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Characteristic of vegetative and resting forms in *Wolffia arrhiza* (L.) Wimm.

II. Anatomy, physical and physiological properties

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Wolffia arrhiza forms in laboratory cultures two kinds of vegetative fronds: floating and immersed ones, which drop to the bottom (Godziemba-Czyż, 1969).

In response to unfavourable conditions of culture e.g. deficiency of mineral compounds, low temperature, unsuitable light intensity — hibernating organs — turions — are formed. Turions of *Wolffia arrhiza* are able to germinate immediately after formation; they must only be transferred onto a fresh nutrient solution and placed at suitable temperature and light intensity. Germinating turions emerge on to the surface and give rise to vegetative floating fronds. A certain percentage of turions germinate at the bottom giving rise to vegetative immersed fronds. These fronds, however, arise frequently from floating vegetative fronds cultured on mineral nutrient solution enriched with sucrose.

All hitherto published papers on the *Lemnaceae* family treat *Wolffia arrhiza* fronds dropping to the bottom of the water body as turions; Landolt (1957), however, mentions that "turions" do not differ externally from vegetative fronds and are able to form new turions.

Genuine turions, obtained in this Laboratory are considerably smaller in size than vegetative fronds and never form secondary members. Thus, the descriptions found in literature concerned most probably immersed vegetative fronds.

With regard to the foregone, it seems most advisable to elaborate a detailed characteristic of both vegetative fronds as well as of turions from the morphological and anatomical point of view, to determine their fresh and dry weight, water content and physiological activity. Considering possible differences it would be justified to treat the turions obtained in this Laboratory as a resting form of *Wolffia arrhiza*.

The present literature concerning *Lemnaceae* does not give any description of two different vegetative forms for any species. Therefore an attempt to determine the role of the developmental forms and their usefulness in maintaining the species seemed to be most purposeful. Only investigations on the resistance to harmful factors could, at least, partly, elucidate this problem.

MATERIAL AND METHODS

Wolffia arrhiza fronds were collected in 1956 from water basins in the vicinity of Kierskie Lake near Poznań. The stock culture was grown under sterile conditions in a light thermostat (light intensity about 1800 lux and temp. 26—28°C) in a liquid Pirson's and Seidel's nutrient solution (Kandeler, 1955) with 1% sucrose. Morphological investigations, determination of fresh and dry weight were performed also for vegetative fronds and turions cultured on a nutrient solution without sucrose and under various light intensities (500, 5300 lux).

Measurements of the large (D) and small (d) diameter of all forms of *Wolffia arrhiza* were made under a microscope (x 40 magnification) provided with an ocular with scale. Mean dimensions for 20 vegetative fronds or turions from individual culture conditions (nutrient solution, light intensity) were calculated.

Material for anatomical examinations was fixed in Navashin's fixing solution. The fixed objects were cut on a microtome; slide thickness 10 μ. Slides were stained with 1% gentian solution according to Newton (Filutowicz & Kużdowicz, 1951).

In order to determine the fresh and dry weight of individual forms of *Wolffia arrhiza*, vegetative fronds or turions from individual culture conditions were counted for the whole series — consisting of about 20 flasks. They were subsequently dried on filter paper and weighed accurately. After 24 hours desiccation at 105°C they were weighed again. As the number of the vegetative fronds or turions was known it was easy to calculate the fresh and dry weight and the water content of one vegetative frond or turion; having obtained their volume (dividing the water amount forced out by vegetative fronds or turions by their number) their specific weight was calculated.

In order to determine the chlorophyll content, 1g of fresh material was ground in a mortar with glass and a certain amount of $CaCO_3$. Chlorophyll was extracted with 80% acetone water solution (V/V). This acetone extract was subsequently filtered on a Shott funnel (G 3) and washed with acetone solution until it was completely decolorised. It was, then, made up acetone to a given volume (50 ml). The values of pigment extraction in the acetone solution were determined by means of a Uvispeck Hilgar spectrophotometer at 663 and 645 nm wavelengths. Readings at 710 nm served as a control of the solution clarity. The whole chlorophyll amount and the amount of chlorophyll a and b (mg per liter) were calculated according to M a c k i n e y ' s 1941 (H o l d e n, 1965) formula. Determination of chlorophyll for each form of *Wolffia arrhiza* was repeated 10 times. The mean contents of chlorophyll per 1 floating, 1 immersed frond and 1 turion were calculated.

For solution and purification of starch P u c h e r ' s *et al.* (1948) and S a m o t u s ' s method was adopted.

One gram of fresh filtered material was mixed with 80% ethyl alcohol and quickly brought to boil. After filtration 2,5 ml distilled water was added and poured into a test tube in the centrifuge. After stirred thoroughly with a glass rod 5 ml of 60% perchloric acid was added and the tube was left for 15 minutes at room temperature, with stirring from time to time. Subsequently 10 ml distilled water was added and stirred thoroughly, the insoluble part being subsequently removed by centrifuging. The solution was then transferred into a flask of 50 ml capacity; the insoluble rest was treated in the same way once again. The combined extracts of the disolved starch were diluted to 50 ml with distilled water. The whole was divided into two test tubes, 25 ml in each, and then each test tube was treated differently. The basic starch solution (25 ml) was mixed with 12,5 ml of 20% sodium chloride and 5 ml of iodine solution in potassium iodate (reagents according to P u c h e r, 1948). After 20 min the iodine starch complex was centrifuged, washed in 10 ml of ethanol NaCl mixture, and centrifuged again. Pure starch was then suspended in distilled water and the flask was filled to a determined volume (for floating fronds 12,5 ml, for immersed ones 50 ml, for turions 1000 ml).

To determine starch the colorimetric method of determination of carbohydrates with anthron was used (S n e l l *et al.*, 1961). The examined solution (2 ml) was poured into a test tube and placed in a water bath at 10°C, 4 ml of the reagent were subsequently added (1g anthron for 1 liter concentrated H_2SO_4). The mixture stirred with a dry glass rod and left for 10 min in a boiling water bath. Optical density was then determined by means of a spectral colorimeter (Spekol — Zeiss Jena) at wavelength 650 nm. This was compared with a control solution (anthron and distilled water). In order to calculate the results, a calibration curve for a model solution of soluble starch (desiccated for 3 hours at 105°C) was prepared. Starch analysis was repeated 10 times for each vegetative form and turions.

Gas exchange activity was measured by means of a microrespirometer (Z u r z y c k i, 1955, and S t a r z e c k i, 1961). The diameter of four reaction chambers and a control one was 7 mm and the activity diameter of the capillary was 0,094 mm². Constant concentration of CO_2 in the chambers was maintained by 0,1 M Warburg No. 10 carbonate buffer in the amount of 40 ml for each chamber. A projection bulb 250 W supplied with 220 V current with a stabilizer and autotransformer constituted the light source. The light concentrated by a set of lenses passed through a liquid filter of Mohr's salt (g/l) which absorbed infrared radiation. The object was illuminated from below, light intensity being regulated by means of a diaphragm and equalling 3, 6, 12, 24, 36, 48, 60 x 10^3 erg $cm^{-2}s^{-1}$. Measurements of light intensity were taken by means of a Kipp and Zonnen's thermopile and Kipp's galvanometer (type A-70, Delft, Holland) using WG-1 and RG-8 filters. Measured values refer to photosynthetic active light (400—700 nm). During measurements the temperature in the microrespirometer was kept at a constant level 26°C by connecting it to an ultrathermostat. Gas exchange was measured every 5 minutes, with 5 readings of production respectively consumption of O_2 each time. The reaction chambers were prepared for measurements by placing 20 vegetative floating and immersed fronds, and 60 turions respectively in a hanging drop on each cover glass (except control). Ten measurements were made for each form at various light intensities and in darkness. The results are given in μl O_2 per hour.

