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## 2. PHYSIOLOGICAL CHARACTERISTICS

### 2.1. GENERAL

Members of the family of Lemnaceae are suitable organisms to investigate physiological processes and effects of different chemical substances. The utilization of Lemnaceae is therefore wide-spread. Many laboratories throughout the world are involved in studies with Lemnaceae. The amount of papers reporting on physiological properties of Lemnaceae is steadily mounting. The specific literature is so voluminous, diverse, and complex that a complete survey on all work done can scarcely be achieved. A concise appreciation of the older literature has been made by HILLMAN (1961a). A more recent survey of the environmental requirements of Lemnaceae (and other water plants) was made by STEPHENSON et al. (1980). For most details of different methods and experimental conditions, the original literature must be referred to.

Sometimes, it is difficult to compare results of different authors due to different species used or to deviating experimental conditions and methods. This might explain some of the non-conformable results. The identity of the Lemnaceae species in the physiological literature is not always unequivocal. Changes in nomenclature have been made in this work without being mentioned. Apparent misidentifications are corrected by reference to the original name in brackets. Many of the chemical compounds used are named by customary abbreviations. These abbreviations are explained at the end of the volume (p. 582).



## 2.2. CULTIVATION

### 2.2.1. Aseptic culture technique

Aseptic culturing of Lemnaceae is generally used today. Methods to obtain sterile fronds have been described by CLARK (1932), SAEGER (1930), LANDOLT (1957), HILLMAN (1961a), BOWKER et al. (1980) and many others. The following method is used at the Geobotanical Institute of the Swiss Federal Institute of Technology (SFIT) in Zürich and is applicable to every species of Lemnaceae:

- 1) Cleaning of the new clone in water and cultivation under near optimal conditions for one to two days. In this way a good number of healthy fronds will be achieved and spores of microorganisms will eventually germinate, thus becoming more sensitive to the sterilization technique. An addition of sugar may be of advantage in some cases in which spores cannot be brought to germination otherwise. However, the cultivation time must be restricted to 1 or 2 days to prevent too much growth of microorganisms.
- 2) Dipping of single fronds in a 0.5% solution of hypochlorite (e.g. NaOCl; bleaching agents such as Chlorex, Purex, Eau de Javelle, diluted 10 times) for 1/2 to 5 minutes.
- 3) Short washing in aseptic H<sub>2</sub>O and transferring to an aseptic nutrient solution.
- 4) After several days, transferring of the fronds which propagate and float in still clear (not cloudy) solution to a new aseptic nutrient solution containing 1% sugar, 0.5% casamino acids and 0.004% yeast extract. This will show up eventual contaminations at once.

If all the fronds die or if the solutions become cloudy or covered by fungi, the treatment has to be begun again. Normally about 1-10% of the fronds succeed in staying alive and become aseptic. Some species (e.g. Wolffiella) need more attempts than others.

Table 2.1. Composition of some nutrient solutions in mg/l used for Lemnaceae growth.

\* originally  $\text{CaCO}_3$  dissolved in  $\text{HNO}_3$

1) Hutner 1/5

3) Hillman

5) Bonner-Devirian

2) Hoagland

4) Pirson and Seidel

Substances	1	2	3	4	5
$\text{KNO}_3$		1000	1515	400	85
$\text{NH}_4\text{NO}_3$	40				
$\text{KH}_2\text{PO}_4$		140	680	200	20
$\text{K}_2\text{HPO}_4$	80				
KCl					60
$\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$	40 *	600	1180		240
$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$				1200	
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	100	500	490	300	40
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	5		5.4	5	
ferritartrate		5			4
tartaric acid			3		
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$		2	3.6	0.3	
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	3				0.1
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	13	0.2	0.2		1
$\text{H}_3\text{BO}_3$	3	3	3	0.5	1
$\text{MoO}_3$		0.07			
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	5		0.12		0.025
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.8	0.08	0.08		0.03
$\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$	0.2				
$\text{Na}_2$ salt of EDTA	100				

### 2.2.2. Nutrient solutions

Some of the most used nutrient solutions are put together in table 2.1. The mineral molarities of these and further solutions are compared in table 2.2. The different nutrient solutions may be characterized as follows:

- 1) **Solution of Hutner.** The solution of HUTNER (1953) is too concentrated for most species of Lemnaceae. According to BOLGIANO (1979), it is especially the high boron content which is toxic. Therefore, the solution was diluted five times for most of our experiments. The diluted Hutner solution is characterized by rather low concentration of the main nutrients, a comparatively high concentration of trace elements, and the addition of the chelating agent EDTA. Chlorine is absent. To supply the plants with Cl, it is recommended to exchange  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  for  $\text{Mn Cl}_2 \cdot 6 \text{H}_2\text{O}$  (Cl content 0.04 mM).
- 2) **Solution of Hoagland.** The solution of Hoagland as used by JACOBS (1947) contains a large amount of nitrogen and potassium, but no sodium, and only few trace elements. To cultivate Lemnaceae on this solution for a longer time, it is necessary to add some NaCl (c. 0.1 mM) to the solution. The iron is added to the solution as tartrate which works as a chelating agent.
- 3) **Solution of Hillman** (M solution). HILLMAN (1961c) modified the solution of Hoagland by increasing the content of potassium, nitrogen, phosphorus, and calcium (and sodium).
- 4) **Solution of Pirson and Seidel.** The disadvantage of the solution of PIRSON and SEIDEL (1950) is the absence of sodium, zinc, molybdenum, copper, and cobalt. In addition, due to the lack of a chelating agent the absorption of trace elements especially iron is hampered.
- 5) **Solution of Bonner-Devirian.** The Bonner-Devirian solution as described in HILLMAN (1959b) has rather low concentrations of elements (especially trace elements except chlorine).
- 6) **Solution of Eyster.** EYSTER (1966) tried to optimize the amount of necessary elements by measuring growth rates and other characteristics of S. polyrrhiza successively varying the different elements. The solution he subsequently proposed to be optimal is distinguished by high content of nitrogen and potassium and relatively small amounts of trace elements using EDTA as a chelating agent.
- 7) **Solution of Datko 4.** DATKO et al. (1980a) worked out a solution for

Table 2.2. Comparison of the molar nutrient content (in mmol/l or mM) of 7 different nutrient solutions

1 Hutner 1/5, 2 Hoagland, 3 Hillman, 4 Pirson and Seidel, 5 Bonner-Devirian, 6 Eyster, 7 Datko, 8 Docauer

Elements	1	2	3	4	5	6	7	8
N	1.4	15	23	4	3	26	5.8	0.58
P	0.4	1	5	1.5	0.15	0.5	0.4	0.04
S	0.4	2	2	1	0.2	2	0.4	0.55
Ca	0.2	4	8	5	1	2	1.4	0.45
Mg	0.4	2	2	1	0.2	0.5	0.8	0.55
K	0.9	11	50	6	2	16.5	1.5	0.74
Na	0.2	0	0.05	0	0.01	0.01	0.11	1.49
Fe	0.02	0.02	0.02	0.02	0.02	0.5	0.06	0.036
Mn	0.02	0.01	0.02	0.002	0.001	0.01	0.01	0.004
Zn	0.08	0.0015	0.0015	0	0.007	0.1	0.003	0.002
B	0.05	0.05	0.05	0.01	0.001	0.001	0.7	0.009
Mo	0.03	0.005	0.005	0	0.0001	0.001	0.006	0.001
Co	0.001	0	0	0	0	0.001	0.005	0.0002
Cu	0.004	0.0004	0.0004	0	0.0001	0.001	0.003	0.000002
Cl	0	0.02	0.04	10	1	0.13	1.2	1.58
EDTA	0.3	0	0	0	0	0.1	0.08	0.16

optimal growth of L. aequinoctialis. The solution has a rather low content of the main nutrients and a relatively high content of trace elements (including chlorine).

- 8) **Solution of Docauer.** DOCAUER (1983) developed a medium which is comparable to natural waters (of Michigan). His solution is relatively poor in P and N and rich in Na and Cl. It also contains EDTA.

If supplemented with the missing elements, the nutrient solutions mentioned work well for most species. Optimization of some of the solutions might be achieved by adding the minimum nutrients or by consistently using the chelating agent EDTA. The content of macro-nutrients of 1/5 Hutner and of Bonner-Devirian is at the lower level of the optimum and should be enhanced. KRZECOWSKA et al. (1975) compared the frond size and protein content of L. minor grown in solutions of Hutner, Hoagland, and Pirson and Seidel (at different concentrations). The protein content was highest in fronds grown in Hoagland solution and lowest in Pirson and Seidel solution. The sequence is in accordance with the content of nitrogen in the different solutions. The frond size (not measured in the solution of Pirson and Seidel) was biggest in 0.4 Hutner and in 0.7 Hoagland solution respectively. It did not differ significantly between these two solutions.

