ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XXXVI — 1
1967

Photosynthesis and respiration of turions and vegetative fronds of *Spirodela polyrrhiza*

M. CZOPEK

Turions of *Spirodela polyrrhiza* (L.) Schleiden are particularly suitable objects for the study of many physiological processes. They are easily obtained in laboratory conditions from sterile cultures of *Spirodela* regardless of the vegetative season. Therefore many aspects of the physiology of *Spirodela* turions are relatively well known, and especially their formation, dormancy state and germination (Jacobs 1947; Henssen 1954; Czopek 1959a, b, 1960; 1962; 1963a, b, c, d; 1964a b, c). Experimental research on photosynthesis and respiration during dormancy or germination states was initiated by Henssen (1954) and Czopek (1964b) respectively.

The present paper summarizes the results of studies on the course of photosynthesis and respiration of turions and vegetative fronds of *Spirodela* in dependence on the thermal and light conditions. The results of this study show the similitudes and differences in the same physiological processes occurring in turions and vegetative fronds of *Spirodela polyrrhiza*.

MATERIAL AND METHODS

Spirodela polyrrhiza was grown in the laboratory in sterile cultures on Pirson and Seidel's nutrient solution supplemented with 1% sucrose (Czopek 1959b, 1963a). The cultures were kept in a light thermostat at 28°C (±1°C) and illuminated with continuous white light emitted by 6 fluorescent 25-W tubes. The light intensity was about 4 300 ergs/cm²sec (i.e. about 1 000 lux). Turions of *Spirodela* which were formed by vegetative fronds were collected and kept in complete darkness for one month in a refrigerator (at 0—3°C); they were immersed in sterile water which was periodically changed (Czopek 1962). For determination of the rates of photosynthesis and respiration the microrespirometric method was adopted (Zurzycki 1955; Starzecki 1961). Warburg's carbonate solution No. 10 was used as a CO_2 source in the microrespirometer. Turions were allowed to germinate in sterile nutrient solution in the light thermostat and were then transferred to the micro-chambers of the microrespirometer. Three turions (4,5 mm² area each) were placed in each of the four micro-chamber in a small drop of nutrient solution. Measurements were performed over 20 min. at 5 min. interval at constant temperature of 28°C (±0,2°C) or at 10, 15, 20, 25, 30, 35 and 40°C. The intensities of the

photosynthetically active radiation (PAR) in the microrespirometer were 2 300, 7 400, 15 800, 27 000, 45 000 and 64 000 ergs/cm²sec for vegetative fronds and 4 600, 14 800, 31 600, 54 000, 90 000 and 128 000 ergs/cm²sec for turions illuminated from both sides. The details of the method adopted for PAR measurement were described by Czopek (1967a, b).

The photosynthesis and respiration of vegetative fronds of *Spirodela* were recorded by means of an infrared gas analyzer (VEB Junkalor, Dessau). The apparatus was a completely closed system adapted for measurement of CO_2 concentration changes in the concentration range 300 — 400 ppm for respiration and 400—300 ppm for photosynthesis (Egle and Ernst 1949; Egle and Schenk 1951; Egle 1960; Koller and Samish 1964). A Petri dish with vegetative fronds on a nutrient solution was placed in the chamber with controlled air temperature. The air stream passed through a drying column with anhydrous $CaCl_2$. The speed of the gas flow indicated by the rotameter was 35 litres per hour. The intensities of photosynthetically active radiation were 1 800, 4 800, 8 600, 18 400, 38 000, 55 000 and 73 500 ergs/cm²sec.

The results of microrespirometric measurements were expressed in microlitres of oxygen and calculated from the amount (mg) of carbon dioxide consumed or produced per one hour and by one turion or one vegetative frond.

RESULTS

The rate of photosynthesis and respiration recorded by means of the microrespirometer in turions of *Spirodela* was determined:

1. just after their formation by vegetative fronds,
2. after one month of dormancy at 0—3°C,
3. On the fourth day of germination (after the vegetative frond had been cut off),
4. after a suitable period of germination and detachment from the vegetative frond.