In order to examine the resistance of vegetative and resting forms of *Wolffia arrhiza* to unfavourable external factors, as e.g. lack of light, experiments were carried out on vegetative fronds and turions cultured on nutrient solution with 1% sucrose or without it in a dark thermostat of constant temperature 26°C. Every 4 days 4 flasks with individual forms of *Wolffia arrhiza* were taken out, the percentage of dead forms and growth increment under light of 1800 lux intensity were calculated. The germination percentage of turions was determined after transferring them onto a fresh nutrient solution. The experiment was repeated 3 times.

For examination of resistance to low temperature, cultures of vegetative fronds or turions were placed in a refrigerator set at 1 and —2°C. Every two days two flasks with immersed vegetative fronds, two with floating ones and two with turions were transferred to a light thermostat (26°C, light intensity 1800 lux). For vegetative fronds the percentage of dead ones and the growth increment of the live ones were calculated. Turions were transferred onto a fresh nutrient solution and the percentage of dead turions and the germination ability of the live ones were determined. The experiment was repeated 3 times. Colourless, almost transparent fronds showing no further growth were considered as dead; as for turions, they were considered dead if they showed no germination ability when transferred onto a fresh nutrient solution.

The material was desiccated in a closed chamber in presence of $CaCl_2$ at a known air moisture. A thermohydrograph TZ-8 was used to register the relative humidity and temperature during the whole period of desiccation. Relative humidity varied within 55—60% and temperature was 20—21°C. At the same time 4 samples, each 1g of fresh weight, were placed for desiccation after being previously dried carefully on filter paper. One sample was weighted every 15 minutes, and from the others a certain number of vegetative fronds or turions was transferred onto a fresh nutrient solution every 15 minutes, and subsequently placed in a light thermostat. After 2 days the number of live vegetative fronds und turions was calculated as percentage. The whole cycle of operations was repeated for every form 3 times, and the mean value was calculated from all measurements.

RESULTS

1. Morphology of vegetative fronds and turions of *Wolffia arrhiza*

Individual vegetative fronds of *Wolffia arrhiza* are ellipsoidal, a little flattened at the tops (fig. 1). Floating fronds are usually paired — sometimes single fronds are found. Immersed fronds can form additionally groups of 3 to 4 members. The average dimensions of the fronds depend on the conditions they were cultured in, i.e. the kind of nutrient solution and light intensity (fig. 2).

The mean dimensions of vegetative floating fronds prove to be smallest (D = 0,89 mm and d = 0,7 mm) on a nutrient solution enriched with 1% sucrose and under lowest light intensity (500 lux). Fronds of largest dimensions (D and d = 1,27 mm), on the other hand, are spherical and are found on nutrient solution without sucrose under highest light intensity (5300 lux) — fig. 1d, 2.

Immersed vegetative fronds show a smaller diversity in shape and dimensions. They always are ellipsoidal and much larger on nutrient solution with sucrose (D = 1,25 mm and d = 1,15 mm); within this nutrient solution they do not change their dimensions with the change of light intensity. The smallest immersed fronds were observed on nutrient solution without sucrose at 1800 lux light intensity (D = 0,75 mm and d = 0,58 mm).

Turions are almost spherical, they appear singly (fig. 1c, 2). Their mean dimensions are much smaller than those of vegetative fronds, D = 0,5 mm and d = 0,42 mm on nutrient solution enriched with sucrose (light intensity 1800 lux).

2. Anatomical structure of vegetative fronds and turions of *Wolffia arrhiza*

Observations on the anatomical structure were carried out on longitudinal and cross sections parallel or perpendicular to the long axis of the vegetative fronds or turions (fig. 3). In individual vegetative fronds two parts can be distinguished: the basal one turned towards the parental member and the apical one. The secondary member grows in reverse direction to the parental one. The upper part of the fronds is flattened and the lower one bulged. Fronds are covered with a one-layer epidermis, the cells of which have straight-lines contours. In the upper epidermis

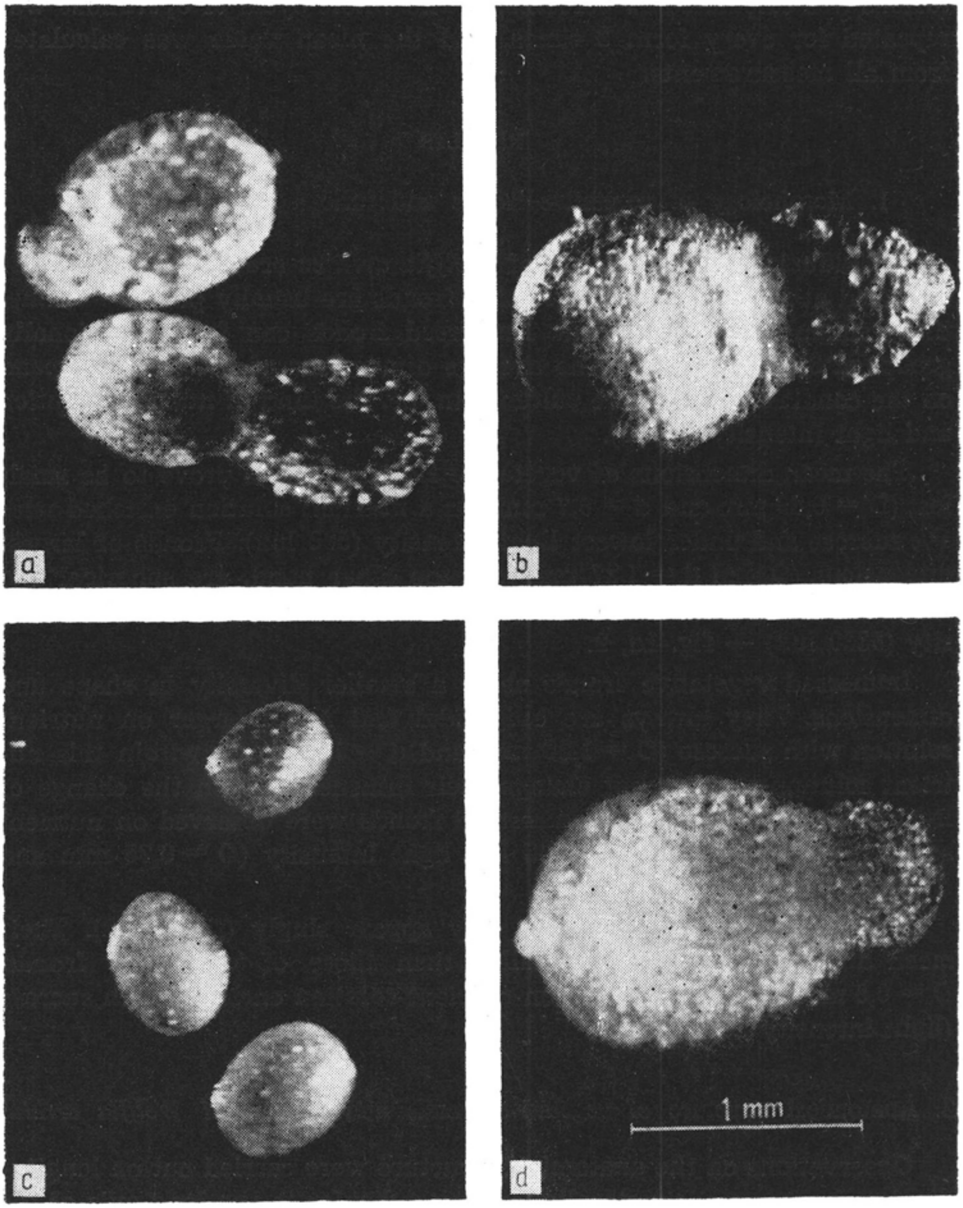

Fig. 1. Morphology of vegetative fronds and turions of *Wolffia arrhiza* cultured (except 1d) on a nutrient solution with sucrose, light intensity 1800 lux,

a — vegetative floating fronds, *b* — vegetative immersed fronds, *c* — turions, *d* — vegatative floating fronds cultured at light intensity 5300 lux without sucrose

