Photosynthetic activity and productivity of the sporophyte of *Funaria hygrometrica* during ontogenesis

J. KRUPA

INTRODUCTION

The structure of a sporophyte, which plays a considerable part in the reproduction cycle of mosses, has already been investigated and described (Goebel 1906; Haberlandt 1918; Janzen 1929 and others). The presence of assimilatory parenchyma and stomatal apparatus in the capsula suggests its photosynthetic activity. This suggestion is supported by an intensive development and a proper differentiation of chloroplasts (Bold 1940) as well as by considerable amounts of chlorophyll, sometimes even more abundant in the sporophyte than in the gametophyte (Haberlandt 1918). On the other hand, however, results of cultures of a sporophyte isolated from the gametophyte (Herzfelder 1923; Bopp 1954) are not univocal and this does not permit to ascertain the complete autotrophy of the sporophyte. It seems that an experimental determination of photosynthetic productivity of the sporophyte based upon measurements of gas exchange can give a basis for a more precise determination of the degree of trophic dependence of both generations of the moss.

The aim of the present paper is to examine the photosynthetic and respiration rate of the *Funaria hygrometrica* sporophyte in various stages of its development. Constant light conditions, applied among others, during the whole developmental period, permitted to determine the rate of photosynthetic production of the sporophyte. The carbon balance calculated on these grounds makes determination of the degree of autotrophy of the sporophyte possible.

MATERIAL AND METHODS

Sporophytes of *Funaria hygrometrica* from laboratory cultures constituted the experimental material. Sporophytes were cultured in glass dishes covered with glass on a medium consisting of humus soil and charcoal. Spores taken from only one ripe capsula were sown out into each dish in order to equalize the material genetically.

The culture was illuminated for the whole time of culturing with 2,5 klux light intensity (from fluorescent tubes Telam 40 W) in daily cycles 12 hours light and 12 hours darkness in turn. Temperature equalled 20°C ($\pm 1^\circ\text{C}$).

In the above given conditions the period of the sporophyte development equaled 14 weeks from the appearance of sporophytes till their full ripeness. A period of 96 days was adopted as the average developmental period. This period of time was divided into 4 sub-periods, each of them corresponding morphologically and ana-

Fig. 1. Developmental stages of sporogonia of *Funaria hygrometrica*

A — appearance of capsula; P — cross section

tomically to the characterized developmental stage (stages I—IV). With certain approximation it may be assumed that these stages are equal as to their length of time; each of them lasting 24 days. The morphological character of the distinguished developmental stages, based upon the developmental picture obtained in the middle of each period (after about 12, 36, 60, 74 days) is as follows: (Fig. 1.).

Ist stage: the cap in the lower part is closely adjacent to the seta, capsula is not marked, no distinct differentiation of the tissue of the capsula in the cross section.

IInd stage: capsula distinctly outlined and situated on the axis of seta the cap often covers half of the capsula, stomatal apparatus appears, all anatomical elements of the capsula are distinguishable, green seta.

IIIrd stage: capsula attains its final dimensions, it is hanging down and green, the cap covers the top of the capsula, cross section does not show any essential qualitative anatomical changes in relation of the previous stage, mother cells of spores divide, green seta.

IVth stage: capsula is yellow-green often without a cap, the inside of the spore sack filled with spores, yellow seta.

After having reached a certain developmental stage the sporophyte was cut off at the base of the gametophyte, immediately weighed and placed in the reaction chamber of the microrespirometer. After completion of gas exchange measurements the sporogonium was transferred into a dessicator (105°C internal temperature) and weighed subsequently. After this period of time the capsulae, respectively whole sporophytes were characterized by constant weight. Fresh and dry weight of the capsulae or the whole sporophyte was determined by means of a micro-balance (Dyakowska and Zurzycki 1959).

Intensity of gas exchange was determined by means of the microrespirometric method (Zurzycki 1955; Starzecki 1961). Reaction chambers described by the above mentioned authors were used for measurements of gas exchange. Measurements of respiration and photosynthesis of the whole sporophyte were carried out by use of chambers the length of which was suitably enlarged for the whole sporophyte to find place in it (Zurzycki and Starzecki 1967). Capsulae respectively the whole sporogonium were illuminated during measurement of photosynthesis from two sides, the illuminating optical arrangements being adapted in such a way as to obtain an equal light intensity measured on the plane of reaction chambers. Tungsten bulbs 1000 W constituted the light source applied in measurements of photosynthesis. A 10 cm thick filter with water solution of Mohr's salt, 150 g per 1 ltr water concentration, was mounted in the illuminating optical arrangement of the respirometer. Light intensity was regulated with neutral filters of black coated copper nets. Temperature during measurements of gas exchange was 25°C.