A true analysis of optimization of the necessary amount of macro- and micro-nutrients as proposed by HOMES (1963) is still missing. Since the optimum amount of an element for best growth lies well within a wide variation (see EYSTER 1966, LUEOEND 1983, BEYER 1983), the differences between the solutions do not necessarily result in differences of growth rates. But for long-lasting experiments without regular changing of the solutions, it is important to have a well balanced medium with the concentration of the elements near the upper limit for optimal growth.

The pH of the solution is best between 5.5 and 6 (see chapter 2.3.3.3). In general, the growth rate is not influenced significantly by smaller variations of the pH. However, if ammonium is the only source of nitrogen, the pH might sink rapidly during the experiment and hamper growth or ultimately cause the fronds to die (chapter 2.3.3.3).

Some difficulties may be caused by undisturbed solutions staying at constant temperatures. As a consequence, the solution layer just below the absorbing lower surface of the fronds becomes poorer in nutrients since the absorption is faster than the diffusion from lower layers (EVANS

1972). Occasional movements of the culture vessels help to distribute the nutrients better. Also the carbon dioxide supply will be facilitated.

It is known that different species of the Lemnaceae have different needs of nutrients (LANDOLT 1957 and unpubl., ZIMMERMANN 1981, LUEOEND 1983, BEYER 1983, DOCAUER 1983). In addition, submerged species (e.g. L. trisulca, W. lingulata, W. oblonga, W. gladiata, W. denticulata) need a source of carbonate to achieve optimal growth (see chapter 2.3.3.4.5) since the diffusion of CO<sub>2</sub> in the water is too weak for good growth.

### 2.2.3. Culture and preservation techniques

Cultivation of Lemnaceae is possible in Erlenmeyer flasks or test tubes containing a suitable nutrient solution (chapter 2.2.2). The disadvantage of this simple method is that the medium has to be changed within relatively short periods in order to avoid exhaustion of certain nutrients and to keep growth rates constant. Installation of steady-state culture might be preferable. This technique is described by ERISMANN and FINGER (1968), EICHHORN and AUGSTEN (1969), STRASSER (1971), ERISMANN and BRUNOLD (1973), DATKO et al. (1978a), TSUDZUKI and KONDO (1979), and ERICSSON et al. (1982). WUESTLING and BOEHM (1980) developed a culture technique of submersed cultivation of W. arrhiza.

The preservation of clones of Lemnaceae is realized by cultivating in test tubes with an agar medium (except for most Wolffiella species which are difficult to grow for any length of time on agar). If 1% sugar and 0.004% yeast extract is added to the medium the cultures are able to grow at low light intensities, and possible infections can be detected quickly. The optimal temperature for storing Lemnaceae is dependent on the species. It should be kept as low as possible to keep growth and evaporation rate low. Species from tropical and subtropical regions need a temperature of about 20°C. The reinoculation to new test tubes with fresh medium has to be done every three months. Within this time the agar dries out and the Lemnaceae fronds begin to die.

It takes about 4 to 6 weeks to get a constant growth rate of Lemnaceae previously kept on agar and transferred to nutrient solution.

Methods of lyophilization or deep freezing are not worked out but urgently needed in order to keep large collections of living Lemnaceae strains for longer periods. In our own experiments, some species of Lemnaceae (e.g. L. minor) were able to survive lyophilization but died after a few days under normal conditions due to the difficulty of cleaning the fronds of glycerol. An adequate method has not been found up to now.

#### 2.2.4. Tissue cultures

Full-grown fronds of L. aequinoctialis transferred to a solution with 10 mg/l 2iP and 0.1 mg/l 2,4-D were able to form callus tissue out of the meristematic region around the node (CHANG and HSING 1978). L. gibba needed a ratio 10 2,4-D to 1 2iP for callus production (CHANG and CHIU 1976, 1978). IAA and NAA instead of 2,4-D did not show any effect. After 4 months of growing, the callus began to form green protuberances which later took on frond-like structure (CHANG and HSING 1978). SLOVIN and COHEN (1985c) generated primary callus derived from the meristematic region of L. gibba using 2,4-D or IAA-L-alanine as an auxine source. The same authors (1986) investigated chemical mutagenesis and soma-clonal variation in L. gibba. Plants regenerated from primary callus exhibit major variations in size and shape of the fronds. Streptomycin treatment produced a number of mutations including a highly gibbous line. NMU mutagenesis resulted in a number of white, yellow, and pale green lines. UV was found to be ineffective.



## 2.3. VEGETATIVE GROWTH

### 2.3.1. Measurements

#### 2.3.1.1. Growth rate (multiplication rate)

The growth of Lemnaceae is nearly exponential provided that the nutrient and CO<sub>2</sub> supply is sufficient and steady. Contrary to bacteria and other unicellular organisms, fronds of Lemnaceae do not propagate by mere doubling. According to species and culture conditions each frond forms up to 20 daughter fronds which become visible within different intervals (several hours to a few days). The determination of the growth rate therefore needs a sufficient number of fronds (at the beginning at least 15-20 fronds) in order to guarantee a more or less statistically based regular growth rate. The simplest method to note the growth rate is to count the visible fronds; daughter fronds are included as soon as they emerge from the pouch of the mother frond. In Lemnaceae the preconditions of cultivation have a much longer lasting effect on the growth rate than in unicellular organisms since the formation of the new buds takes place many days before their appearance. As a rule, the experimental conditions should be kept constant for at least 4 weeks before beginning the growth rate measurements. Since the appearance of new daughter fronds is enabled by the elongation of the cells, short-time change in the culture conditions (e.g. short fluctuation of temperature, replacement of the nutrient solution) may show up in a short-term change of growth rate.

The counting of fronds in the closed culture vessel may sometimes be difficult if the number of fronds is very great. Periodical photographs of the cultures might help.

The growth rate is expressed as the difference of the logarithms of the final frond number ( $F_d$ ) and the initial number ( $F_o$ ) divided by the number of days of growth. If  $\log_{10}$  is used, the result must be multiplied by 1000 to achieve a full value. The growth rate is then represented by the formula:

$$K = \frac{\log_{10}(F_d) - \log_{10}(F_o) \times 1000}{d}$$

A K of 300 means a doubling of frond number within 24 hours, a K of 150 a doubling within 2 days. If the logarithmus naturalis ( $l_n$ ) is applied the multiplication rate k is expressed as

$$k = \frac{l_n(F_d) - l_n(F_o)}{d} = \frac{K}{1000 \times 0.43} = \frac{K}{430}$$

A doubling of fronds within 24 hours results in a k of 0.7.

The average doubling time  $T_d$  (EYSTER 1966) is  $\frac{K}{300} = 0.0033 \times K = 1.4 \times k$ .

As soon as some factors (e.g. nutrients, light,  $CO_2$ ) become scarce or if some waste substances concentrate too much, the growth is no longer exponential and the growth rate not steady. Therefore, the experiments with Lemnaceae have to be made in frequently changed solutions or in steady-state culture (see chapter 2.2.3). To be sure that growth is measured within the exponential phase, the counting should be done several times successively. In this way, it should be possible to calculate a regression line according to the following formula:

$$g = \ln(F_o) + bt \quad (\text{cf. LUEOEND 1980})$$

g corresponds to the ln of the frond number at a time t. b is approximately identical with k.

Different authors (e.g. PIRSON and GOELLNER 1953, HENSSEN 1954, BORNKAMM 1966a, TILLBERG et al. 1979) observed seasonally different growth rates or other varying characteristics of Lemnaceae. To minimize seasonal effects it is advisable to check the chemical composition of the air and the light intensity periodically. For investigations which last for more than one season a control experiment with the same clone and under the same conditions should be performed periodically to detect possible deviations due to different seasonal conditions or annual periodicities. The maximal growth rates of Lemnaceae are species specific. Highest growth rates measured amount to total K of 300-315 ( $k = 0.70-0.74$ ) in L. aequinoctialis (LANDOLT 1957, DATKO et al. 1980a) and of 280-340 in W. microscopica (VENKATARAMAN et al. 1970). This growth rate corresponds to a doubling time of frond numbers between 20 and 24 hours. Lowest maximal growth rates are observed in Lemnaceae in submerged species (L. trisulca, W. lingulata, W. oblonga, W. gladiata, W. denticulata; LANDOLT 1957

and unpubl.). The limiting factor of submerged species in solutions without sugar is the carbon dioxide supply (cf. chapter 2.3.2.1.).

The cellular multiplication rate in meristematic tissue of L. minor was measured by ROMBACH (1976) as 2.7 to 3.0 times higher than the multiplication rate of fronds.

#### 2.3.1.2. Dry weight of fronds

The dry weight of a Lemnaceae frond is species specific, clone specific, and dependent on the environmental conditions. Especially under conditions which favour the storage of starch (e.g. high light intensity and high CO<sub>2</sub> concentration), the dry weight of the frond is enhanced. There is no direct correlation between dry weight of the frond and growth rate. If enough light is present, the accumulation of starch takes place even under poor nutrient conditions or low temperatures which result in slow growth. Under constant culture conditions, the increase of dry weight within a certain period is a measure for the growth rate. However, TILLBERG et al. (1979) report on big fluctuations of mean fresh weight in L. gibba at different times even under the same controlled conditions.