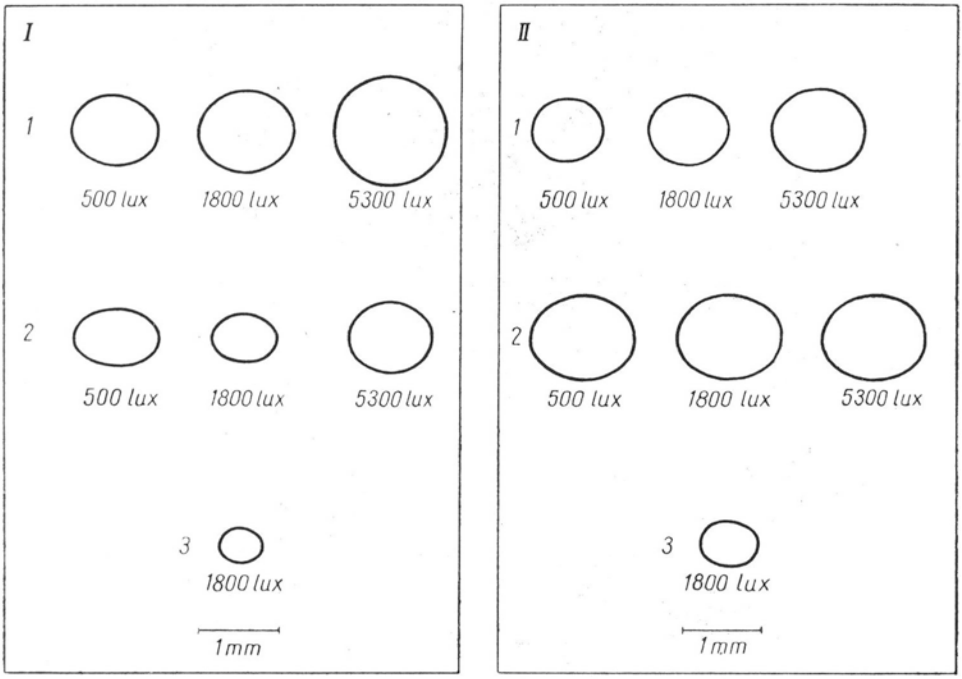

Fig. 2. Scheme of average dimensions of vegetative fronds and turions of *Wolffia arrhiza* in dependence on the kind of nutrient solution and light intensity

I — nutrient solution without sucrose; *1* — floating vegatative fronds, *2* — immersed vegatative fronds, *3* — turions.

II — nutrient solution with 1% sucrose. Notation as under I (fig. 2)

stomata are irregularly situated, without any additional cells. No raphides, druses and no anthocyanin cells are present. The parenchyma is differentiated into two layers. Under the upper epidermis there is a parenchyma layer occupying $^1/_4$ of the whole hight of the frond. This parenchyma is built of smaller cells rich in chloroplasts and starch. Intercellular spaces are in this part also smaller. This parenchyma changes gradually into the lower part of the parenchyma built of large cells and large intercellular spaces. These cells contain less chloroplasts and less starch, but starch grains are bigger especially in the immersed fronds. In the basal part of the frond there is a round opening on the side wall to a funnel-shaped pocket from which the secondary members grow.

The anatomical structure of the floating and immersed vegetative fronds is very similar. The greatest differences concern starch content and formation of intercellular spaces. Floating fronds are poor in starch which is mainly localized in the small-cell parenchyma of young fronds. The diameter of individual grains is about 5 μ. The intercellular spaces are extensive. Immersed fronds, on the other hand, have a much higher starch content; the starch grains being about 11 μ in diameter and inter-

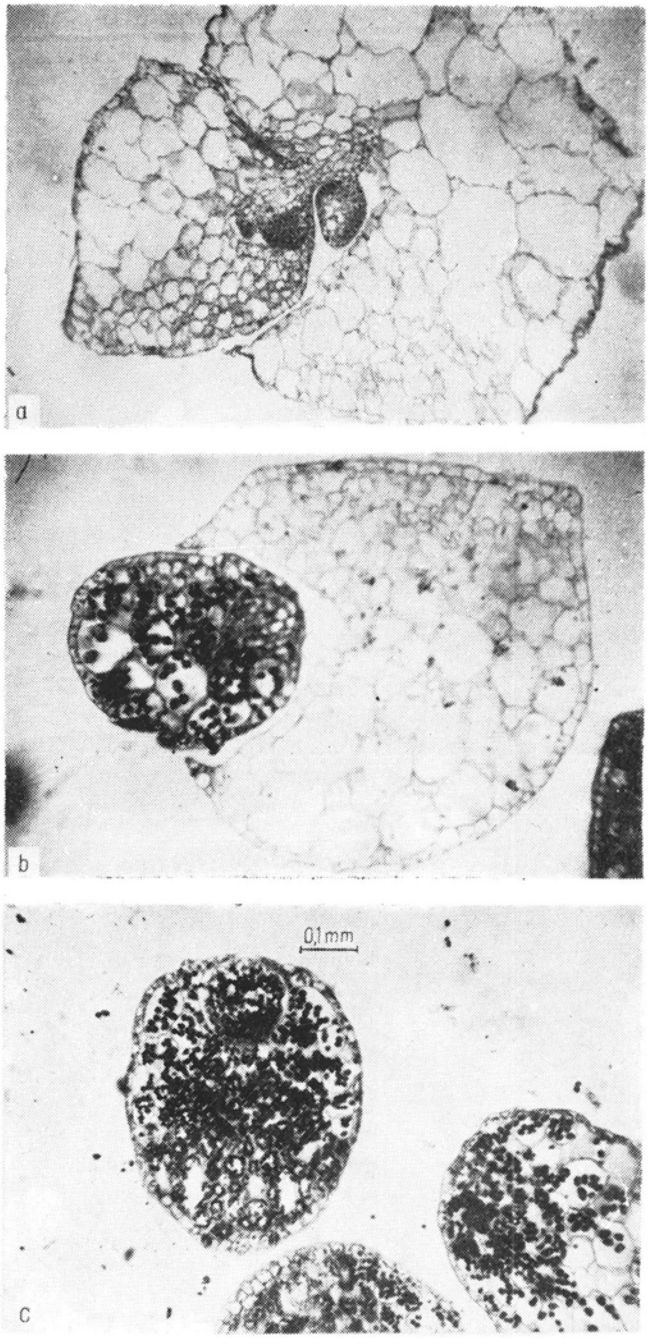

Fig. 3. Anatomical structure of vegetative fronds and turions of *Wolffia arrhiza* (longitudinal section, parallel to the long axis of fronds and turions)

a — floating parental member with secondary members (section in medial plane), *b* — immersed parental member with secondary members (section perpendicular to upper surface), *c* — turions (section parallel to upper surface). Stomata denoted by arrow.

cellular spaces being smaller. In floating fronds stomata are widely opened, reversely as it is the case with immersed fronds and turions in which stomata are only slightly opened.