Intensity of gas exchange for individual developmental stages of a capsula was calculated for 1 mg dry weight. In view of the small differences in gas exchange intensity between the sporogonium and the capsula as well as for methodical reasons the majority of measurements of photosynthetic and respiration intensity refers to the intensity of these processes in the capsula itself (see results).

The calculation of photosynthetic production and carbon balance was based upon photosynthetic intensity in light of 2,5 klux (i.e. such intensity in which the culture was grown). On the grounds of gas exchange intensity and the established changes in dry weight the carbon balance was calculated during individual developmental stages of the sporophyte. Photosynthetic and respiration intensity were measured in $\mu\text{l O}_2/10 \text{ min}$ and calculated and expressed in terms of mg of hexose, whereas dry weight was treated as 100 % hexose.

RESULTS

Changes occurring during the development of the sporophyte of *Funaria hygrometrica* can be characterized by a differentiation of external and internal morphology of the capsula. Developmental stages distinguished on these grounds (see method) are characteristic of certain periods in the development of the sporophyte. The period of the development of the sporophyte, which in experimental conditions lasts usually 96 days counting from the moment the sporophyte appears on the gametophyte, till the moment the capsula grows yellow, was divided into 4 sub-periods each 24 days long. The first one, when the cap is adjacent to the seta in its lower part is characterized by a quick elongation of the seta. No differentiated elements of the capsula are visible on the cross-section. These are formed only in the second developmental stage. Differentiation of the elements is closely connected with the growth in thickness of the capsula and bursting of the cap which gets shifted towards the top of the capsula. In the IIIrd phase anatomical elements of the capsula develop further till by the end of this phase a rather intensive division of mother cells and appearance of spores take place. The shape of the capsula becomes dorsal-ventral

Fig. 2. Photosynthesis of *Funaria hygrometrica* capsulae.

Axis — x — light intensity in klux, axis — y — gas exchange intensity in $\mu\text{l O}_2 \times 10^{-2} / 1 \text{ mg dry weight} / 10 \text{ min}$: — $\triangle$ — in stage I, — $\circ$ — stage II, — $\bullet$ — stage III, — $\blacktriangle$ — stage IV

and changes its position in respect to the axis of the seta. Seta is still green and does not show any elongation growth. In phase IV a yellowing of the capsula and seta takes place and the cap easily falls off. The inside of the sporogonium is filled with spores. The IVth phase ends in a considerable loss of water, the capsula getting folded and brown. The four stages distinguished on the grounds of morphological changes characterize a certain developmental period and exhibit considerable differences in gas exchange intensity. Curves presented in fig. 2 show the intensity of photosynthesis of respective developmental stages of the capsula illuminated with light of various intensities. It follows from the diagram that the compensation point is reached for stages I, II, III within the limits of about 1 klux, whereas, for stage IV this value shifts towards higher light intensities (to about 7 klux). Analogically the saturation point for stages I, II, III is reached in light intensity of about 4 klux, whereas, for stage IV in 10 klux. Intensity of apparent photosynthesis is

different in individual developmental phases of the capsula. In the first three stages of the development of the capsula the intensity of apparent photosynthesis exceeds considerably the respiration rate in the whole range of applied light intensities. The highest photosynthetic rate, calculated for 1 mg dry weight is observed in the IIth developmental stage and the values attained in light intensity of 6 klux exceed almost 2,5 times the intensity of this process in stage I. The IVth stage is characterized by a decrease of photosynthetic as well as respiration rate.

Fig. 3. Respiration intensity of capsulae (lower curve) changes in water content in various developmental stages (upper curve)

The data presented in fig. 3 concerning respiration rate in individual developmental stages of the capsula (lower curve) indicate that the intensity of this process in stages I, II and III does not undergo any considerable changes: showing only some slight decreasing tendency intensified in stage IV. Changes in the water content in one unit of dry weight are considerable during the growth of the sporophyte (smallest changes occur in stages II and III), whereas, a considerable loss of water is noted only in the end phase of stage IV.