1. Turions formed under light and already detached from the vegetative frond respire intensively (Fig. 1). The photosynthesis rate in this stage is only slightly higher than respiration rate. The value of the compensation point is about 11 000 ergs/cm²sec and that of the light saturation point about 32 000 ergs/cm²sec. Light is without effect on the intensity of photosynthesis exceeding the last value. Because of the thickness of the turions it was necessary to illuminate separately their lower and their upper surface with light of the same intensity. In the dormancy state the thickness of a turion amounts to 0.25—0.35 mm and rises in the germination stage to 0.35—0.45 mm (Jacobs 1947).

2. In the dormancy state at low temperature (0—3°C) in darkness, the respiration first decreases to a minimum value. Then the respiration rate rises to higher rates than before the transfer of the turions to the refrigerator; on the contrary the apparent photosynthesis is decreases. The compensation and the light saturation points attain about 13 000 ergs/cm²sec and about 32 000 ergs/cm²sec respectively.

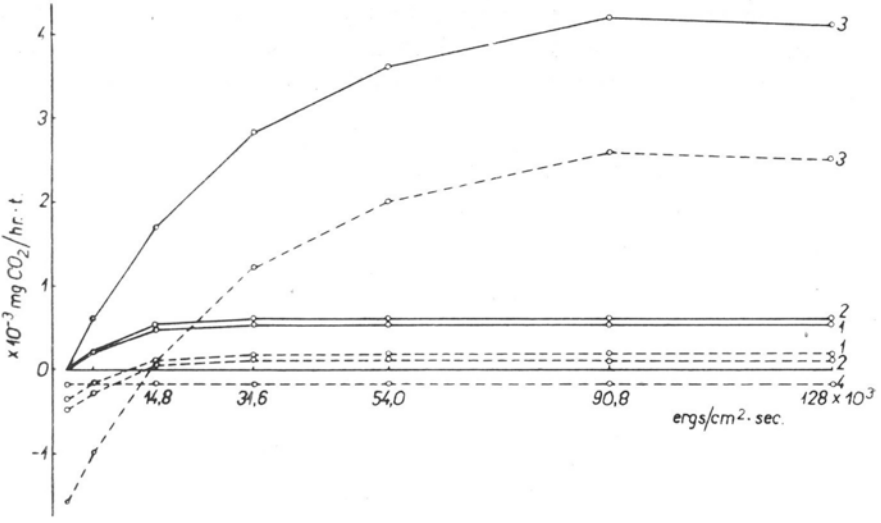

Fig. 1. Intensity of photosynthesis in turions of *Spirodela* as a function of light intensity.

Abscissae — light intensity $\times 10^3$ ergs/cm^2 sec; ordinates — intensity of photosynthesis $\times 10^{-3}$ mg CO_2/hr turion. *1* — photosynthesis in newly formed turions by vegetative fronds; *2* — after one month of dormancy at the temperature 0—3°C; *3* — the fourth day after germination; *4* — after germination and detachment from the vegetative frond. Solid line — true photosynthesis, broken line — apparent photosynthesis.

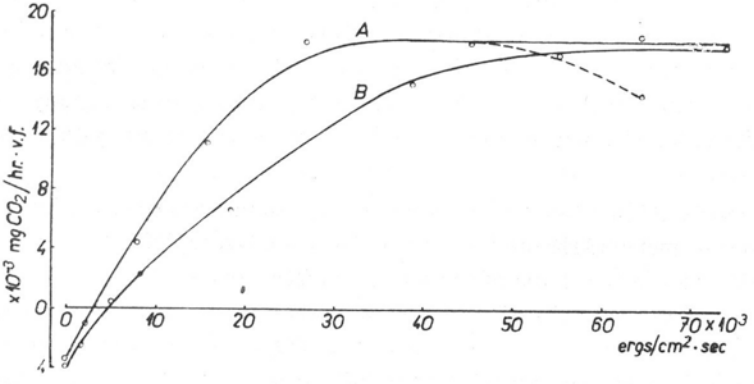

Fig. 2. Intensity of apparent photosynthesis in vegetative fronds of *Spirodela* as a function of light intensity.