The anatomical structure of turions resembles that of immersed vegetative fronds. Here too two parenchyma layers are distinguishable: the intercellular spaces, however, are small. All cells including those of the epidermis are filled with starch grains about 14 μ in diameter. In the pocket there is usually one secondary frond which closes tightly the opening to the pocket. The side walls of the pocket do not deflect as is the case with vegetative fronds but fit closely to the secondary frond. The secondary frond is in all turions at the same developmental level.

3. Physical properties of vegetative fronds and turions of *Wolffia arrhiza*

The fresh and dry weight of vegetative fronds of *Wolffia arrhiza* depends upon the conditions of the culture, i.e. composition of the nutrient solution and light intensity (fig. 4).

Fresh and dry weight of floating vegetative fronds cultured on a nutrient solution with sucrose increase with the rise of light intensity from $44{,}2 \times 10^{-5}$g fresh and $96{,}6 \times 10^{-7}$g dry weight (500 lux) to $75{,}2 \times 10^{-5}$g and 190×10^{-7}g fresh and dry weight respectively (5300 lux).

The average water content is about 97% and undergoes small changes (about 0,3%). On the basis of morphological data it can be stated that the increase in weight is strictly connected with the increase of dimensions of individuall members (fig. 2). On a nutrient solution enriched with sucrose the fresh and dry weight of floating vegetative fronds decrease slightly, with the increase of light intensity, from $68{,}9 \times 10^{-5}$ g and 171×10^{-7} g (500 lux) to $54{,}3 \times 10^{-5}$ g and 165×10^{-7} g fresh and dry weight respectively (5300 lux). The average water content is 97% (about 0,7% variation limits). As the average dimensions of individual fronds increase on this type of nutrient solution with the increase of light intensity, and the percentage of water content does not change, it may be supposed that these fronds become flattened. An analysis of the results permits the conclusion that nutrient solution with sucrose is, under low light intensities, more favourable for the increase of fresh and dry weight of vegetative fronds; at a certain light intensity (2750 lux for fresh weight) the weight of fronds in both kinds of nutrient solution becomes equal, and at higher light intensities, the fresh and dry weight of floating fronds cultured on a nutrient solution with sucrose is much higher.

Vegetative immersed fronds cultured on a nutrient solution without sucrose show a weak growth, therefore fresh and dry weight were not determined. The value of fresh and dry weight decreases from $115{,}4 \times 10^{-5}$g and 250×10^{-7}g (500 lux) to $65{,}3 \times 10^{-5}$g and 129×10^{-7}g fresh and dry

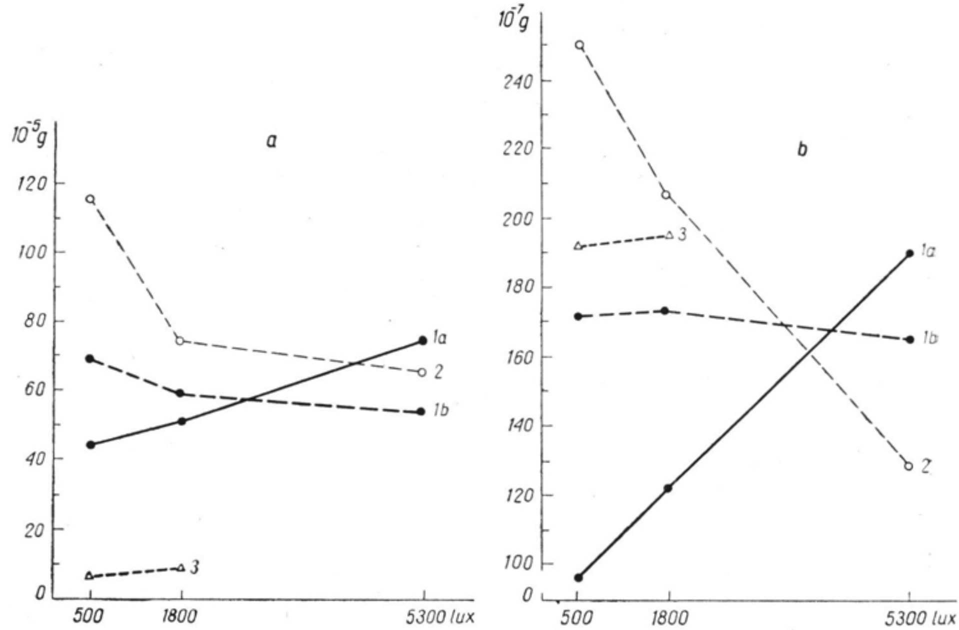

Fig. 4. Course of fresh weight (fig. 4a) and dry weight (fig. 4b) changes of individual vegetative fronds and turions in dependence on the kind of nutrient solution and light intensity.

Axis x — shows light intensity in lux, axis y — fresh or dry weight in g of an individual vegetative frond or turion.

1a — floating fronds — nutrient solution without sucrose, *1b* — floating fronds — nutrient solution with 1% sucrose, *2* — immersed fronds — nutrient solution with 1% sucrose, *3* — turions — nutrient solution with 1% sucrose.

weight, respectively, with the increase of light intensity (5300 lux) in cultures on a nutrient solution enriched with 1% sucrose. The water content is similar to that of floating fronds i.e. 97% on the average (0,9% variation limits). As the dimensions of immersed fronds within the applied light intensities are very similar, it may be supposed that the fronds become flattened with the decrease of light intensity as is the case with floating fronds cultured on a nutrient solution with sucrose.

The lowest fresh weight is that of turions ($9,2 \times 10^{-5}$g) owing to their small size. The dry weight of one turion (195×10^{-7}g) is however, considerably high because their water content is only 78,9%. In the range of light intensity 500—1800 lux the change in fresh and dry weight is small. As formation of turions requires specific conditions their fresh and dry weight on a nutrient solution with sucrose was determined only for two light intensities.

The mean specific gravity of one vegetative floating frond (culture conditions: nutrient solution with sucrose, 5300 lux) is 0,9145 G/cm³, that of one immersed vegetative frond in the same culture conditions 0,9986 G/cm³ and of one turion 1,21 G/cm³. The specific gravity of the

Pirson's and Seidel's nutrient solution with 1% sucrose is 0,9956 G/cm³ (temp. 26°C).

The difference between the specific gravity of the nutrient solution and the immersed vegetative frond is very small and so individual fronds float in the nutrient solution and drop only very slowly to the bottom.

4. Biochemical investigations of the three morphological forms of *Wolffia arrhiza*

a) analysis of chlorophyll

The chlorophyll content calculated in mg per 1g of fresh and dry weight and per one vegetative frond on turion is compiled in table 1. The greatest amounts of chlorophyll are contained in vegetative floating fronds, somewhat less — in immersed ones and the least amounts of chlorophyll are present in turions. Only when calculated per 1g fresh weight have turions a higher content of chlorophyll than immersed fronds, because of their lower water content. The ratio of chlorophyll a to b is equal for all forms of *Wolffia arrhiza* and equal 1:2,6.