For of methodical reasons (see method) most measurements of gas exchange rate were carried out on the capsulae only. The differences between respiration and photosynthetic rate of the capsulae and whole sporogonia are very small. The respiration intensity of the capsula in stage II equals e.g. $48,9 \times 10^{-2} \mu\text{O}_2/10 \text{ min}/1 \text{ mg dry weight}$, whereas, that of the whole sporophyte 47,2 and analogically the photosynthetic rate in light intensity 10 klux equals $74,5 \times 10^{-2} \mu\text{O}_2/10 \text{ min}/1 \text{ mg dry weight}$ respectively. In a similar way values are arranged for the IIIrd developmental stage when the respiration rate of the capsula equals $41,1 \times 10^{-2} \mu\text{O}_2/10 \text{ min}/1 \text{ mg dry weight}$ and 39,9 for the whole sporogonium, whereas the photosynthetic rate in light intensity 6 klux is $62,98 \times 10^{-2} \mu\text{O}_2/10 \text{ min}/1 \text{ mg dry weight}$ and $58,73 \times 10^{-2} \mu\text{O}_2/10 \text{ min}/1 \text{ mg dry weight}$ for the capsula and sporogonium respectively.

Only in stage I some differences in the gas exchange rate of the whole sporogonium and the capsula alone occur in favour of the whole sporogonium; and so e.g. in this phase the respiration rate of the sporogonium is by $3,42 \times 10^{-2} \mu\text{O}_2/10 \text{ min}/1 \text{ mg dry weight}$ higher than in the capsula and equals $51,8 \times 10^{-2} \mu\text{O}_2/10 \text{ min}/1 \text{ mg dry weight}$. The rate of apparent photosynthesis in this stage is e.g. in light intensity of 10 klux higher for the sporogonium and equals $45,5 \times 10^{-2} \mu\text{O}_2/10 \text{ min}/1 \text{ mg dry weight}$ and $38,5 \times 10^{-2} \mu\text{O}_2/10 \text{ min}/1 \text{ mg dry weight}$ for the capsula alone.

A small contribution of the seta to the general gas exchange is suggested by its low dry weight which constitutes only a small percent of the total mass of the spo-

rogonium, the differences varying from 0,006 mg to 0,016 mg for the stage I and III, respectively. The balance of the carbon utilization calculated on the grounds of the gas exchange rate and increase of dry weight was expressed in terms of hexose and presented in fig. 4.

Assuming cellulose, the main structural material of the cell walls, and starch the storage material, calculation of dry mass in terms of hexose seems most suitable.

Fig. 4. Carbo balance of sporogonium of *Funaria hygrometrica*.

Axis y — mg hexose; P — photosynthetic production; R — utilization of hexose during respiration; M — increase of dry weight in mg hexose; S — difference between photosynthetic production and respiration and increase of dry weight

When calculating carbon balance, the process of glucose utilization (respiration within 24 hours and the increase of dry weight) and its production process (photosynthesis in light intensity 2,5 klux 12 hours daily) were compared. The established predominance of the former process over the latter requires an appropriate amount of assimilates to be transported from the gametophyte, whereas, the predominance of the photosynthetic production over the utilization of assimilates suggests their transport to the gametophyte. For calculation of the carbon balance numerical data established for the typical stage (middle of the period in question) — as representative for the whole period of time — were adopted. It follows from the diagram that the photosynthetic production exceeds slightly the dissimilatory process only in the IInd developmental stage of the sporophyte and suffices to cover the increase of dry weight. The deficit in the photosynthetic production in the IIIrd stage is rather small and equals 0,028 mg hexose. In the other developmental stages and specially in the IVth one the photosynthetic production does not compensate the consumption of assimilates for dissimilatory processes and the increase of dry weight; this indi-