Abscissae — light intensity $\times 10^3$ ergs/cm^2 sec; ordinates — intensity of photosynthesis $\times 10^{-3}$ mg CO_2/hr. veg. frond. *A* — the course of photosynthesis observed in the microrespirometer; *B* — the course of photosynthesis observed in the infrared gas analyzer. Solid line is the result of a measurement made at the beginning of experiment, broken line is the result of measurement at the end of experiment.

3. After the dormancy state the turions were allowed to germinate under continuous white light. On the fourth day of germination, when the rates of photosynthesis and respiration were the highest (Czopek 1964b) the vegetative fronds were removed by means of pincers and the subsequent experiments were performed only on turions. The results of measurements show that the rate of photosynthesis increases

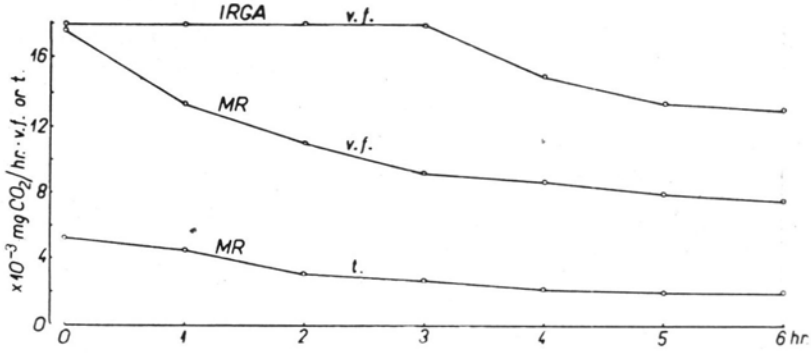

Fig. 3. Course of apparent photosynthesis in vegetative fronds (*v.f.*) and in turions (*t*) as a function of the experimental time in measurements made with the infrared gas analyzer (IRGA) and the microrespirometer (MR).

Abscissae – time in hours; ordinates – intensity of photosynthesis $\times 10^{-3}$ mg CO_2/hr veg. frond or turion.

with increasing light intensity. The compensation and the light saturation points attain about 15 000 ergs/cm²sec and about 91 000 ergs/cm²sec respectively. Turions require higher intensities for the saturation of the photosynthetic process; this may be connected with their structure (Czopek 1964 c).

4. In turions which formed already 2—3 vegetative fronds a consumption of storage substances was observed and accordingly the respiration rate reached a very low level. The turions were no more capable of photosynthesis because of decay of chlorophyll. The vegetative fronds formed in this period were already capable of independent life. The detached turions fell on the bottom of the culture flask and finally died.

The course of photosynthesis of vegetative fronds was measured by means of a microrespirometer (MR) and an infrared gas analyzer (IRGA). The results show some differences in the shape of the photosynthetic curves, although the final values attain the same level (Fig. 2). In the microrepirometer the compensation point attains about 30 000 ergs/cm²sec and in the CO_2 analyzer it has a much higher value above 60 000 ergs/cm²sec. The differences in the shapes of the photosynthetic curves should be ascribed to differences in the spectral composition of the light emitted by the light sources. In the microrespirometric studies the light source was a projector Tungsram 250 W, 220 V lamp characterised by intensive red radiation and weaker blue. In the CO_2 analyzer the light source was a 250 W, 220 V, mercury lamp, which emits intensive blue and weak red radiation.

The decrease of the photosynthetic rate observed during the first hours in experiments made with the microrespirometer should be ascribed to the exhaustion of CO_2 caused by the high intensity of photosynthesis despite the use of Warburg buffer No. 10. Point 1 on the curve of photosynthesis (Fig. 2) corresponds to the beginning of experiments and point 2 to the final experiments.