Highest dry weight of a Lemnacean frond was observed in S. polyrrhiza with 0.6 mg (LANDOLT 1957). Possibly, S. intermedia is able to reach values of frond weight up to 1 mg dry weight. The average dry weight of the smallest Lemnaceae frond (W. angusta or W. globosa) might be as low as 0.01 mg.

#### 2.3.1.3. Size of frond and other frond characteristics

Size of frond, length/width ratio, thickness of frond, number and length of roots, number of cohering fronds, formation of anthocyanin, number and size of papules etc. are further characteristics of Lemnaceae which have been measured to check the influence of certain experimental factors. Especially the size of the frond is a frequently used characteristic in experimental studies. A simple method of measuring the size of the frond is by photography. Cultures of Lemnaceae are pictured from above using a light from below shining through the culture. On a photoelectric cell the light intensity which is proportionate to the total frond area can be measured at once (ASHBY and OXLEY 1935). There is also

the possibility of taking a photograph. In the black-white negative, the fronds are white and the surroundings black. The light which penetrates the negative is proportional to the total size of the frond and can be measured by a photo cell (GORHAM 1941). The size of S. polyrrhiza fronds reached 39 mm<sup>2</sup> in its highest (LANDOLT 1957) under the following conditions: 32°C, 1000 lux, 1/3 Hutner solution with 1% sucrose. In herbarium samples sizes of up to 60 mm<sup>2</sup> could be observed in S. polyrrhiza. The smallest full-grown fronds of W. globosa show a size of less than 0.1 mm<sup>2</sup> (own observations).

## 2.3.2. Effects of gaseous substances

### 2.3.2.1. General remarks

Gaseous substances are absorbed by Lemnaceae through the open stomata from the air or through the lower epidermis by diffusion from the nutrient medium. The normal composition of the air allows optimal growth for Lemnaceae if circulation of air is guaranteed. Toxic gases in the air influence the growth of Lemnaceae in many ways (see chapters 2.3.2.3 to 2.3.2.6.).

NEY (1960) calculated a gas exchange rate of 2.0 mg per 100 cm<sup>2</sup> and minute for L. minor. This corresponds to a production of 11 l oxygen per m<sup>2</sup> and hour. WARD et al. (1963) measured an oxygen production of 4.4 l per m<sup>2</sup> and hour.

### 2.3.2.2. Carbon dioxide

#### 2.3.2.2.1. Influence of the CO<sub>2</sub> concentration

Autotrophic growth of L. minor was not possible at a CO<sub>2</sub> concentration of 40 ppm (0.004%) but was at 65 ppm (MUELLER et al. 1977). Under sub-optimal conditions of CO<sub>2</sub> (65 and 100 ppm), the fronds are light green and develop 1-3 cm long roots. At 330 ppm CO<sub>2</sub>, a concentration which corresponds to the normal air composition, they are dark green, have 4-6 cm long roots and a much faster growth. A supply of 9000 ppm CO<sub>2</sub> (0.9%) does not enhance the growth rate but raises the dry weight, the content of starch, free sugars, free nitrate, and free amino acids; the roots become longer and twisted like a corkscrew. The starch content at 9000 ppm CO<sub>2</sub> is 5 times higher than at 100 ppm. BJOERNDAL and NILSEN (1985) compared growth of L. gibba at a high photon flux rate in normal and in CO<sub>2</sub> enriched air (1500 ppm CO<sub>2</sub>). The high CO<sub>2</sub> content results in an increase of the net assimilation rate and in a decrease of the photosynthetic area per unit dry weight. However, it did not affect the growth rate. A significant increase of dry weight and growth rate of L. gibba was observed by ANDERSEN et al. (1985) if CO<sub>2</sub> was enriched up to 6000 ppm. This high CO<sub>2</sub> optimum was explained by the presence of non-functional stomata. The response to high CO<sub>2</sub> was less or absent following four days growth in 2% O<sub>2</sub>. The photosynthetic rate was increased by

CO<sub>2</sub> enrichment up to 1500 ppm showing only small increases with further CO<sub>2</sub> enrichment up to 5000 ppm. Figure 2.1 shows the different responses of Lemna multiplication at two concentrations of CO<sub>2</sub> and at several light intensities. EYSTER (1966) improved the dry weight increase by aeration with CO<sub>2</sub> enriched air (best results at 50000 ppm = 5%). Aeration with normal air did not bring any better results than no aeration at all. Some authors (e.g. ASHBY and OXLEY 1935, at light intensities of 15000 lux, and LANDOLT 1957 at 2500 and 6000 lux) did not achieve higher growth rates when air was bubbled through the culture flasks. On the other hand, GORHAM (1945) achieved a 10-15% higher growth rate of S. polyrrhiza when he aerated the cultures with 4% CO<sub>2</sub> (40000 ppm) at light

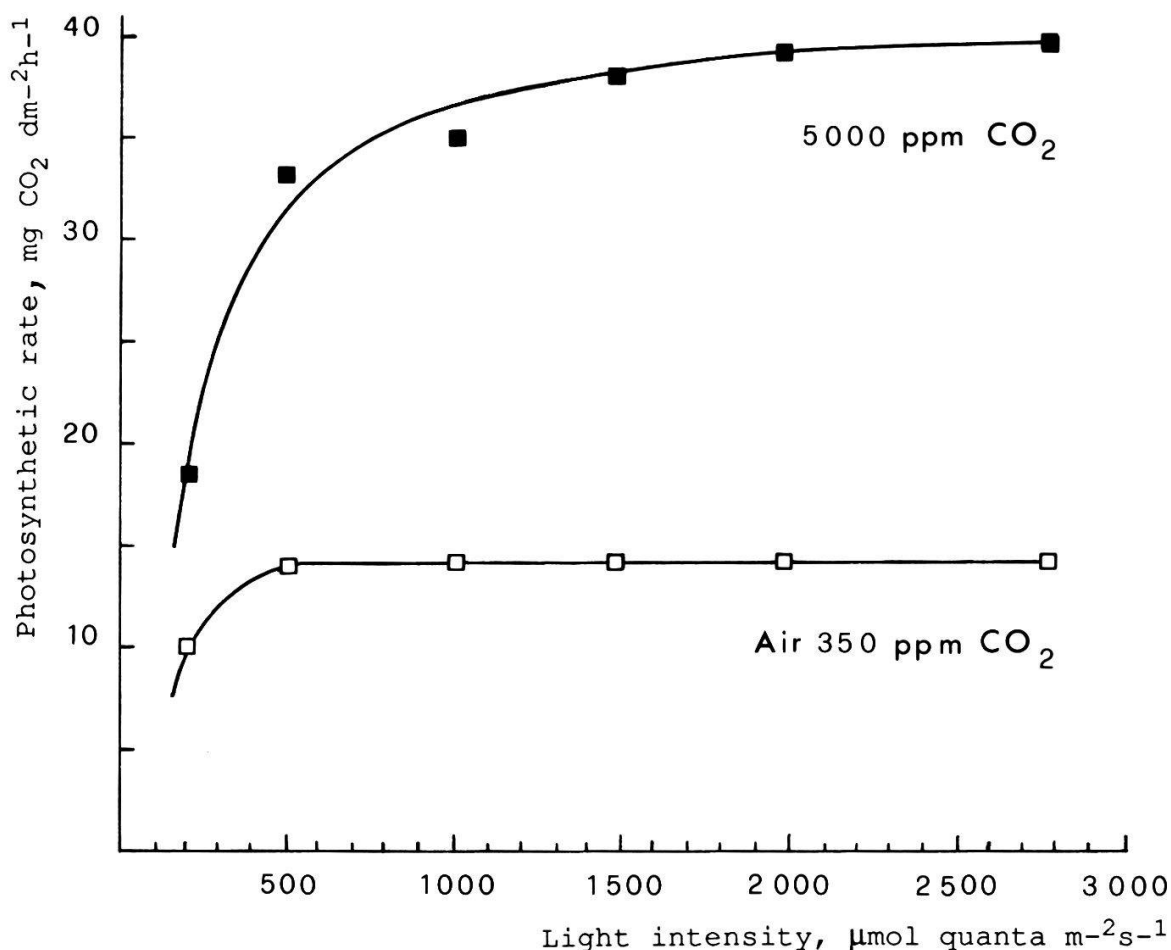


Fig. 2.1. Light response curves for photosynthetic rates of Lemna gibba measured in air and in CO<sub>2</sub> enriched air at 5000 ppm CO<sub>2</sub> (from ANDERSEN et al. 1985)

intensities of 3000 lux; at 1000 lux no growth acceleration was observed. GORHAM used test tubes for his cultures. Possibly, the diffusion of  $\text{CO}_2$  within the tubes was not sufficient to supply the necessary  $\text{CO}_2$ . A steady intermixing of the nutrient solution as a consequence of the aeration might also lead to a better supply of nutrients. In a similar way, STEINBERG (1946) was able to enhance the growth rate in crowded cultures by aeration. The growth rate of L. aequinoctialis was enhanced by aeration with 0.3%  $\text{CO}_2$ ; no further acceleration was achieved with 5%  $\text{CO}_2$  (DATKO et al. 1980a). The differences in the results with aeration are probably due to different measuring of growth rates. The growth rate measured as frond increase is not much affected by aeration with normal or  $\text{CO}_2$  enriched air except at high light intensities and under crowded conditions. In non-aseptic cultures (as used by ASHBY and OXLEY 1935) on the other hand, the  $\text{CO}_2$  might be continuously replaced even at high light intensities due to the respiration of heterotrophic organisms. However, if the frond weight and the photosynthetic rate are considered, the values increase with increasing  $\text{CO}_2$  content.