cates the necessity of supply of organic compounds from the gametophyte during these stages. In the general carbon balance, during the whole period of formation of the *Funaria hygrometrica* sporophyte, a supply of assimilates from the sporophyte is necessary in an amount twice exceeding the dry weight of a ripe sporophyte. In spite of this participation of photosynthesis in the development of a sporophyte is essential; if the process were excluded the amount of assimilates which would have to be supplied to compensate the increase of dry weight and dissimilatory processes would exceed 4,5 times the dry weight of a mature sporophyte (fig. 4). A sporophyte produces 0,732 mg hexose of the total amount of 1.175 mg hexose accumulated in dry weight and utilized in the respiration process. It should be stressed that the above given calculation is true only for given culturing conditions and under the above mentioned assumptions. It seems most unlikely that other, more detailed assumptions of calculation (e.g. division of the developmental period into more sub-periods) could change the results decisively. However, more advantageous developmental conditions (a longer exposure to light per day, higher light intensity) may cause a certain increase of photosynthetic production of the growing sporophyte.

DISCUSSION

Young capsules of many species of mosses possess a well developed photosynthetic apparatus. Presence of the assimilatory parenchyma which sometimes contains elements of palisade and spongy parenchyma (Haberlandt 1918; Janzen 1929) as well as the occurrence of stomatal apparatus connected with intercellular spaces gives evidence not only of the photosynthetic possibilities but suggests, moreover, that this productivity can be comparatively high (Haberlandt 1918; Janzen 1929). Some species are known, among mosses, (*Euxbaumia*, *Ephemeropsis*) which show a considerable reduction of the gametophyte; this would indicate a full autotrophy of the spore generation being possible (Denning 1929). On the other hand, attempts of culturing isolated *Funaria hygrometrica* sporophytes (Haberlandt 1918; Herzfelder 1923) would confirm the suggestion that this generation is autotrophic if not during the whole developmental period, at any rate during some stages (Bopp 1954).

It has been frequently found that the role of photosynthesis in nutrition of sporophytes is not properly appreciated (Bold 1940). There were, however, no precise experimental data concerning photosynthetic and respiration rate as well as increase of dry weight of the sporogonium which would permit to draw a decisive conclusion as to the edaphic relations between these two generations. The above mentioned structure of the photosynthetic apparatus permits to suppose that the sporophyte is adapted to active photosynthetic production. Efficiency of this apparatus will depend on various factors. The established fact that the sporophyte is much richer in chlorophyll than the gametophyte (Haberlandt 1918) can by no means justify any conclusion concerning the autotrophy of the former. Examination of

photosynthetic intensity and its role in dependence on morphological differentiation and developmental stage of the sporophyte seems most appropriate. The distinguished developmental stages characterized by morphological changes correspond with certain stages described by Janzen (1929). The performed measurements of gas exchange intensity show that photosynthetic intensity coincides with morphological changes in the capsula. It may be concluded from measurements of gas exchange in individual developmental stages of the capsula that changes in photosynthesis intensity are initially (I and II stage) connected with the development of the photosynthetic apparatus. Specially in stage II, when the increase of dry weight is small the photosynthetic production compensates for the dissimilatory process and fully covers the requirements resulting from the increase of dry weight. A small increase of dry weight and of the size of the capsula as well as its imbibition indicate that in this period of time mainly elongation and differentiation of cells take place. In the IIIrd stage — when all the elements of the capsula are properly differentiated the photosynthetic intensity slightly drops. Differences in its intensity, however, calculated for 1 mg dry weight are small and lie within the limits of error. Yellowing, dehydration and formation of spores initiated in the final phase of the IIIrd stage explain clearly the decrease of photosynthetic activity in the IVth stage of the sporophyte development.

The above given results cast some light upon the possibilities of culturing isolated sporogonia of *Funaria hygrometrica* on a mineral substratum only in certain developmental stages (Bopp 1954). The present results are in agreement with the results of Bopp (1954) who proved that isolated sporophytes of *Funaria hygrometrica* can be cultured on a mineral substratum only in certain developmental phases. It follows from the above data that a sporophyte is fully autotrophic only during two developmental stages. Analogy in structure and development of sporangia between *Funaria hygrometrica* and other species of mosses permits to assume that edaphic relations between the gametophyte and sporophyte will be largely similar though, not identical. The degree of edaphic dependence of the sporophyte on the gametophyte depends both on the size and morphological differentiation of the generation as well as on ecological conditions.

