnium* culture in which only erect sporophytes were seen was placed on April 11, at 12 : 00 m, in a horizontal position and illuminated with light reflected from the sky in a direction parallel with the long axes of the sporophytes. After 20 hours several showed slight bends near the tips, in different directions, some upwards, two laterally, others downwards. On April 13, five out of twenty-two individuals were inclined downwards, three upwards, two laterally, the remainder maintaining the horizontal position, presumably not yet having reached that stage in their development at which they became sensitive to gravity. On April 16, eight showed a downward curvature, three pointed obliquely downward, two pointed upward, nine were still horizontal. Three had become capable of perception and motion since April 13, and had responded by a downward curvature. On April 17, ten curved downward, the two laterally directed individuals had changed position so as to point downward, two still pointed upward. On April 19, all had taken a position in which the sporophytes pointed downward.

From this experiment, it appears that for a time the young sporophytes are not perceptive to gravity and maintain for days in a horizontal position an „Eigenrichtung" derived possibly from the relation to the sporophyte. As they become capable of perception and reaction, in spite of illumination parallel with their long axes, the rudiments respond by curvatures directing the apices toward the earth.

Thus it appears probable that *Mnium* sporophytes take on the curvature characteristically made in response to geotropic induction.

In the hope of getting further light on the question of the part played by gravitation and light, a culture of *Mnium* was placed in a perpendicular position, illuminated from above by diffused light.

On April 12, all sporophytes were perpendicular, on April 13, thirteen out of thirty showed a distinct curvature near the base of the calyptra. On April 15, fifteen were still erect, the others showing curvatures varying from a few degrees to as high as 90° from the perpendicular. On April 18, all were more or less inclined, and in various radii, indicating no marked directive influence. Here, as bevore, a period of inability to perceive or react is followed by perception and reaction.

From the experiments sketched here in scant outline, it appears probable that the curvature of the seta in the species is due to a growth reaction on the part of the young sporophyte to the stimulus of gravity. It appears probable, also, in view of the directive influence of lateral illumination and the absence of uniformity in symmetric illumination, that the radius in which the sporophyte falls in carrying out this curvature is determined by the direction of the strongest illumination.

In the hope of getting further light on the question, several attempts were made to carry through to maturity cultures in a horizontal position on the klinostat, but unfortunately I was balked each time by the occasional failure of the apparatus to perform its duty. In spite of these discouraging features, a pot of *Mnium* capsules was obtained in which, instead of the usual pendulous position, an average inclination of 30 ^{0} from the perpendicular was seen at maturity.

Experiments with *Funaria* gave results very similar to those above described. The *Funaria* sporophytes showed themselves more quickly responsive to lateral illumination than *Mnium*. An unknown species of *Webera*, found on a somewhat exposed hillside, was more sensitive to the directive influence of light than either *Funaria* or *Mnium*.

In view of the partial failure of the klinostat experiments, it was deemed desirable to supplement the observations described with some further evidence to show the relative effectiveness of light and gravity in calling forth responses in the sporophyte, and the following studies were made in the spring of 1901, ad Harvard University.

Two experiments were carried out with *Mnium cuspidatum*, a form found in great abundance in the open woods near Arlington Heights. Colonies of this moss were transplanted March 22 with the least possidisturbance to flower pots filled with woodsoil and taken to the greenhouse laboratory at the Botanical Garden. The sporophytes had already made considerable progress but were still erect and showed no indication of a capsular enlargement.

In the first experiment, the desire was to ascertain the behaviour of the growing sporophytes when placed in a horizontal position with perpendicular illumination. These conditions were obtained by sinking the pot containing the *Mnium* culture on its side in a larger pot filled with sphagnum which was held away from the plants by another flower pot placed with its mouth over the open end of that containing the moss. Light was admitted by breaking out a piece of the empty pot 1 inch wide by about 3—12 inches long. After placing the moss tuft in the position described, the containing pot was put in bright diffused light. After forty-eight hours, nearly all of the sporophytes had made a more or less marked curvature upwards toward the light. This curvature was some distance from the free end of the sporophyte and seemed to be clearly located in its growing zone. Another curvature much sharper and more localized was also noted, especially in the more advanced individuals.

immediately below the calyptra or perhaps partly within it. This second curvature was, like the first, in the plane of the incident rays, but in the opposite direction, depressing the tip of the sporophyte. In many cases, the capsular rudiment was maintained by those two exposed curvatures in an approximately horizontal position. In the more advanced cases, the ends of the sporophytes were pointed nearly downward, that is, away from the light.

Twenty-four hours later, the long upward curve of the setal portion was somewhat more marked, and in the older individuals in which the capsular rudiments were furthest advanced, and constituted a very well developed, narrowly elliptical body, the sharp curve just below the calyptra had so far progressed as to bring the capsular rudiments into a nearly perpendicular position. In the younger sporophytes the general upward curve brought the distal ends of the sporophytes into a nearly perpendicular position. Immediately below the calyptra no curve was seen.