At very high light intensities, L. gibba is photoinhibited. The percentage of inhibition rises with the lowering of the  $\text{CO}_2$  content from less than 30% (1000 ppm  $\text{CO}_2$ ) to 50% (350 ppm corresponding to normal air) and 70% (100 ppm) (NILSEN and DANIELSEN 1985).

#### 2.3.2.2.2. Intensity of $\text{CO}_2$ uptake

GAPONENKO and STAZHETSKII (1969) measured a  $\text{CO}_2$  uptake per area in S. polyrrhiza at light saturation and 0.5%  $\text{CO}_2$  of 3 g per  $\text{m}^2$  and hour. LINDEMAN (1972) recorded the following  $\text{CO}_2$  uptake for L. minor (table 2.3):

Table 2.3.  $\text{CO}_2$  uptake of Lemna minor at different light intensities and 5%  $\text{CO}_2$  (according to LINDEMAN 1972)

Light intensity in W per $\text{m}^2$	$\text{CO}_2$ uptake in g $\text{CO}_2$ per $\text{m}^2$ and hour
10	0.6
70	3.1
100	3.6

Table 2.4. CO<sub>2</sub> uptake of Spirodela punctata, Lemna minor and L. valdiviana at a light intensity of 240 m<sup>-2</sup> s<sup>-1</sup> and at different CO<sub>2</sub> concentrations (from LOATS et al. 1981).

Concentration of CO <sub>2</sub> in ppm	CO <sub>2</sub> uptake in mg CO <sub>2</sub> per g fresh weight and hour		
	S. punctata	L. minor	L. valdiviana
300	105	160	140
500	150	205	215
1000	160	250	300

LOATS et al. (1981) measured the CO<sub>2</sub> uptake per fresh weight for three different species (table 2.4).

A low O<sub>2</sub> content (1%) enhances the netto photosynthesis rate of L. minor within the first days. Afterwards growth is impeded and the fronds eventually die if nitrate is used as a nitrogen source. In ammonium solutions, fronds become smaller but reproduce further at the same rate (ERISMANN and MARTI 1986).

#### 2.3.2.2.3. Mode of CO<sub>2</sub> uptake

Lemnaceae are generally thought to be C<sub>3</sub> plants. This is true for S. polyrrhiza, L. minor, and L. trisulca (ERISMANN 1972, BAUER et al. 1976, HOUGH and WETZEL 1977), and L. gibba (RAGHAVENDRA and DAS 1976) (compare chapter 2.5.1.1). According to RAGHAVENDRA and DAS (1976) and HOUGH and WETZEL (1977), Lemnaceae lack Kranz anatomy. WEDGE and BURRIS (1982) mention that Lemnaceae are not typical for C<sub>3</sub> plants in respect to light saturation and optimal temperature of CO<sub>2</sub> assimilation which are both higher than in C<sub>3</sub> plants, also the low CO<sub>2</sub> compensation point is characteristic rather for C<sub>4</sub> plants. The CO<sub>2</sub> compensation point in axenic L. minor is attained at 0.95 ppm CO<sub>2</sub> in the air (FILBIN and HOUGH 1985). However, these authors found that the activity of the PEP carboxylase and levels of the C<sub>4</sub> acids in early fixation products are very low and clearly within the range normal for C<sub>3</sub> plants.

SABAT and SAHA (1986) measured dark carbon dioxide fixation in S. polyrrhiza. It amounts to 74 pmole per g and hour at 25°C. Submerged aquatic plants fix 20 to 100 times more CO<sub>2</sub> in the dark. The fixed carbon was



found to about equal parts in the alcohol-soluble and -insoluble fractions.

#### 2.3.2.2.4. CO<sub>2</sub> uptake from the water

In aseptic cultures of some Lemnaceae species, the CO<sub>2</sub> content is a minimum factor because the diffusion of CO<sub>2</sub> in water is extremely slow. This results in a growth stop of L. trisulca and a very slow growth rate of Wolffiella species as W. gladiata, W. denticulata if sucrose or another carbon source is not added to the solution (Hutner solution does not contain carbonate) (LANDOLT 1957 and unpubl., see also chapter 2.3.3.4.5). Under natural conditions, CO<sub>2</sub> will scarcely be a minimum factor for submerged species because heterotrophic microorganisms produce CO<sub>2</sub> by respiration. Moreover, the submerged species mentioned generally grow in waters containing carbonate.

S. polyrrhiza and L. minor which float on the surface of the water are capable of utilizing carbon compounds from the air as well as from the water (WOHLER 1966). WOHLER measured a C uptake from the water in the field between 11 a.m. and 2 p.m. in mg C per g dry weight and hour for different Lemnaceae: 1.6 for S. polyrrhiza, 1.4 for L. minor, 1.9 for L. trisulca, and 2.7 for W. gladiata. Also SATAKE and SHIMURA (1983) observed in S. polyrrhiza CO<sub>2</sub> fixation from air and water. The carbon assimilation from water is comparable to that from the air under normal pH conditions (see table 2.5.). FILBIN and HOUGH (1985) state that in nature (small hard water lake in southern Michigan) 86% of carbon fixed

Table 2.5. Carbon uptake from water in Spirodela polyrrhiza in mg C per g dry weight and hour (after SATAKE and SHIMURA 1983). To compare the results of table 2.5 and tables 2.6 the values of table 2.5 have to be multiplied by a factor of ca. 2800.

pH	uptake in water	uptake in air
9.2	0.0044	0.0093
7.0	0.033	0.0079
5.0	0.051	0.0050
4.0	0.059	0.0035

Table 2.6. Carbon uptake from water in Spirodela polyrrhiza in nmol C per g fresh weight and minute (after ESHEL and BEER 1986)

nm not measured

pH	uptake in water	uptake in air
8.0	24.8	518
7.2	22.7	600
5.9	5.6	495
4.9	nm	479

by L. minor originated from aqueous inorganic carbon whereas in carbonate-free Hutner solution (in the laboratory) under saturating illumination, 63% of the carbon used in photosynthesis came from the atmosphere. The results of SATAKE and SHIMURA (1983) are questioned by ESHEL and BEER (1986) who point to some principal as well as methodological faults in these investigations. In contrast to SATAKE and SHIMURA (1983) they found, in S. polyrrhiza, only up to 5% of the carbon used in assimilation as coming from the water (table 2.6). In the water they measured the ratio of free CO<sub>2</sub> to bicarbonate ions at pH 5 as 1 : 0.04 and at pH 7 as 1 : 3.8 (see also fig. 2.9).

ALBERGONI and BASSO (1985) described an apparatus for the simultaneous measurements of photosynthetic <sup>14</sup>CO<sub>2</sub> fixation by a large number of leaf samples (tested with fronds of L. minor).

#### 2.3.2.3. Sulfur dioxide (SO<sub>2</sub>)

SO<sub>2</sub> concentrations in the air of up to 0.3 ppm do not have any direct influence on the growth of L. minor. However, the starch content of the fronds diminishes from 0.15 ppm SO<sub>2</sub> upwards. A concentration of 0.6 ppm SO<sub>2</sub> resulted initially in a reduction of the growth rate of about 25% and in signs of chlorosis. Interestingly, the tested clone of L. minor was able to adapt to the high SO<sub>2</sub> concentration within 50 days, and the normal growth rate was achieved again but the fronds remained smaller (FANKHAUSER et al. 1976). Similarly, LOATS et al. (1981) were not able to detect an influence of 0.5 and 0.75 ppm SO<sub>2</sub> on the growth rate of L.

minor and L. valdiviana. In contrast, the growth rate of S. punctata was slightly reduced. The rate of photosynthesis did not change in L. minor but became diminished in the other two species. The dry weight per frond was enhanced in S. punctata and L. valdiviana but not in L. minor under  $\text{SO}_2$  influence. SCHAEERER et al. (1975) observed an inhibition of sulfate uptake in L. minor at  $\text{SO}_2$  concentrations of 0.15, 0.32, and 0.61 ppm. YOON et al. (1981) investigated the effect of 0.5 ppm  $\text{SO}_2$  on chromosomes of L. minor.  $\text{SO}_2$  induced chromosomal nondisjunction and lowered the mitotic index. The same was found by STOECKLI et al. (1975). The authors conclude from their results that  $\text{SO}_2$  in the air leads to mutagenous effects.