Table 1

Chlorophyll content in vegetative fronds and turions of *Wolffia arrhiza*

Kind of fronds	Vegetative fronds		Turions
	floating	immersed	
per 1 g fresh weight in mg	0,293	0,174	0,422
per 1 g dry weight in mg	9,156	6,214	1,623
per 1 vegetat. frond or turion $\times 10^{-5}$ in mg	17,3	12,9	3,9
% of fresh weight	0,029	0,017	0,042
% of dry weight	0,916	0,621	0,162

b) analysis of starch

Starch content calculated per 1g of fresh and dry weight, and per one vegetative frond or turion is shown in table 2. An analysis of the results

Table 2

Starch content in vegetative fronds and turions of *Wolffia arrhiza*

Kind of fronds	Vegetative fronds		Turions
	floating	immersed	
per 1 g fresh weight in mg	0,35	0,95	26
per 1 g dry weight in mg	10,95	34,02	98,8
per 1 vegetat. frond or turion $\times 10^{-4}$ in mg	2	7	24
% of fresh weight	0,035	0,095	2,6
% of dry weight	1,1	3,4	9,9

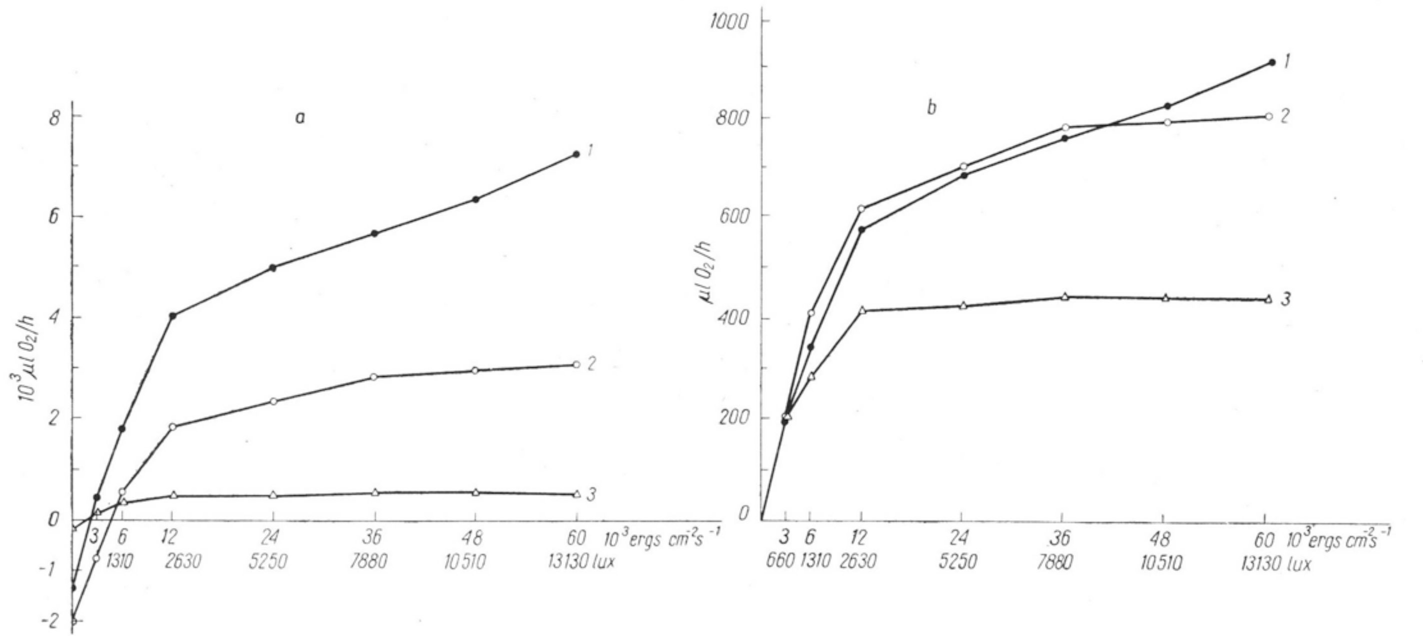

Fig. 5. Respiration and photosynthesis intensity of vegetative fronds and turions of *Wolffia arrhiza:* 5a — per 1g dry weight, 5b — intensity of true photosynthesis per 1 mg chlorophyll

Light intensity in erg cm^{-2} s^{-1} and in lux shown on x axis, y axis — consumption or production of oxygen in η l/hour. *1* — floating vegetative fonds, *2* — immersed vegetative fronds *3* — turions.

shows that turions have consistently the highest starch content, constituting almost 10% of their dry weight. Immersed vegetative fronds contain 3 times less starch per 1g dry weight (3,4%) whereas, floating vegetative fronds 9 times less (1,1%).

5. Physiological examinations of vegetative fronds and turions of *Wolffia arrhiza*

a) gas metabolism — respiration, photosynthesis

The respiration and photosynthetic rate were expressed in μl of the consumed or emitted oxygen within 1 hour per 1g dry weight and 1 mg chlorophyll (fig. 5a and 5b). An analysis of the curves in the diagrams shows that floating vegetative fronds reach the compensation point at 2400 erg $cm^{-2}s^{-1}$ light intensity. In the range of light intensities applied the value of photosynthesis increases steadily up to 60 000 ergs $cm^{-2}s^{-1}$; without saturation being reached. Immersed vegetative fronds reach the compensation point at a somewhat higher light intensity of 4800 erg $cm^{-2}s^{-1}$; at highest light intensities 36 000 — 60 000 erg $cm^{-2}s^{-1}$ photosynthesis increases only slightly, approaching saturation. The lowest gas exchange occurs in turions which reach the compensation point at a 2100 erg $cm^{-2}s^{-1}$ light intensity, and the lowest light intensity necessary for attaining the maximum photosynthetic intensity (saturation point) equals 36 000 erg $cm^{-2}s^{-1}$.

The highest photosynthetic activity is observed in vegetative floating fronds; immersed fronds show a higher respiration intensity which results in a lower photosynthetic production. The lowest respiratory and photosynthetic activity was recorded for turions. The intensity of photosynthesis recalculated to one chlorophyll unit (1 mg) gives the photosynthetic efficiency of chlorophyll. These results show that at the same light intensity the photosynthetic efficiency both for immersed and floating fronds is similar, whereas, it is much lower for turions.

b) resistance to harmful factors

Vegetative floating fronds kept in darkness for 32 days remain alive in 80%, and when transferred to light they resume growth. Vegetative immersed fronds, on the other hand, survive in darkness only in 20% and are incapable of growth when transferred to light. All turions remain alive and transferred onto a fresh nutrient solution germinate normally.

When nutrient solution with 1% sucrose is used for the culture, floating fronds survive for 32 days in darkness in 55%, and the immersed ones in 67%. All living fronds undertake growth when transferred to light. The increase in the number of immersed fronds is during an eight-day long exposure to light twice as high as that of floating fronds. This intensive increase is most probably connected with the kind of nutrient