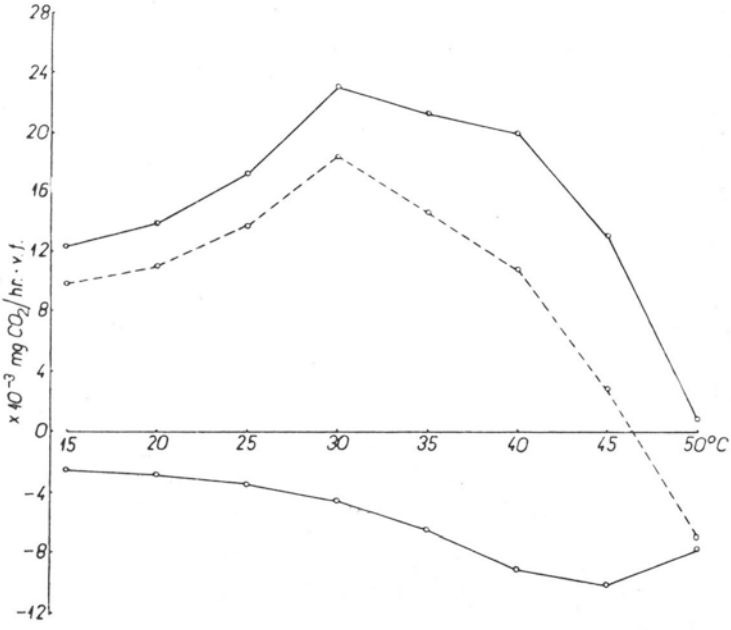

Fig. 4. Intensity of photosynthesis and respiration in vegetative fronds as a function of temperature. Abscissae — temperature; ordinates — intensity of photosynthesis and respiration $\times 10^{-3}$ mg CO_2/hr veg. frond. Solid line — true photosynthesis, broken line — apparent photosynthesis. The experiments were made with the use of an infrared gas analyzer.

The course of photosynthesis measured in the CO_2 analyzer does not show any fall in the first hours of the experiments (Fig. 3). After this period the photosynthesis begins to decrease. This has been attributed to the inactivation of the assimilatory apparatus. In turions of *Spirodela* the decrease of photosynthesis in experiments of longer duration is much lower (Fig. 3) and should be ascribed either to the low intensity of photosynthesis in turions or to smaller requirement of the carbon dioxide.

The photosynthesis of a mature vegetative *Spirodela* frond (about 32 mm^2) is more than four times higher than the intensity of photosynthesis of one germinating turion (about 4.5 mm^2). If, however, these values are reported per unit area it appears that the intensity of photosynthesis of a germinating turion is nearly twice that of a vegetative frond.

The results of study on the effect of temperature on the course of photosynthesis and respiration measured by means of the CO_2 analyzer show that the intensity of photosynthesis of mature vegetative fronds increases with temperature up to 30°C

(Fig. 4). At higher temperatures the photosynthesis begins to decrease at first slowly and from 40°C abruptly. Photosynthesis at 50°C is already hardly discernible. The intensity of respiraiton increases with temperature up to 45°C and then decreases.

Microrespirometric measurements show that also the intensities of photosynthesis and respiration depend upon the thermal conditions (Fig. 5), more specially