#### 2.3.2.4. Hydrogen sulfide ( $\text{H}_2\text{S}$ )

Growth rate of L. minor was reduced by 15% under the influence of 6 ppm  $\text{H}_2\text{S}$ . The gas impedes the absorption of  $\text{SO}_4^{2-}$  from the nutrient solution (BRUNOLD and ERISMANN 1970, 1974, 1975). The photosynthesis rate is lowered by 10 to 14% if the  $\text{H}_2\text{S}$  content is enhanced from 6 to 60 ppm. At 120 ppm  $\text{H}_2\text{S}$  the reduction amounts to 88% but is still reversible if the  $\text{H}_2\text{S}$  aeration is stopped. The content of sulfate and cysteine within the frond is enhanced after treatment with  $\text{H}_2\text{S}$ .

#### 2.3.2.5. Ozone ( $\text{O}_3$ ) and ozonated hexene

WANGERMANN and LACEY (1952) reported a damaging effect of UV radiation on L. minor which they attribute to the action of developing ozone. Ozone damages L. minor and reduces the chlorophyll, protein, and RNA content. The sensibility towards ozone is dependent on the composition of nutrients in the medium. A high amount of nitrogen increases the loss of chlorophyll caused by ozone, a high content of iron diminishes it. The damages of ozone are less if the nutrient solution contains no copper salts (CRAKER 1971, 1972). A concentration of 0.1 ppm ozone lowers growth rate and suppresses flowering in L. aequinoctialis (FEDER and SULLIVAN 1969). According to TODD et al. (1956) and ERICKSSON and WEDDING (1956) the ozonated hexene (0.2 ppm) called synthetic smog has a stronger reducing effect on the photosynthesis rate of L. minor than ozone (1 ppm). Hexene alone in comparable concentrations had no effect. It is assumed that ozone and ozonated hexene effects the semipermeabili-

ty of the cell membranes. 100 ppm DPX, an ozone protectant, is able to counterbalance the toxic effect of 0.5 ppm O<sub>3</sub> on L. aequinoctialis (FEDER et al. 1980).

#### 2.3.2.6. Further gases

**Carbon monoxide.** According to HILLMAN (1954) CO lowers the growth rate of L. minor. However, DALY and BROWN (1954) report that Lemna takes up more oxygen under the influence of CO.

**Nitrogen.** Photorespiration of L. gibba is blocked and photosynthesis and nitrate reductase activity is reduced by aeration with 100% N<sub>2</sub> (JARCZYK et al. 1984).

**Hydrogen fluoride (HF).** HF which dissolves in water is taken up passively by turions of S. polyrrhiza by permeation of the undissociated HF through biomembranes and a subsequent dissociation to the impermeable F<sup>-</sup> ion (KRONBERGER 1983).

**Illuminating gas.** SAEGER (1933b) reports on the toxicity of illuminating gas on S. polyrrhiza.

**Tobacco smoke.** 5 blows of 35 ml tobacco smoke into an Erlenmeyer flask brought death to L. gibba plants within 48 hours. The effective substances within the smoke have been detected as dimethylnitrosamine and methylbenzopyrene. Benzantracene, the concentrate of smoke and the hydrocarbon fraction of the smoke did not show any effect on the growth rate (BHALLA and SABHARWAL 1974).

### 2.3.3. Effects of dissolved chemical compounds

#### 2.3.3.1. Absorption of dissolved compounds from the water

Water and nutrients are mainly absorbed by the lower epidermis of the fronds of the Lemnaceae which are immersed in water. The roots of Spirodela and Lemna are not very effective in taking up water and dissolved compounds. If the lower epidermis of L. minor is made impermeable with lanolin, the growth rate is greatly reduced. No effect is observed if only the upper surface is covered with lanolin. Cutting off the roots does not influence the growth rate (GORHAM 1941). BLACKMAN and ROBERTSON-CUNNINGHAME (1955) were able to reduce the toxic effect of 2,4-D on L. minor by covering the lower surface of the frond with lanolin regardless of whether the roots were intact or removed. According to SARGENT (from HILLMAN 1961a), there is no transport of water through the root of L. minor if the frond is lifted above the surface of the water with the root tip still dipping in the water. However, FERNANDEZ et al. (1972) report that in S. intermedia the absorption of 2,4-D is achieved mostly through the roots (for explanation see chapter 2.5.2.3.2).

#### 2.3.3.2. Concentration of the nutrient solution

Lemnaceae generally are able to grow within a wide range of nutrient concentrations. Even in deionized water they have been observed growing after 2-3 days (SHARP 1978). But, though Lemnaceae may remain alive in very diluted solutions for days or weeks, using the accumulated nutrients of the older fronds, a long-lasting constant growth rate is only possible in solutions with relatively high nutrient content. LANDOLT (1957) showed that different species behave differently in respect to the minimal, optimal, and maximal concentration of nutrients in the medium. In an experiment with 9 species (LANDOLT unpubl. results) the growth rate was compared in Hutner solution 1/5 and 1/20. S. polyrrhiza, L. gibba, L. aequinoctialis, W. hyalina, W. neotropica, and W. borealis developed as fast in 1/5 Hutner as in 1/20. However W. lingulata, W. oblonga, and W. gladiata had a much higher growth rate in 1/20 Hutner than in 1/5 and looked more healthy. A different utilization of nutrient concentration by 2 species (L. gibba and W. arrhiza) is shown in fig. 2.2 (from LANDOLT 1957). At higher concentrations, L. gibba is much faster

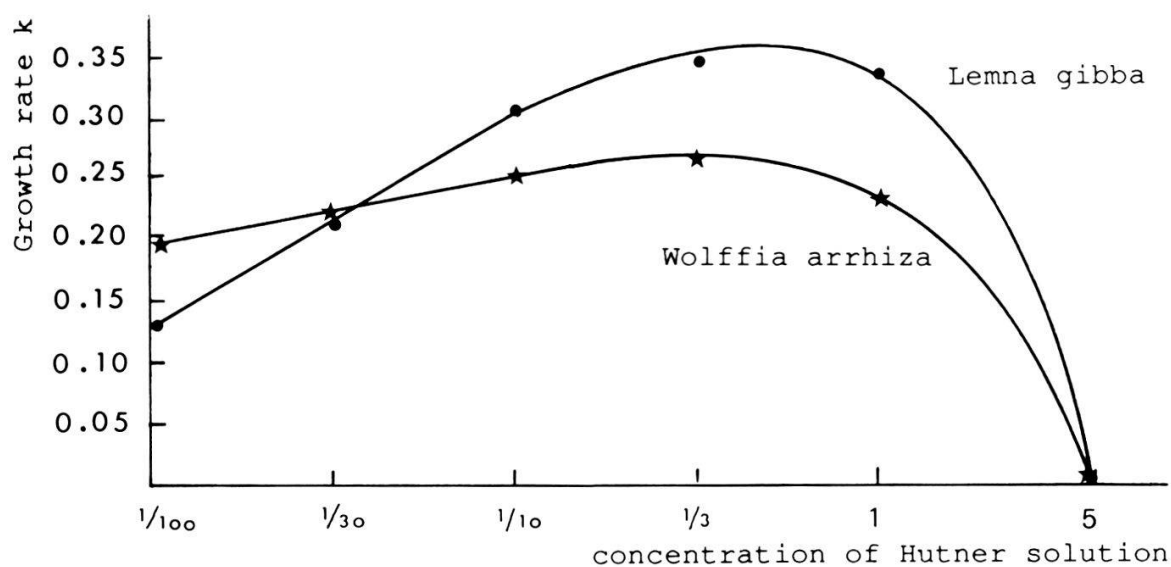


Fig. 2.2. Growth rate of *Lemna gibba* and *Wolffia arrhiza* in relation to the concentration of Hutner solution (from LANDOLT 1957)

Table 2.7. Comparison of growth rates of 15 species of *Lemnaceae* grown in Hutner solution 1/3 and 1/100. The ratio of both rates is given. Each value corresponds to a single clone (LANDOLT unpubl. results)

Species	growth rate
<i>L. gibba</i>	2.3; 1.6
<i>L. turionifera</i>	2.1; 1.8
<i>W. hyalina</i>	1.8
<i>S. punctata</i>	1.6
<i>L. minor</i>	1.6; 1.4; 1.3
<i>S. polyrrhiza</i>	1.4
<i>L. aequinoctialis</i>	1.2
<i>W. arrhiza</i>	1.2
<i>W. borealis</i>	1.1
<i>L. minuscula</i>	1.1; 1.1; 1.1
<i>W. neotropica</i>	1.0
<i>W. lingulata</i>	1.0
<i>W. gladiata</i>	1.0
<i>W. columbiana</i>	1.0
<i>W. globosa</i>	1.0

growing than W. arrhiza. In 1/30 or 1/100 Hutner, the growth rate of W. arrhiza surpassed that of L. gibba. In a similar way, CHANG et al. (1977) reported that W. globosa (named as W. arrhiza) was still able to grow in 1/50 Hutner solution and had its optimum between 1/10 and 1/20 whereas S. polyrrhiza and L. aequinoctialis achieved good growth at 1/5 and 1/10 but not in lower concentrations. LANDOLT (1957 and unpubl. results) compared growth rates of 15 species in Hutner solution 1/3 and 1/100. The ratios of growth rates  $K_{1/3}$  to  $K_{1/100}$  are shown in table 2.7.