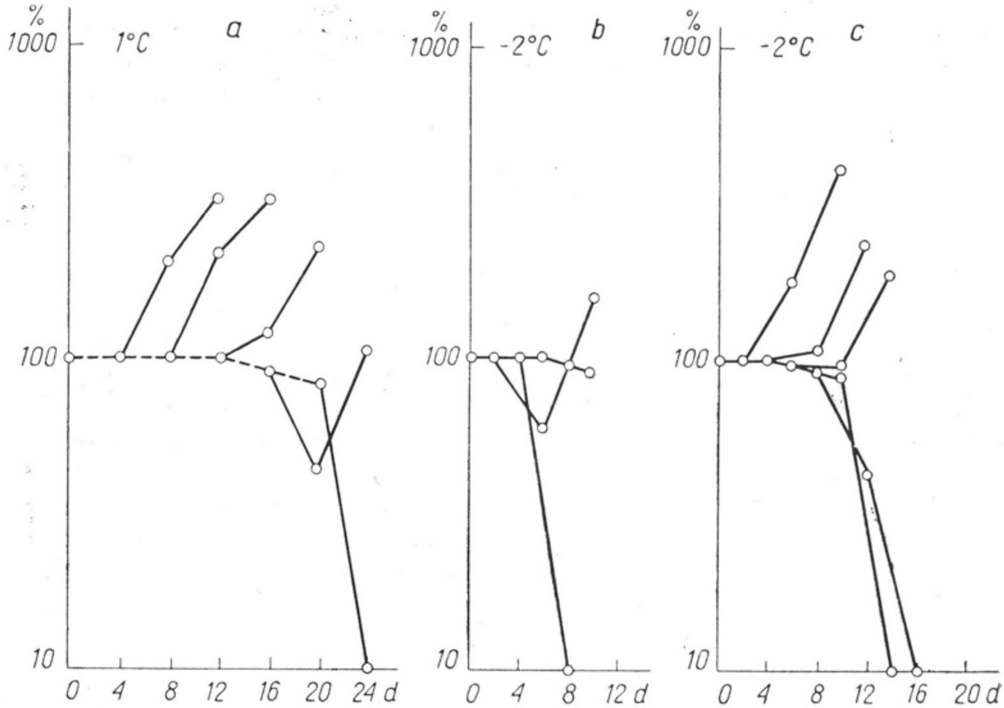

Fig. 6. Resistance of floating vegetative fronds to low temperature

x axis — time in days (24 hours), y axis — percentage of living fronds in logarithmic scale. Dashed line — percentage of living fronds at low temperature, continuous line — percentage of living fronds after transfer to 26° C.

6a — temperature 1° C, nutrient solution with sucrose, *6b* — temperature —2° C, nutrient solution wih sucrose, *6c* — temperature —2° C, nutrient solution without sucrose.

solution applied as addition of sucrose exerts a positive influence on immersed vegetative fronds.

Floating vegetative fronds (nutrient solution with sucrose) tolerate well a low temperature of 1°C for 12 days, and when transferred to 26°C they start normal growth. A 16-day exposure to this low temperature causes a slight decrease of the percentage of living fronds, however, 56% of fronds still resume growth at 26°C. After a 20-day treatment with a temperature of 1°C all fronds die (fig. 6a). A drop of temperature to —2°C kills the whole culture already after 4 days (fig. 6b). Floating fronds cultured on a nutrient solution without sucrose show a somewhat higher resistance to low temperatures; they survive at —2°C as long as 6 days (fig. 6c). Immersed vegetative fronds die when kept at temperature 1°C for 16 days (fig. 7a). A decrease of temperature to —2°C shortens this period to 10 days but after 6 days already 40% of these fronds are incapable of further growth (fig. 7b). All turions remain alive for 6 days at 1°C, but after a 2-day exposure to this temperature their germination power decreases. They cease to germinate after 14 days and die after an 18-day exposure to 1°C (fig. 8a). A temperature of —2°C

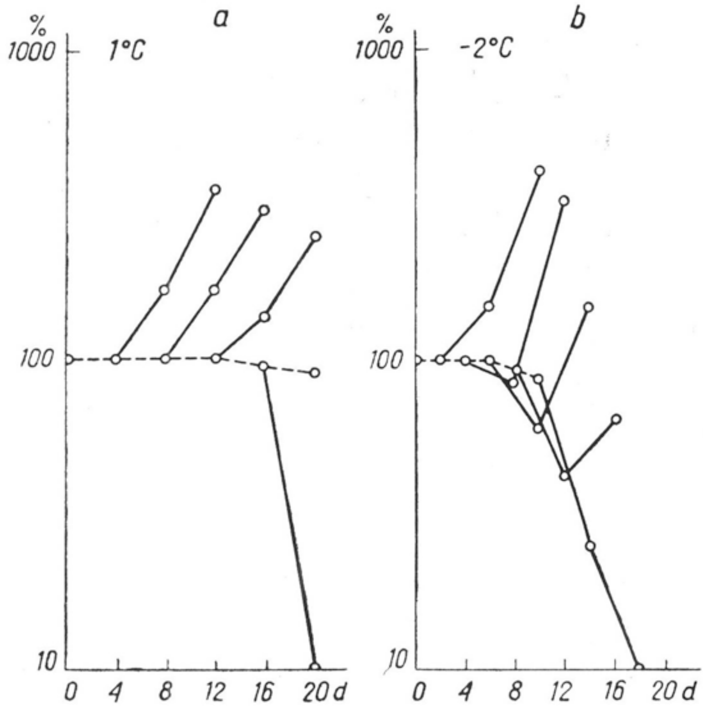

Fig. 7. Resistance of immersed vegetative fronds to low temperature (nutrient solution with sucrose). Notations as in fig. 6.
7a — temperature 1° C, 7b — temperature —2° C.

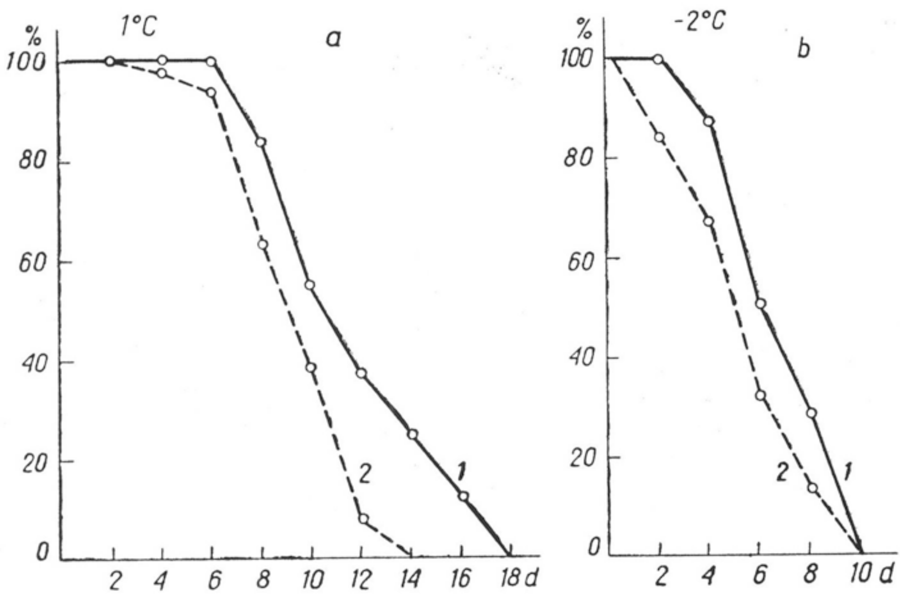

Fig. 8. Resistance of turions to low temperature (nutrient solution with sucrose) x axis — time in days (24 hours), y axis — percentage of turions, 1 — percentage of living turions capable of germination.
8a — temperature 1° C, 8b — temperature —2° C.

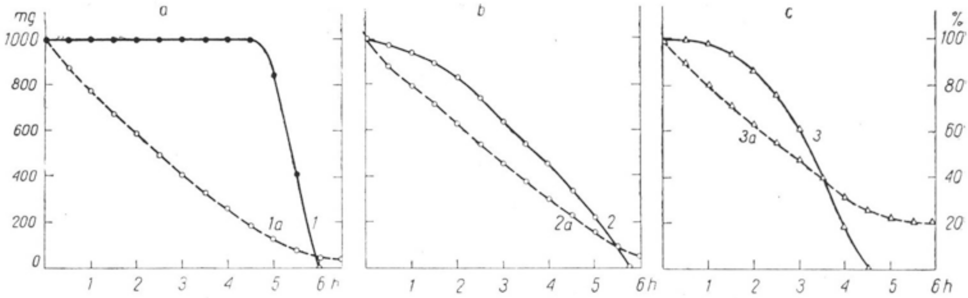

Fig. 9. Resistance of vegetative fronds and turions of *Wolffia arrhiza* to desiccation
x axis — time in hours, y axis — fresh weight in mg and percentage of living vegetative fronds or turions.
9a — resistance of floating fronds to desiccation, *1* — percentage of living fronds, *1a* — loss in fresh weight.
9b — resistance of immersed fronds to desiccation; *2* — perentage of living fronds, *2a* — loss in fresh weight.
9c — resistance of turions to desiccation, *3* — percentage of living turions, *3a* — loss in fresh weight.

causes a rapid decrease of the percentage of germinating turions, and after 10 days turions perish (fig. 8b).