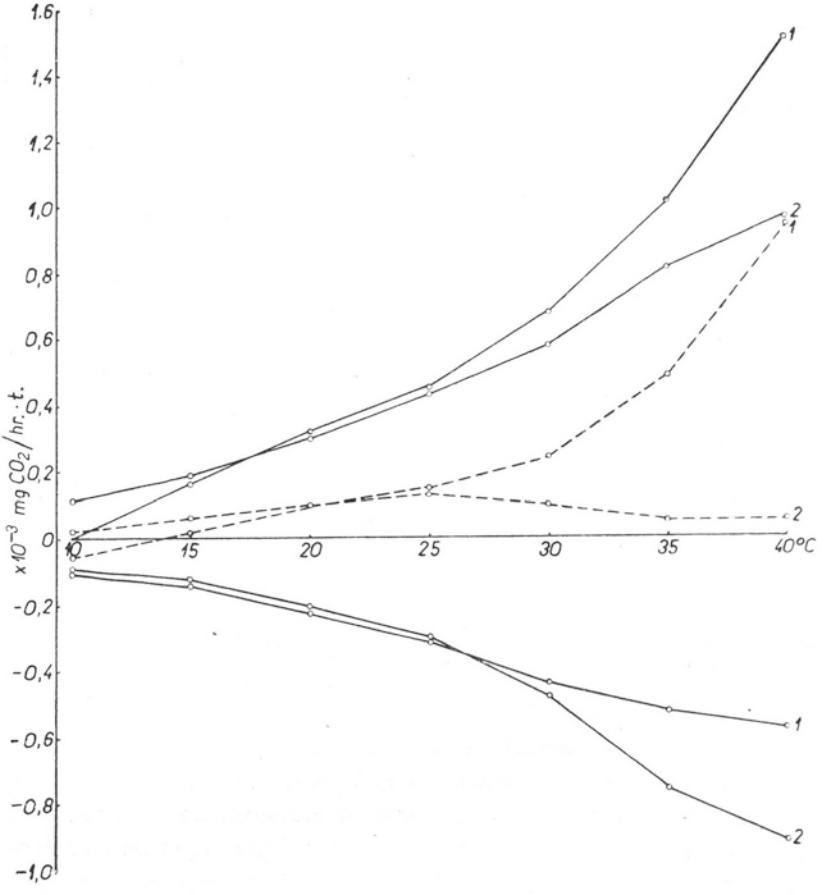

Fig. 5. Intensity of photosynthesis and respiration in turions as a function of temperature.
Abscissae — temperature; ordinates — intensity of photosynthesis and respiration $\times 10^{-3}$ mg CO_2/hr turion. *1* — turions just after their formation by the fronds; *2* — turions after one month of dormancy at low temperature.

upon the length of the dormancy stage. For 30°C and higher temperatures, the turions just after their formation by the vegetative fronds show a higher rate of photosynthesis and a lower rate of respiration than turions which spent a month in a state of dormancy at low temperature. These differences indicate that changes in the physiological activities take place during the dormancy stage of the turions. In germinating turions (Fig. 6) the rates of photosynthesis and respiration are

6—10 and 3—5 times higher respectively than in the dormancy state. On account of technical difficulties it was not possible to perform microrespirometric measurements above 40°C.

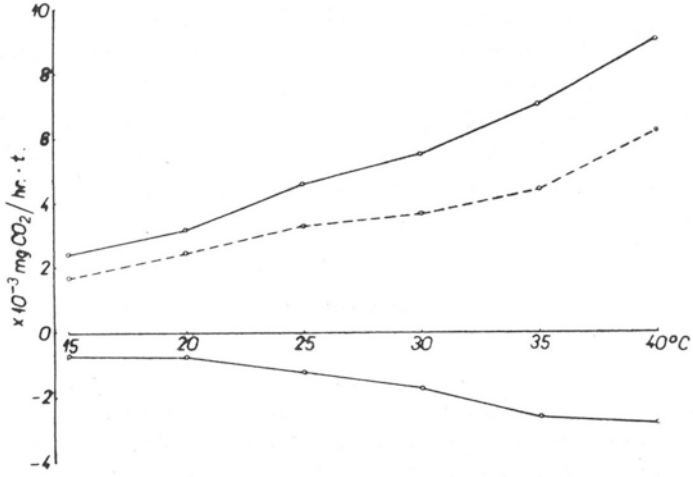

Fig. 6. Intensity of photosynthesis and respiration of a turion on the fourth day after germination as a function of temperature. For explanation see Fig. 5.

DISCUSSION

The results of this study on the dependence of photosynthesis and respiration on the thermal and light conditions led to the establishment of similitudes and differences manifested in these physiological processes by turions and vegetative fronds of *Spirodela*. The application of two methods — that is, the microrespirometric and infrared gas analyzer, leads to similar results. The photosynthesis of turions just formed by vegetative fronds is only slightly higher than that of respiration. After one month of dormancy at low temperature in darkness, the respiration attains higher rates and the intensity of photosynthesis slightly decreases. Maximum intensities of photosynthesis and respiration are attained in the fourth day after germination. Higher light intensities are required by the turions for saturation of the photosynthetic apparatus (about 91 000 ergs/cm^2 sec).