The different behaviour of L. gibba, L. turionifera, and W. hyalina on the one hand, and of L. minuscula and most Wolffiella and Wolffia species on the other, is remarkable. It seems that low concentrations can be well utilized by species with great absorbing surface in relation to dry weight (thin fronded and submerged species).

The normal Hutner solution is too concentrated for most species (containing very high amounts of trace elements). HILLMAN (1960a) was able to neutralize the toxic effect of the full Hutner solution on W. columbiana by adding kinetin.

Spirodela and Lemna form longer roots in diluted solutions; in the same way, the appendage of W. hyalina and the conical projection of W. microscopica prolongate; also, the fronds get larger and thinner (LANDOLT, unpubl. results). HILLMAN (1954) made a similar observation with L. minor. He was able to reverse the effect by adding sucrose to the medium. Lactose or mannitol had no effect. The exact reason of the prolongation effect of low nutrient concentration is not known. Similar effects occur in solutions where only nitrate or phosphate are in low concentrations (see chapter 2.3.3.4.4.).

### 2.3.3.3. pH of the nutrient solution

#### 2.3.3.3.1. Optimal pH for growth

The influence of the pH on the nutrient absorption of water plants is complex. Especially the solubility of phosphate and of certain trace elements (e.g. Fe, Mo, Zn, Mn) changes with different pH. If chelating agents are used, the limits of pH within which an optimal ion uptake is assured are greatly extended. Good long-lasting growth of 15 species is possible between pH 5 and 8 (higher pH was not investigated) without

significant reduction of the growth rate (LANDOLT, unpubl. results), if EDTA is present in the solution. DATKO et al. (1980a) invariably noted the same growth rate of L. aequinoctialis between pH 4.8 and 7.0. Similar good growth was noted by EYSTER (1966) between pH 4 and 7 for S. polyrrhiza. The weight of the frond is generally higher at high pH (7-8.5) than at lower pH (CHANG et al. 1977). PIETERSE (1972) achieved good growth of W. gladiata in 1/3 Hutner solution between pH 5.5 and 9.0. The growth was stopped at higher and at lower pH and the fronds became yellowish. The relatively high lower pH limit for this submerged species is probably due to the difficulty to get sufficient CO<sub>2</sub> at low pH from the water (cf. chapter 2.3.3.4.5). If sugar is added, W. gladiata is able to grow at pH 4 (see chapter 2.3.3.3.2).

Especially in earlier publications, the pH tolerances for good growth of Lemnaceae often appear much narrower. These results are probably based on cultures with inadequate nutrient solutions (without chelating agent) as is the case in the study of HICKS (1932b) who checked pH tolerances in Knop nutrient solution which yielded good growth of different species only within the following pH limits: S. polyrrhiza (6.4-7.6), L. minor (6.1-6.7), L. trisulca (6.1-6.7), L. minuscula (named as L. cyclostasa) (6.1-6.7), W. gladiata (5.0-6.4), W. borealis (6.4-7.6), W. columbiana (6.4-7.0). In experiments with purified waste water, best growth for S. polyrrhiza was observed at pH 6, slightly less at pH 7.8 and 5. No growth occurred at pH 3. McLAY (1976) estimated pH optima for S. punctata with 7.0, for L. minor with 6.2, and for W. australiana with 5.0.

#### 2.3.3.3.2. Lower and higher pH limit for growth

YOSHIMURA (1950) was able to culture S. polyrrhiza at a pH as low as 3.8. When he added 1-10 mM CaCl<sub>2</sub>, MgCl<sub>2</sub>, or Al<sub>2</sub>K<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> the plants still developed at a pH of 3.2 to 3.4. It is supposed that these salts have an antagonistic effect on the H<sup>+</sup> ions. Three species of Lemnaceae from New Zealand (S. punctata, L. minor, and W. australiana) were grown in Hoagland solution by McLAY (1976). The solution was changed daily. S. punctata propagated between a pH near 3 and 10.5, L. minor and W. australiana (named as W. arrhiza) between a pH of 3-4 and 10.5. LANDOLT (unpubl. results) investigated the lower pH limit for growth of 30 Lemnaceae species in 1/10 Hutner solution (table 2.8). To lower the pH successively, NH<sub>4</sub><sup>+</sup> ions were used as sole nitrogen source. The fronds take up NH<sub>3</sub> by



diffusion and, therefore, the solution rapidly become acidified. The pH at which the cultures die is supposed to represent the lower pH limit. For each clone 3 replications were made. The variations within these replications rarely exceeded 0.1.

The lower limits of pH tolerance vary between the species, but also between clones of the same species. Species with a generally high limit (3.7->4.0) are: L. gibba, L. disperma, W. hyalina, W. microscopica, L. trisulca, S. polyrrhiza. The following species tolerate rather low pH (2.8-3.2): W. gladiata, S. punctata, W. lingulata, W. oblonga, W. denticulata, W. angusta. The first group contains mostly species which do not colonize waters poor in bases; species of the second group, however, are found occasionally in waters with a low concentration of Ca and Mg.

In nature, Lemnaceae can be observed in waters which have at least temporarily a pH of as low as 3.5 and as high as 10 (LANDOLT and WILDI 1977, LANDOLT unpubl.). It is assumed that organic substances in most of the waters with Lemnaceae have a certain chelating effect.

DOCAUER (1983) measured pH after cultivating species in nutrient solution with  $\text{NO}_3^-$  as sole nitrogen source. His cultures did not die but finally reached a pH of 10.5 (for W. columbiana), 9.5 (for L. turionifera, W. borealis) and 9.0 for S. polyrrhiza.

#### 2.3.3.3.3. Special pH effects

KOPP et al. (1974b) investigated the influence of pH on the absorption of nitrate and ammonium in L. minor. The growth rate in a medium with nitrate as sole nitrogen source is the same at pH 7.1 and 7.7 but 15% lower at pH 6.1. In nutrient solutions with ammonium as sole nitrogen source, the growth rate is similar at pH 6.2 and 7.0 but 15% lower at pH 4.7. The nitrogen content per mg dry weight is correlated with the growth rate but the starch content per mg dry weight is higher at lower pH.

A lower pH facilitates the absorption of growth regulators as the weak acids PAA and TIBA in L. minor (BLACKMAN and SARGENT 1959, BLACKMAN et al. 1959) (compare chapter 2.5.2.3.2). In a similar way the uptake of phenoles and 2,4-D is easier at a low pH than at a higher one (SIMON et al. 1952, SIMON and BLACKMAN 1953). The lethal dose of 2,4-D for L. minor is 45 ppm at a pH of 4.6 and 457 ppm at a pH of 6.1. In contrast, weak bases such as BA, are more active at a higher pH (6.2) than at a lower pH (4.2) (HILLMAN 1954, 1955).

Table 2.8. Lower pH limits for growth of Lemnaceae (LANDOLT unpubl. results)

Species	no. of clones	lower pH limits clonal values	mean value
<i>S. intermedia</i>	7361,7342,8410,7792, 7291,7178,7357,7702, 7355	3.3, 3.3, 3.3, 3.4, 3.5, 3.5, 3.5, 3.6, 3.7	3.5
<i>S. polyrrhiza</i>	7222,7551,7110,7549, 7235	3.5, 3.6, 3.7, 3.7, 3.8	3.7
<i>S. punctata</i>	6725,7111,7488,7248, 7760	3.0, 3.0, 3.0, 3.0, 3.1	3.0
<i>L. gibba</i>	6861,7218,7135,6745, 7245,7611,7107,7705, 6566,7240,7021,7179, 7007,7533,7320,7922, 7262,7257,7922a	3.4, 3.5, 3.6, 3.6, 3.7, 3.8, 3.8, 3.9, 3.9, 4.0, 4.0, 4.0, 4.1, 4.1, 4.1, 4.2, 4.2, 4.2, 4.6	3.9
<i>L. disperma</i>	7276,7818,7268,7782, 7259,7223,7190	3.6, 3.6, 3.8, 3.9 3.9, 3.9, 4.0	3.8
<i>L. minor</i>	6591,6570,7022,6580 7189,7436,7538,7295, 7210,7008,6578,7194, 6728,7018,6579	3.1, 3.1, 3.1, 3.2, 3.2, 3.2, 3.3, 3.3, 3.3, 3.3, 3.3, 3.3, 3.4, 3.6, 3.6	3.3
<i>L. japonica</i>	7182,7951,7471,8339, 8653	3.3, 3.4, 3.4, 3.4, 3.4	3.4
<i>L. obscura</i>	7982,8107,7134,7133, 7352,7599,7856,7143,	3.3, 3.3, 3.3, 3.4, 3.4, 3.4, 3.5, 3.5	3.4
<i>L. turionifera</i>	6601,6619,6853,8194, 8117,8000,7693,7137, 6735	3.1, 3.2, 3.4, 3.4, 3.4, 3.4, 3.4, 3.4, 3.5	3.4
<i>L. trisulca</i>	6624,7794,7615,8137, 7315	3.7, 3.8, 3.8, 3.8, 4.0	3.8
<i>L. perpusilla</i>	8539,8473,7507,8017, 8612	3.0, 3.1, 3.3, 3.3, 3.3	3.2
<i>L. aequinoctialis</i>	7122,8715,7476,6746, 7304,7610,7643	2.9, 2.9, 3.2, 3.3, 3.4, 3.4, 3.4	3.2
<i>L. valdiviana</i>	7005,7227,7703,8375, 7614	3.2, 3.2, 3.3, 3.4, 3.4	3.3