Floating vegetative fronds proved most resistant to desiccation. They easily tolerate a decrease of water content of as much as 80% fresh weight; when transferred to nutrient solution they resume normal growth. A further desiccation causes a rapid decrease of the number of living fronds, but only a 95% decrease of fresh weight i.e. a 6-h long desiccation causes their death (fig. 9a). Immersed vegetative fronds can resist suffering no serious consequences — a loss of about 5% fresh weight; a further desiccation causes a decrease of the number of living fronds, which upon 96% loss of fresh weight — i.e. after 5 hours and 45 minutes desiccation — drops to zero — fig. 9b). Turions show a slightly higher resistance in this respect than vegetative fronds and survive a loss of about 10% fresh weight; a further loss of water results in a rapid drop of the number of live turions reaching zero when the loss is about 75% fresh weight, i.e. after 4 hours and 30 minutes (fig. 9c).

Taking into consideration the water content in fresh weight, the lethal deficit of water can be calculated as percentage of water content on the basis of the above given data. It is 92,7%, 68,4% and 73,4% for floating vegetative fronds, immersed ones and turions, respectively.

DISCUSSION

The morphological descriptions of vegetative fronds of *Wolffia arrhiza* and their average dimensions given by Landolt (1957), Daubs (1965) and Sculthorpe (1967) are very similar to those reported in the present paper for floating vegetative fronds. Their average dimensions are within the range given by Daubs (1965) 0,5—1,2 mm length and 0,4—1,0 mm width.

The reduction of size and form in genus *Wolffia* is connected with a simplification of anatomic structure. A rather detailed description of the anatomical structure of vegetative fronds of *Wolffia arrhiza* was given by Hegelmaier (1868). This author distinguished a small-cell upper layer of parenchyma and a lower one built of large cells with wide intercellular spaces. The epidermis, unlike that of other *Lemnaceae* is built of cells with straight-line contours. The fronds have a funnel-shaped pocket with a round centrally situated opening. A similar description of the anatomical structure of vegetative fronds of *Wolffia arrhiza* was given by Lawalrée (1943), Daubs (1965) and Sculthorpe (1967).

Observations of anatomical structure were carried out in order to determine the differences between individual fronds of *Wolffia arrhiza.* These differences are small and concern only the number and size of starch grains, the degree of opening of stomata, the size of intercellular spaces and degree of development of secondary fronds.

The difference in water content is an important feature distinguishing vegetative fronds from the resting forms. In *Spirodela polyrrhiza* vegetative fronds contain 94,6% water and turions only 61,8% (Czopek, 1959). Our investigations proved that floating and immersed fronds of *Wolffia arrhiza* contain about 97% water, whereas turions only 78,9%. Further studies aiming at characterizing *Wolffia arrhiza* forms covered biochemical examinations — analysis of chlorophyll and starch.

Werner (1967) report 10,98 mg chlorophyll for *Lemna minor* per 1g fresh weight. The present analysis established the value of 9,16 mg chlorophyll per 1g fresh weight for floating fronds of *Wolffia arrhiza;* these results are in good agreement. Wide divergences are observed in starch content. Turions of *Spirodela polyrrhiza* cultured on a nutrient solution with 1% sucrose contain 0,55 mg starch per 1g dry weight (Henssen, 1954). In a nutrient solution with the same amount of sucrose turions of *Wolffia arrhiza* contain 98,8 mg starch per 1g dry weight (own results). Such wide divergences are caused presumably by the application of different methods of starch analysis.

Individual starch grains in turions are very large — Hegelmaier (1868) reports for *Spirodela polyrrhiza* an average size of 22,5 μ and in turions of *Wolffia arrhiza* it is 13,8 μ (own results).

Respiration of turions of *Spirodela polyrrhiza* is very intensive just after formation and dropping to the bottom. Photosynthesis does not compensate this process. The specific gravity of turions decreases owing to the consumption of storage substances (Czopek, 1964). As Henssen (1954) reports, the respiratory of turions of *Spirodela polyrrhiza* drops during the first 14 days, in the following months, however, it remains at a constant level. Respiration is limited almost to minimum when turions are kept in darkness and low temperature (0—3°C). When conditions are changed, respiration increases but only as late as after 40 hours does it attain the value from before the resting period. Respiration increases in the first days of germination, reaches the maximum value on the 4th day, and subsequently drops slowly. Respiration of the forming frond is considerably more intensive than that of a turion (Czopek, 1964). Optimal temperature for respiration of vegetative fronds of *Spirodela polyrrhiza* equals 45°C and for photosynthesis 30°C (Czopek, 1967). Henssen (1954) found that photosynthesis of turions of *Spirodela polyrrhiza* is lower as comparised with that of vegetative fronds; in muddy water it is very low. Under optimal laboratory conditions it can exceed 5 to 20 times the respiration rate.

Investigations on gas metabolism in *Wolffia arrhiza* were carried out on one-month-old turions. These were kept under culture conditions, i.e. light intensity 1800 lux, temperature 26°C. On the basis of Czopek's (1964) investigations it would seem that these turions were in a stage when respiration already stabilized at a low level for some months. After illumination turions become immediately photosynthetically active. This proves that they have not passed through true resting period. Under 2100 erg $cm^{-2}s^{-1}$ light intensity turions reach the compensation point, and at 3600 erg $cm^{-2}s^{-1}$ the saturation point. The photosynthetic rate is almost 3 times that of respiration. Floating vegetative fronds reach the compensation point at light intensity 2400 erg $cm^{-2}s^{-1}$ and saturation point at 60 000 erg $cm^{-2}s^{-1}$. At this light intensity photosynthesis exceeds respiration more than 5 times.

Turions as hibernating organs should be more resistant to unfavourable factors prevailing in natural conditions such as low temperature and desiccation. Gluck (1906) reports that turions of *Utricularia vulgaris* were still alive after 12 days exposure to freezing; turions of *Hydrocharis morsus-ranae* and *Myriophyllum verticillatum* — survived a 10-day period of freezing. In *Lemnaceae* turions can still be formed at 4°C (Landolt, 1957). Turions of *Spirodela polyrrhiza* can survive —4°C for 3 months (Jacobs, 1947), whereas, vegetative fronds die after a 3-week exposure to 7°C. Turions of *Wolffia arrhiza,* on the other hand, are less resistant to low temperature. They tolerate 1°C for 18 days, but —2°C for 10 days only; they do not differ in this respect from vegetative fronds (own results).

Turions of *Wolffia arrhiza* also proved to be sensitive to desiccation. This conforms with Jacobs' (1947) results obtained for turions of *Spirodela polyrrhiza.* A period of darkness (32 days), on the other hand, does not exert a harmful influence on turions of *Wolffia arrhiza.*

A comparison of the morphological and anatomical structure, physical, biochemical and physiological properties of the described forms shows considerable differences in many respects existing between turions and vegetative forms. These differences justify the differentiation of turions of *Wolffia arrhiza* as an individual developmental form.

Wolffia arrhiza is a fresh water plant very rarely met in Poland. In most cases it appears in very muddy old fish ponds or in pits formed after peat exploitation.