On March 29, about 170 hours after the beginning of the experiment, every capsular rudiment, now in most cases about one-half the mature size, hung in a perpendicular position through a sharp curve immediately at the base, near the edge of the calyptra. This curvature, in the plane of the incident rays, directed the apex of the rudiment away from the light, toward the center of the earth. The general upward curve was found to have remained as before.

On April 1, about 240 hours after the beginning of the experiment, the capsules, normal and nearly full sized, were seen in every case to have the sperculum directed downward. The general setal curve had been made permanent by the internal tissue development.

Further observations showed that normal capsules developed without any further changes in position.

From the data described, it seems justifiable to draw the following conclusions: The young sporophyte, prior to the development of a distinct capsular rudiment, is either positively heliotropic or negatively geotropic, and tends to assume a perpendicular position which is fixed by the further development of the tissues. When in the young sporophyte a capsular rudiment has begun to develop, this setal curvature is not marked, possibly because of the rigidity of the seta through the development of mechanical tissue. A sharp curvature appears just below the capsule, turning the capsular rudiment into the perpendicular position as a result of either a positively geotropic or of a negatively heliotropic reaction. It appears that these two opposed responses seen in the two curvatures can for a time take place synchronously. The question of the part played by light and by gravity is, however, not settled.

In the second experiment, an attempt was made to make this clear. On March 27, a healthy culture of *Mnium cuspidatum* was potted and packed as above described and placed with the sporophytes in a horizontal position. Illumination from below was secured by means of a mirror placed about 8 inches below the culture which reflected light from the eastern sky upon the horizontal sporophytes. Light was admitted through an opening 1—1/4 inches wide and 2 inches long, made by breaking a place in the pot opposite the sporophytes. Except in the forenoon, when some direct sunlight was reflected upon the plants, strong diffused light was supplied.

On March 29, the young sporophytes in many cases showed a slight gradual bend in the distal half in an upward direction, in a manner not markedly different from that seen in the preceding experiment. At the distal end a slight downward direction is noticeable, brought about by a curvature of smaller radius near the lower edge of the calyptra. In a few cases, the sporophytes were practically horizontal except at the distal end, where a downward curve was seen.

On April 1, no increased curvature in the setal was noticed. A sharp downward curvature at the lower edge of the calyptra, however, was conspicuous, bringing the now clearly enlarged capsular rudiments into a position approaching the perpendicular. With but three exceptions in about thirty-five individuals, the tips of the calyptrae pointed almost directly downwards, therefore, against the incident light rays.

On April 10, the capsules were found to have developed normally and were approaching their full size. In every case, the long axis of the capsule was perpendicularly directed, and stood at approximately a right angle to the axis of the seta. The plants seemed to be entirely healthy.

The experiments above described seem to make it clear that in the case of *Mnium*, and probably of *Funaria* also, the „nodding“ of the capsules is brought about by the stimulating action of gravity, since the direction of the illumination does not interfere with the tendency of the capsules to assume the „nodding“ or, in the case of *Mnium*, the pendulous position, seen in nature. The partial success of the klinostat experiments points in the same direction.

The directive influence of illumination is clearly marked in determining the plane in which the capsular rudiment shall fall. Sometimes the apex of the capsule falls toward the source of light, sometimes against it. This conclusion has been tested many times by the study of *Mnium* in its usual habitat, and, with very rare exceptions, it is possible to parallel the results seen in the laboratory. Occasional exceptions may be accounted for by considering the foliage conditions and the position of the sun in the heavens during the day at the time of the year when the sporophytes are developing. Various obstacles interfering with light may thus be located.

In an early growing stage, the young sporophytes seem to react somewhat to gravity in a negative sense, tending to bend upward toward the perpendicular whether lighted from above or from below.

In the course of the study of the sporophyte, as above sketched, a number of interesting relations were observed pertaining especially to the calyptra.

In *Funaria*, the calyptra is a more highly developed structure than in *Mnium* and seems to perform so much more perfectly the protective function which has been assumed for the calyptra. This appears reasonable when one bears in mind the nature of the dangers incident to these two types of moss. *Funaria* grows on the ground in dry, exposed situations, in the full blaze of the direct sunlight and is exposed during the fortnight or three weeks required for the maturation of the spores to the danger of desiccation. During the first week after the appearance of the growing archegonium among the perichaetial leaves, desiccation is fatal to the young sporophyte, and it is only after the capsular rudiment has reached about one-half of its mature bulk that it is able to survive the degree of desiccation resulting from a few days of hot, dry weather. Hence, the season for sporophyte formation falls in the moist season of the year when the temperature is sufficiently high to allow rapid growth.

The calyptra is an added factor of great ecological significance for this moss.