Table 2.8. (continued)

Species	no. of clone	lower pH limits clonal values	mean value
<i>L. minuscula</i>	8370,6711,6717,7921,	3.1, 3.2, 3.2, 3.3	3.2
<i>W. neotropica</i>	7290,7279,7609	3.0, 3.0, 3.3	3.1
<i>W. Welwitschii</i>	7644,7468	3.2, 3.3	3.3
<i>W. hyalina</i>	7378,7426,8640,7555	3.7, 3.9, 4.0, 4.2	4.0
<i>W. lingulata</i>	8041,7655,7360,7725, 8175	2.9, 3.0, 3.1, 3.2, 3.4	3.1
<i>W. oblonga</i>	7166,7923,7732,7997, 8393	2.9, 3.0, 3.2, 3.2, 3.2	3.1
<i>W. gladiata</i>	8261,7852,8066,8350, 9392	2.8, 2.8, 2.9, 2.9, 2.9	2.9
<i>W. denticulata</i>	8221,7454	3.1, 3.2	3.2
<i>W. microscopica</i>	8359	4.1	4.1
<i>W. brasiliensis</i>	7466,7925,8319,7500, 7897	3.4, 3.4, 3.4, 3.5, 3.5	3.4
<i>W. borealis</i>	7788,8592,7566,7690, 7867	3.4, 3.4, 3.5, 3.5 3.5	3.5
<i>W. australiana</i>	7605,7543,7267,7773	3.4, 3.4, 3.5, 3.5	3.5
<i>W. angusta</i>	8713,8714,7480,7274	3.1, 3.1, 3.2, 3.2	3.2
<i>W. arrhiza</i>	7323,7687a,7014,7347 7651	3.4, 3.4, 3.5, 3.5, 3.6	3.5
<i>W. columbiana</i>	7463,7467,7574,7310, 7616	3.4, 3.4, 3.5, 3.6, 3.6	3.5
<i>W. globosa</i>	8245,6592,8341,7233	3.3, 3.4, 3.5, 4.4	3.5

### 2.3.3.4. Inorganic substances

#### 2.3.3.4.1. General remarks

The requirement of a certain nutrient is partly dependent on the culture conditions. Especially the concentration of other nutrients is important. Certain antagonists might neutralize each other; some elements might be replaced by others; in addition, it is possible that pH and organic substances influence the uptake of trace elements.

Apart from C, O, and H, the following elements proved to be essential for Lemnaceae: K, Ca, Mg, N, P, S, Cl, Fe, Mn, Mo, and B. In addition, a promoting effect is attributed to Na, Li, Cu, Co, Ni, Ga, and Sr. Lemnaceae have a certain capacity to prevent the net uptake of most elements (e.g. K, Ca, Na, P, N, S, Cl). If the concentration of an element in the water reaches a certain optimal level, the content of the element no longer rises within the frond (ALLENBY 1981).

A survey of necessary elements for growth of S. polyrrhiza is given in table 2.9, distinguishing between the minimal amount which is needed for constant growth rate, the optimal amount necessary for a maximal growth rate and the maximal amount which still permits a steady growth rate.

Table 2.9. Nutrient requirements of Spirodela polyrrhiza in mM  
(according to EYSTER 1966)

Elements	minimal amount	optimal amount	maximal amount
N as NO <sub>3</sub>	<0.5	10 - 30	100
as NH <sub>4</sub>	<0.5	2.5 - 5	75
P	0.005	0.2 - 1	50
S	0.001	0.5 - 20	50
K	0.005	1 - 20	50
Na	<0.00001	0 - 60	>60
Ca	0.2	0.5 - 10	50
Mg	0.01	0.2 - 4	50
Cl	0.0001	0.01 - 1	>10
Fe	0.0005	0.01 - 0.1	1
Mn	0.00001	0.001 - 0.06	1
Zn	0.002	0.02 - 0.5	5
B	<0.00001	0.1 - 1	10
Mo	<0.000001	0 - 0.3	3
Cu	<0.00001	0.001	0.1

The effects of the different nutrients on growth of S. polyrrhiza are presented in figs. 2.3 and 2.4. It is evident that with other species (or clones) and under other conditions, the values will be different.

The effect of some inorganic toxical substances will be shown in chapter 2.3.3.5.6.6.

The addition of 50% deuterium oxide to water has an inhibiting effect on the growth of L. minor. 80% of deuterated water prevents growth (BOUDET et al. 1975). Deuterium oxide impedes protein synthesis and enhances protein degradation (2-3 times at 20% and 9-10 times at 50%) in L. minor (COOKE et al. 1979a, 1980b).

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#### **2.3.3.4.2. Monovalent alkali metals (K, Na, Rb, Cs, Li)**

##### **2.3.3.4.2.1. Potassium (K)**

Good regular growth of L. minor was achieved with 0.5-10 mM K (STRAUSS 1976). In S. polyrrhiza, the optimal amount of K was found to be between 1 and 20 mM (EYSTER 1966). At 40 mM K the growth rate is much retarded, and at 60 mM it is very low (fig. 2.3). At 0.005 mM K the growth rate of S. polyrrhiza was found to be very low too, and the fronds showed chlorotic symptoms. According to WHITE (1936a, 1938, 1939) the requirement for potassium in L. minor is dependent on the given light intensity. The lowest optimal amount of K varies between 0.05 mM at 600 lux (60 ft-c) and 5 mM at 3000 lux (300 ft-c).

Lack of potassium expresses itself in a reduced dry weight per frond in S. polyrrhiza (EYSTER 1966), in a decrease of the size of the frond, length of the root, photosynthesis rate (WHITE 1938, 1939), cell length, and time of plasmolysis (PIRSON and SEIDEL 1950) in L. minor, and in a higher Mg content in L. gibba (LIEBERT 1986b) (table 2.10).



Potassium is absorbed in exponentially growing L. minor at a constant rate. If the K content of the solution is reduced during an experiment, the uptake of K will be faster at first but will finally become slower than in the solution with a higher K content. If the K content is raised the process is inverted (YOUNG and SIMS 1972). Studies with ATP and CCCP suggest that the K uptake is an active process, and is located at the plasmalemma where the ATPase activity can be shown (YOUNG and SIMS 1973).

The influence of Ca and Mg on the uptake of K in L. minor was studied by AYADI et al. (1974). The daily periodical uptake of K (as well as of Mg,  $\text{PO}_4$ , and  $\text{NO}_3$ ) in continuous light is dealt with in chapter 2.5.9. The absorption of K in concentrations below 1 mM is linear to the logarithm of the content of K ions, above 1 mM it is much faster. Ca reduces the K uptake in concentrations of K above 1 mM (AYADI 1971).

ECHLIN and HAYES (1986) studied the distribution of elements (K, Na, Mg, Ca, P, S, Cl) within the roots. They concluded that potassium is the only element which might be not only taken up locally by the roots but transported from the roots to the aerial parts of the plants.