In older turions, which have already developed one or two vegetative fronds the rate of photosynthesis show a steady decline; also, owing to the exhaustion of nutrient substances, the respiration rate decreases and finally the turion dies.

The differences in the shapes of the curves of photosynthesis for the vegetative fronds obtained in experiments performed with the microrespirometer or the infrared gas analyzer are in part attributable to differences in the spectral composition of the corresponding light sources.

A disadvantage of the microrespirometric method is the possibility of CO_2 exhaustion in the gas phase in experiments of longer duration. In experiments made with the

help of the gas analyzer, the decrease of rate of photosynthesis is not observed before 3 hours, with fronds exposed to high light intensities (about 73 500 ergs/cm^{2}sec). This decrease is ascribable to light inactivation of the photosynthetic apparatus.

The photosynthesis of a mature vegetative frond of *Spirodela* is four times higher than that of one germinating turion. Calculated, however to unit area, the intensity of photosynthesis of a germinating turion is twice that of a vegetative frond. Optimum temperature for photosynthesis in vegetative fronds is 30°C and for respiration about 45°C. Above 40°C the photosynthesis decreases rapidly. Above 30°C the respiration of turions after one month of dormancy is higher and the photosynthesis lower than in freshly formed turions.

SUMMARY

1. Investigations were carried out on the course of photosynthesis and respiration of turions and vegetative fronds of *Spirodela polyrrhiza* in dependence on thermal and light conditions. The experiments were made with the use of a microrespirometer (MR) and infrared gas analyzer (IRGA). The results of experiments show the similitudes and differences in the investigated physiological processes manifested by turions and vegetative fronds.

2. Maximum intensity of photosynthesis and respiration is attained on the fourth day following germination. Turions require high light intensity for saturation of the photosynthetic apparatus.

3. The shapes of photosynthesis curves obtained in experiments performed with the microrespirometer and the infrared gas analyzer show differences although the final values attain the same level. The causes of these differences are discussed.

4. Owing to the possibility of CO_2 exhaustion in the microrespirometer, the gas analyzer is better adapted to measurements of longer duration than the microrespirometr.

5. The intensity of photosynthesis in mature vegetative fronds is at least four times as high as that in turions.

6. In vegetative fronds optimum temperatures for photosynthesis and respiration are 30° and 45°C respectively.

The author is indebted to Professor F. Górski and Professor J. Zurzycki for their guidance in the course of the work.

Department of Plant Physiology
Polish Academy of Sciences
Cracow, Poland

REFERENCES

Czopek M., 1959a, Cultivation of Polish *Lemnaceae* species in laboratory conditions, Acta Biol. Crac. Ser. Bot. 2:13—22.

Czopek M., 1959b, Researches on the physiology of formation and germination of turions in *Spirodela polyrrhiza* (L.) Schleiden, Acta Biol. Crac. Ser. Bot. 2:75—90.

Czopek M., 1960, Ekologiczno-fizjologiczne badania nad zakwitaniem gatunków z rodziny *Lemnaceae*, Wiad. Bot. 4:263—280.