The archegonium after the fertilization of the egg. grows until a length of about 4,2 mm is reached. Separation from the gametophyte results at this stage, and measurements at subsequent stages showed that it makes no further growth. It consists of three distinct parts: (1) a long, slender, beak-like distal portion comprising, about onehalf of the total length which passes by a somewhat abrupt expansion into (2) an enlarged sac-like portion which in the younger stages is traversed lengthwise by longitudinal folds. This sac contracts sharply at the base of the calyptra into (3) a short, basal, collarlike portion which clasps the seta very tightly.

The calyptra is a loose bag, drawn together tightly at its mouth in which the entire dividing and growing regions of the young sporophyte are enclosed. Young, still erect, sporophytes from which the calyptrae were removed with the greatest care rarely succeeded in developing into mature sporophytes, even though giving no evidence of injury in the removal. It was not until the capsular rudiment had reached about half its mature size that the removal of the calyptra failed to produce untoward effects on the sporophytes. All this seems to point to the great importance of the protection afforded by the calyptra, from mechanical injury as well as from undue desiccation. It seems probable that the removal of the calyptra at the later stages is less detrimental because of the increased cuticular development and because of the greater resisting action of the solid masses of tissued formed.

Another fact observed may serve to explain the great efficiency of this protecting structure. Although torn away from its place of growth at an early stage in the life of the sporophyte, the cells of the calyptra are found to be living until shortly before it is ruptured by the increased size of the enclosed capsule. Having no organic connection during this long period with any source of food supply, it must maintain itself independently. Since each cell contains a good number of chloroplasts, this method of support is easily surmised. The source of the neccessary steady water supply is doubtful. On removing the calyptra from the sporophyte, minute drops of water were usually seen adhering to the sporophyte, and the examination under low magnification of the sporophyte bearing the calyptra in position revealed the presence of water more or less filling the upper part of the calyptra. Whether this water is excreted by the sporophyte and maintained in place by the calyptra, or whether the calyptra itself has the ability to condense or absorb water through its much folded outer surface, can hardly be said. At all events, the calyptra is doubtless supplied with the substances and conditions necessary for carbohydrate formation, and for its nearly independent, temporary nutrition. Thus in the developing sporophyte, three organicalli unconnected structures are present, each to a considerable extent capable of independent nutrition.

In *Mnium*, growing on the damp leaf-mould in the woods, the danger of desiccation is minimal and the calyptra seems to reflect this in its structure. It is pointed at the distal end and widens somewhat rapidly toward the base. There is no close clasping of the seta at the base, and the calyptra fall off at a much earlier stage than in *Funaria*. It seems to me that a thorough study of the structure of the calyptra and its ecological relations to the sporophyte offers an interesting problem in ways and means in Archegoniates.

Another question of some interest was raised in connection with this study. It was noted that in the young stages of the *Funaria* sporophyte, the distal end, containing the regions of cell division and most of the zone of elongation, was thrust into the calyptra to the very extreme of the long, beak-like portion and was not withdrawn to the capacious sac-like part until the enlargement of the capsular rudiment was about to begin or was already to be traced. This withdrawal of the end of the sporophyte to the more roomy portion of the calyptra could be seen in progress before any noticeable enlargement of the latter had taken place. Usually, however, the curvature of the seta began contemporaneously with the beginning of the withdrawal. In what way this withdrawal was effected was not easily explained. It seems probable that the inner surface of the beak-like portion of the calyptra is moist and offers little friction to the smooth surface of the slender sporophyte. (5) As the curvature in response to the stimulus of gravity is formed in the growing zone, therefore, in te sac-like portion of the calyptra, a leverage is gradually exerted by the bending zone against the shoulder marking the point of the sudden transition between the upper regions of the calyptra. This taken in connection with the action of the tightly clasping base has the effect of slowly pulling that part of the sporophyte in which the capsular enlargement is seen to take place back into the roomy bag-like part of the calyptra where growth may go on until all danger from desiccation is past before the increasing bulk of the capsular rudiment ruptures the calyptra and interferes with its efficiency as a protection against the loss of water.

The true relations of the curvature formation seem to suggest that the formation of this bond in the seta may serve a purpose quite different from that traditionally ascribed to the curvature. It may aid in advantageously arranging the relations of sporophyte to calyptra as well as later when it assumes the nodding position, aiding in spore dissemination.

The conduct of the calyptra in forms having erect capsules would be of interest in this connection.

Bibliography.

1) Wichura, M., Beiträge zur Physiologie der Laubmoose. (Jahrb. f. wiss. Bot. III. 1860. p. 193.)
2) Goebel, K., Organographie der Pflanzen. Tl. I. Jena 1898. p. 203.
3) —: Organographie der Pflanzen. Tl. II. 1898. p. 372.
4) Spalding, V. M., The traumatropic curvature of roots. (Annals of Bot. Vol. VIII. 1894. p. 423.)
5) Goebel, K., Organographie der Pflanzen. Tl. II. p. 372.
see also
Goebel, K., Flora. 1895. p. 474.