Landolt (1957) carried out laboratory investigations on *Wolffia arrhiza* clones from European habitats in Germany and Holland. Landolt (1957) and Daubs (1965) give as a typical localization of *Wolffia arrhiza* in Europe the zone of the Atlantic and Mediterranean climate, especially Italy. It still occurs in France and Hungary, but is completely unknown on the western hemisphere. Besides Europe habitats of *Wolffia arrhiza* have been found in Africa (Algeria, Angola), Asia (India, Indonesia, Japan, Philippines) and in Australia.

When analysing the characteristic features of individual forms of *Wolffia arrhiza,* and especially their low resistance to cold it may be assumed that the factor limiting the occurences of *Wolffia arrhiza* is a low mean annual temperature. The plant is found in small muddy water bodies with peaty bottom. Turions embedded in mud have the chance to hibernate in unfrozen state. In the water of high peatbogs many living algae can be found in spite of low temperatures. In higher plants (Gessner, 1955) the deficiency of nitrogen characteristic of high peatbogs increases the resistance to low temperature by raising the protoplasm viscosity. Mean annual temperatures of the air in January (of 50 years) are for Polish habitats as follows: Poznań —1,4°C, Warszawa —2,9°C, Bydgoszcz —2,1°C (Statistical Annual 1948). Landolt (1957) reports for Hanover (Germany) 0,4°C, for Utrecht (Holland) 1,2°C. In spite of their low resistance to cold turions of *Wolffia arrhiza* are resting organs. When kept in the nutrient solution in which they were formed at 26°C in a light thermostat they can reserve their germinating power for 6 months. They easily bear a lack of mineral substances owing to their very high starch content. Their lower water content is another factor indicating their resting character. The rate of photosynthesis in turions is in natural conditions insignificant because turions drop to the bottom of very muddy water bodies. Turions, however, contain photosynthetically active chlorophyll, therefore, when light conditions are favourable, e.g. in February and March, when the translucency of water is at its highest, 40—50% of the over-all intensity at the depth of 1m (Pearsall, Ullyott, 1934 cit. Sculthorpe, 1967), they can presumably undertake photosynthesis. The respiration rate of turions is very low — characteristic of a dormancy.

Floating vegetative fronds of *Wolffia arrhiza* are adapted to all growth and developmental functions. Under optimal conditions they cover the water surface with several layers, and in this way can block the irrigation systems and obstruct water communication. In such cases they must be controlled by means of herbicides. They are a good food for fish, water birds and mammals (beavers, swine and cattle) Sculthorpe (1967). The such intensive growth of vegetative fronds is possible owing to a high photosynthetic rate. Floating fronds are very resistant to drought. This kind of adaptation permits them to survive periods when small water bodies dry up. In unfavourable conditions they form turions.

Immersed vegetative fronds have been described on the basis of laboratory culture of *Wolffia arrhiza*. The question whether this form exists in natural conditions can be answered only by carrying out field investigations. In view of their high photosynthetic activity a part of the floating fronds — those which have produced a greater amount of starch may presumably, drop to the bottom of the reservoir and if they find suitable conditions, they may form secondary members. The chances of survival are greater for immersed vegetative fronds than for the floating ones. Floating fronds — cultured under the same conditions — perish already after 6 weeks. In immersed fronds the parental member dies and becomes transparent; then small bright green secondary fronds can be seen in the pocket. They can remain alive for a long time. When transferred onto a fresh nutrient solution the secondary fronds grow very slowly and form normally developed immersed fronds.

SUMMARY AND CONCLUSIONS

1. The present paper gives a comparison of three developmental forms of *Wolffia arrhiza* with regard to morphological and anatomical structure, some physical, biochemical and physiological properties.

2. *Wolffia arrhiza* was cultured on Pirson's and Seidel's mineral nutrient solution with and without sucrose (1%) at light intensity 500, 1800 and 5300 lux. Temperature was kept constant during the whole culture at 26°C.

3. Vegetative fronds are ellipsoidal in shape, flattened at the top. Average dimensions of fronds depend upon culture conditions. Turions are considerably smaller, almost spherical.

4. The anatomical structure of vegetative fronds and turions is roughly similar. Differences concern the opening of stomata, size of intercellular spaces, number and size of starch grains and the degree of development of the secondary member.

5. The fresh and dry weight of vegetative fronds and turions depends upon the culture conditions, i.e. composition of nutrient solution and light intensity. Water content varies slightly and equals 97% for vegetative fronds and 79% for turions.

6. As results from the analysis of chlorophyll and starch content the highest chlorophyll content is found in floating vegetative fronds, and starch content is highest in turions.

7. Results of gas metabolism measurements showed the highest photosynthetic value in floating vegetative fronds, respiration is more intensive in immersed ones. Lowest gas metabolism rate was found in turions.

8. Vegetative fronds and turions differ in their resistance to harmful factors such as low temperature, darkness and desiccation. Floating fronds proved most resistant to desiccation and turions to lack of light. All forms show a similar not too high resistance to the influence of low temperature.

9. As a rule vegetative floating and immersed fronds are similar in many respects whereas the corresponding features of turions differ widely. This seems to indicate that turions should be considered as a developmental resting form.

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Charakterystyka form wegetatywnych i spoczynkowych Wolffia arrhiza (L.) Wimm.

II. Anatomia, własności fizyczne i fizjologiczne

Streszczenie

1. Celem pracy było porównanie trzech form rozwojowych *Wolffia arrhiza* pod względem budowy morfologicznej, anatomicznej, niektórych własności fizycznych, biochemicznych i fizjologicznych.

2. Hodowlę *Wolffia arrhiza* przeprowadzono na pożywce Pirson'a i Seidel'a mineralnej bez sacharozy i wzbogaconej sacharozą (1%) w intensywności światła 500, 1800 i 5300 lux. W czasie hodowli utrzymywano stałą temperaturę w granicach 26—28°C.

3. Pędy wegetatywne mają kształt elipsoidalny, są spłaszczone od góry. Średnie wymiary pędów zależne są od warunków w jakich przeprowadzono hodowlę. Turiony są znacznie mniejsze, prawie kuliste.

4. Budowa anatomiczna pędów wegetatywnych i turionów jest zasadniczo podobna. Różnice ograniczają się do stopnia rozwarcia szparek, wielkości przestworów międzykomórkowych, liczby i wielkości ziaren skrobii i stopnia rozwoju pędu potomnego.

5. Wartość świeżej i suchej masy pędów wegetatywnych i turionów zależna jest od warunków hodowli, tzn. od składu pożywki i intensywności światła. Zawartość wody ulega małym wahaniom i wynosi około 97% dla pędów wegetatywnych i 79% dla turionów.

6. Na podstawie analizy ilości chlorofilu i skrobii stwierdzamy, że najwięcej chlorofilu posiadają pędy wegetatywne pływające, a najwięcej skrobii turiony.

7. W wyniku przeprowadzonych pomiarów przemiany gazowej okazało się, że najwyższą wartość fotosyntezy stwierdzono u pędów wegetatywnych pływających, pędy zanurzone intensywniej oddychają. Najsłabszą przemianę gazową wykazały turiony.

8. Pędy wegetatywne i turiony różnią się odpornością na czynniki szkodliwe jak niska temperatura, ciemność, wysuszanie. Najodporniejsze na wysuszanie okazały się pędy pływające, turiony najlepiej znoszą brak światła. Wszystkie formy wykazują dosyć podobną, niezbyt dużą odporność na działanie niskiej temperatury.

9. Na ogół pędy wegetatywne pływające i zanurzone są do siebie zbliżone pod względem wielu cech, natomiast analogiczne cechy turionów znacznie od nich odbiegają. Są to dowody przemawiające za wyodrębnieniem turionów jako spoczynkową formę rozwojową.

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