Czopek M., 1962, The oligodymanic action of light on the germination of turions of *Spirodela polyrrhiza* (L.) Schleiden, Acta Soc. Bot. Pol. 31:703—722.
Czopek M., 1963a, Die oligodynamische Wirkung des Lichtes auf die Keimung der Turionen von *Spirodela polyrrhiza*, Intern. Symp., Greifswald, **B**, bI.
Czopek M., 1963b, Studies on the external factors inducing the formation of turions in *Spirodela polyrrhiza*, Acta Soc. Bot. Pol. 32:199—211.
Czopek M., 1963c, Wpływ światła chromatycznego na kiełkowanie nasion, Wiad. Bot. 7:11—23.
Czopek M., 1963d, Metody hodowli *Lemnaceae*, Wiad. Bot. 7:153—164.
Czopek M., 1964a, The action of Kinetin, Gibberellic Acid and Red Light on the Germination of Turions of *Spirodela polyrrhiza*, Bull. L'Acad. Pol. Sci. 12:177—182.
Czopek M., 1964b, The Course of Photosynthesis and Respiration in Germinating Turions of *Spirodela polyrrhiza*, Bull. L'Acad. Pol. Sci. 12:463—469.
Czopek M., 1964c, Przegląd wyników badań nad powstawaniem turionów *Spirodela polyrrhiza*, Wiad. Bot. 8:59—78.
Czopek M., 1967a, Preliminary studies on photosynthesis and respiration of some plant species in *Fagetum carpaticum*, Studia Naturae, Ser. B, 1(7).
Czopek M., 1967b, Ecophysiological studies on photosynthesis and respiration of some plant species in meadow ecosystem, Acta Soc. Bot. Pol. 36:73—86.
Egle K., 1960, Methoden der Photosynthesemessung — Landpflanzen, Handbuch der Pflanzenphysiologie 5:115—163.
Egle K., U. Ernst A., 1949, Die Verwendung des Ultrarotabsorptionsschreibers für die vollautomatische und fortlaufende CO_2 — Analyse be: Assimilations — und Atmungsmessungen an Pflanzen. Z. Naturforsch. 4 b:351—360.
Egle K. u. Schenk W., 1951, Die Anwendung des Ultrarotabsorptionsschreibers in der Photosyntheseforschung, Ber. dtsch. bot. Ges. 64:181—197.
Henssen A., 1954, Die Dauerorgane von *Spirodela polyrrhiza* (L.) Schleid. in physiologischer Betrachtung, Flora 141:523—566.
Jacobs D. L., 1947, An ecological life history of *Spirodela polyrrhiza* (greater duckweed) with emphasis on the turion phase, The Bot. Rev. 27:437—469.
Koller D. and Samish Y., 1964, A null-point compensating system for simultaneous and continuous measurement of net photosynthesis and transpiration by controlled gas-stream analysis, Bot. Gaz. 125:81—88.
Starzecki W., 1961, An improved microrespirometer and of its application over plants with big leaves, Acta Soc. Bot. Pol. 30:327—343.
Zurzycki J., 1955, Chloroplast arrangement as a factor in photosynthesis, Acta. Soc. Bot. Pol. 24:27—63.

Fotosynteza i oddychanie turionów i pędów wegetatywnych Spirodela polyrrhiza

Streszczenie

1. Przedmiotem badań było określenie przebiegu intensywności fotosyntezy i oddychania turionów i pędów wegetatywnych *Spirodela polyrrhiza* w zależności od warunków termicznych i świetlnych. Doświadczenie przeprowadzono za pomocą mikrorespirometru (MR) i gazowego analizatora podczerwieni (IRGA). Wyniki badań wskazują na podobieństwa i różnice w przebiegu tych fizjologicznych procesów tak u turionów jak i pędów wegetatywnych.

2. Maksymalne natężenie fotosyntezy i oddychania turionów przypada na czwarty dzień kiełkowania. Turiony potrzebują stosunkowo wysokich intensywności światła do wysycenia aparatu fotosyntetycznego.

3. Różnice w przebiegu krzywych fotosyntezy pędów wegetatywnych wynikają z zastosowania dwóch różnych metod (mikrorespirometru i gazowego analizatora CO_2), jakkolwiek końcowe wartości były na tym samym poziomie.

4. Czas trwania pomiarów fotosyntezy może być dłuższy w gazowym analizatorze podczerwieni niż w mikrorespirometrze.

5. Natężenie fotosyntezy dojrzałych pędów wegetatywnych jest przeszło cztery razy wyższe niż u turionów.

6. Fotosynteza pędów wegetatywnych osiąga najwyższą wartość przy temperaturze 30°C a oddychanie przy 45°C.