SUMMARY

1. Photosynthetic intensity of the sporogonium of *Funaria hygrometrica* in various developmental stages and various light intensities has been examined. In the distinguished 4 developmental stages covering the whole developmental cycle of the sporogonium photosynthetic intensity is connected with morphological changes occurring during ontogenesis.

2. Respiration intensity calculated for 1 mg dry weight does not change essentially in stages I, II, III whereas in the IVth stage intensity of this process decreases considerably.

3. Changes in water content and dry weight occurring during growth and development of the sporogonium have been examined.

4. The carbon balance has been calculated basing upon the intensity of gas exchange and changes in dry weight in individual developmental stages. It has been established that photosynthetic production satisfies the demand for organic substances resulting from the increase of dry weight and dissimilatory processes.

5. In order to compensate for the deficit resulting from dissimilatory processes and increase of dry weight the sporogonium of *Funaria hygrometrica* requires in the general carbon balance a transport of organic substances from the gametophyte in an amount twice exceeding the dry weight of a ripe sporophyte.

I should like to thank professor dr Jan Zurzycki for his guidance in elaborating this problem

Department of Plant Physiology
of the Higher Pedagogical School
Cracow

(Entered: May 17, 1968.).

REFERENCES

- Bold H. C., 1940, The nutrition of the sporophyte in the mosses, *Amer. J. Bot.* 27: 312—322.
Bopp W., 1954, Untersuchungen über Wachstum und Kapselentwicklung normaler und isolierter Laubmoossporogone, *Z. Bot.* 42: 331—352.
Dyakowska J. and Zurzycki J., 1959, Gravimetric Studies on Pollen, *Bull. de l'Acad. Pol. des Sci.*, 7: 11—16.
Goebel K., 1906, Archegoniatenstudien, *Flora Allg. Bot. Zeit.*
Dening K., 1929, Entwicklungsgeschichtliche Untersuchungen an Gametophyten von *Buxbaumia aphylla*, *Verh. natur. Ver. J. Rheinland.*
Haberlandt G., 1918, Physiologische Pflanzenanatomie, Leipzig.
Herzfelder H., 1923, Experimente an Sporophyten von *Funaria hygrometrica*, *Flora Allg. Bot. Zeit.*, 116: 476—489.
Janzen P., 1929, Die Laubmoose Europas, *Rep. speciorum nivarum regni veg.*, Berlin: 94—111.
Starzecki W., 1961, An improved microrespirometer and extension of its application over plants with big leaves, *Acta Soc. Bot. Pol.*, 30: 327—343.
Zurzycki J., 1955, Chloroplasts arrangement as a factor in photosynthesis, *Acta Soc. Bot. Pol.* 24: 27—63.
Zurzycki J. and Starzecki W., 1966, Mikrorespirometr, wyd. Z.F.R. P.A.N.: 1—23.

Aktywność i produktywność fotosyntetyczna sporofitu Funaria hygrometrica w trakcie ontogenezy

Streszczenie

1. Zbadano intensywność fotosyntezy sporogonu *Funaria hygrometrica* w różnych okresach rozwoju i przy różnych natężeniach światła. W wyróżnionych 4 studiach rozwojowych obejmujących cały okres rozwoju sporogonu natężenie fotosyntezy wiąże się z zmianami morfologicznymi zachodzącymi w trakcie ontogenezy.

2. Intensywność oddychania przeliczona na 1 mg suchej masy nie zmienia się zasadniczo w I, II i III stadium natomiast w IV okresie natężenie tego procesu wyraźnie spada.

3. Przebadano zmiany w zawartości wody i suchej masy zachodzące w czasie wzrostu i rozwoju sporogonu.

4. Na podstawie intensywności przemiany gazowej i zmian suchej masy w poszczególnych okresach rozwoju obliczono bilans węglowy. Stwierdzono, że w II i III stadium rozwoju sporogonu produkcja fotosyntetyczna pokrywa zapotrzebowanie na substancje organiczne wynikające z przyrostu suchej masy i procesów dysymilacyjnych.

5. W ogólnym bilansie węglowym sporogon *F.h.* wymaga dostarczenia z gametofitu substancji organicznych do pokrycia deficytu wynikającego z procesów dysymilacyjnych i przyrostu suchej masy w ilości dwukrotnie przekraczającej suchą masę dojrzałego sporofitu.