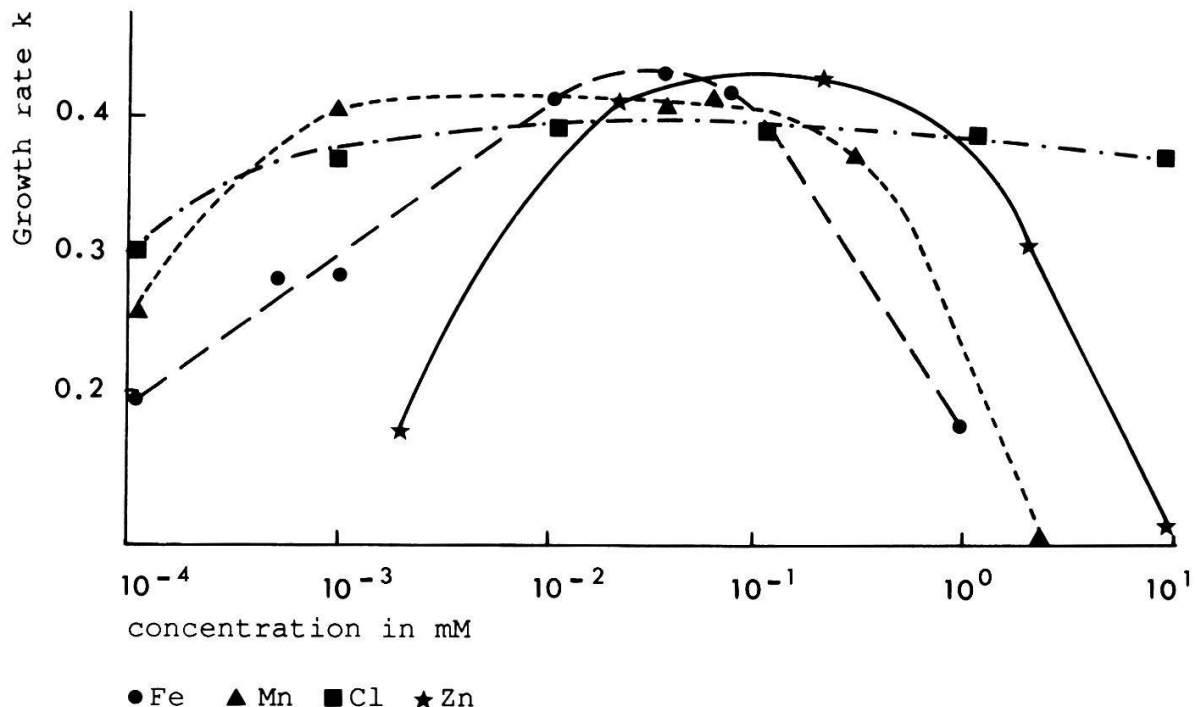


Fig. 2.4. The effect of the concentrations of iron, manganese, zinc, and chloride on the growth rate of Spirodela polyrrhiza (after data in EYSTER 1966)

#### 2.3.3.4.2.2. Rubidium (Rb), Lithium (Li), and Caesium (Cs)

**Rubidium** (contrary to Caesium) is able to replace potassium for longer times. Growth rate and photosynthesis of L. minor do not change, however the length of the root and the size of the frond are reduced (BIERHUIZEN 1954). The uptake and distribution of rubidium into the root tips of L. minor was studied by ECHLIN et al. (1982c). AYADI et al. (1971), LASSALLES et al. (1973) and YOUNG and SIMS (1973) investigated the reciprocal competition between K and Rb during the nutrient uptake in L. minor. The influence of Ca on the Rb uptake of L. minor was demonstrated by AYADI and THELLIER (1970). Substitution of K at a level of 70% or more by Rb extends the period of uptake rhythm in L. gibba G<sub>3</sub> (KONDO 1984b).

The need of **Lithium** for growth of Lemnaceae has never been proved. However, an addition of 0.15-0.72 mM Li to the nutrient solution promoted the growth of L. minor (STRAUSS 1976). Similar results are reported for S. polyrrhiza and L. minor by WIEWIORKA and SAROSIEK (1986). If 3.6 mM Li was added, the growth declined. Addition of 0.2 mM Li or more prolonged the rhythm of K uptake in L. gibba and lowered the amplitude of the rhythm. Na (contrary to Rb, Ca, or Mg) at a very low concentration (20 mM) almost completely cancelled the effect of Li (KONDO 1984a). BIELENSKI et al. (1984a,b) report on the transport of Li in L. gibba. LiCl (1 mM) inhibits flower production in the long-day plant L. gibba and stimulates it in the short-day plant L. aequinoctialis under long-day conditions (KANDELER 1970).

**Caesium** is not essential for Lemnaceae. The uptake of Cs was studied in L. minor by BERGAMINI et al. (1979). It is faster at pH 5 than at higher pH (EL-SHINAWY and ABDEL-MALIK 1980).

#### 2.3.3.4.2.3. Sodium (Na)

It is controversial if Lemnaceae need sodium for constant growth or not. However, a stimulation of the growth rate has often been observed. According to STRAUSS (1976), L. minor needs 5-10 mM Na (0.3-0.6 o/oo NaCl) for good growth. HALLER et al. (1974) achieved best growth of L. obscura (named as L. minor) by adding 14-43 mM Na to the solution. At lower Na concentrations the growth rate was considerably lower. STANLEY and MEADWELL (1976a) working with L. obscura (named as L. minor) also obtained a maximal growth rate with 25-60 mM Na. At 100 mM (6 o/oo NaCl) growth was



greatly reduced and at 150 mM Na (8.8 o/oo NaCl) nearly stopped. HALLER et al. (1974) observed a slight retardation in growth rate at 110 mM Na and attained the lethal concentration at 280 mM Na (16.6 o/oo NaCl).

HOWARD-WILLIAMS (1979) investigated the growth rate of S. polyrrhiza, L. aequinoctialis, and W. hyalina in solutions with different contents of Na (added as NaCl together with  $\text{Na}_2\text{CO}_3$  in the following concentration of Na in mg per l: 5, 20, 100, 250, 500, 750, and 1000). Best growth was achieved for S. polyrrhiza at 250 mg/l, for L. aequinoctialis at <100 mg/l and for W. hyalina at 1000 mg/l. It is not clear if this effect is due to the sodium or to the chloride concentration. However, the high optimal concentration of NaCl for W. hyalina is remarkable.

EYSTER (1966) was not able to promote growth of S. polyrrhiza by adding NaCl to the Na free solution. He measured only dry weight (and not growth rate). At a concentration of 60 mM Na and higher the total dry weight was distinctly lower than in solutions without Na.

In many chemical substances and in the glass of the Erlenmeyer flasks used, Na impurities are present. Therefore, it is difficult to prove the necessity of sodium for Lemnacean growth. In addition, it is not always certain if a positive effect of NaCl is not due to the Cl (see chapter 2.3.3.4.6). According to HUBER and SANKHLA (1979) an addition of 17-85 mM NaCl to the solution stimulates different photosynthetic enzyme activities in L. minor. However, photosynthetic fixation of  $\text{CO}_2$  is impeded at NaCl concentrations of 43-85 mM Na. The Hill reaction of isolated chloroplasts of L. minor shows a promotion at 17 mM NaCl but a slight or strong reduction at 43 and 85 mM, respectively. The internal Na content within the fronds is enhanced sixfold after addition of 85 mM NaCl, the chlorine content only twofold. The ABA level rises to 150% after addition of NaCl.

The addition of NaCl (20, 40, and 80 mM) to the nutrient solution decreased the activities of nitrate reductase, glutamate synthase and glutamine synthetase in L. minor. The activity of glutamate dehydrogenase was slightly stimulated (HUBER 1982). The author points to the fact that the ability of L. minor to assimilate ammonium via the glutamine synthetase / glutamate synthase pathway is inhibited under salt stress conditions. The direct entry of ammonium to glutamate may still be possible (HUBER 1982).

Sodium can partly replace potassium in Lemnaceae (BIERHUIZEN 1954, STRAUSS 1976), however, the sodium ion competes with the potassium ion and slows down the uptake rate of potassium (YOUNG and SIMS 1973).

#### 2.3.3.4.3. Bivalent alkali metals (Ca, Mg, Sr, Ba)

The optimal amount of **calcium** for Lemnaceae growth depends greatly on the content of **magnesium** in the solution and vice versa. Best growth with S. polyrrhiza was achieved with 0.5-10 mM Ca and 0.2-4 mM Mg (EYSTER 1966). Under optimal supply of Mg the lethal dose of Ca is 50 mM; the same limit was observed for Mg under optimal supply of Ca. The minimum amount is 0.1 mM Ca in solutions with 20 mM Mg, and 0.01 mM Mg in solutions with 10 mM Ca. Fronds in solution lacking calcium, are very small, yellowish to brown, and grow in clusters. The highest dry weight per frond is produced in solutions with 5 mM Ca and 0.4 mM Mg. STRAUSS (1973) observed the highest growth rate of L. minor at 0.2-20 mM Ca and at 0.05-10 mM Mg concentrations. The requirements for Ca and Mg by S. polyrrhiza, L. gibba, L. minor, and L. minuscula were investigated by ZIMMERMANN (1981). Good growth for all species was achieved with the simultaneous supply of 0.01-15 mM Ca and the same amount of Mg (higher concentrations have not been studied). 0.002 mM Ca and Mg did not suffice for growth. With a constant supply of 0.3 mM Mg, 0.1 mM Ca was not enough for growth. A slow growth rate was obtained with 0.6 mM Ca for S. polyrrhiza, L. minor, and L. minuscula and with 3 mM Ca for L. gibba. Specific differences between the different species showed up if the Ca content was kept at 0.3 mM and the Mg content varied. A slow growth rate was achieved at 0.001 mM Mg in S. polyrrhiza, L. minor, and L. minuscula, at 0.002 mM Mg in L. gibba. At 5 mM Mg L. gibba and L. minuscula still grew fairly well whereas L. minor and S. polyrrhiza showed a much reduced growth rate. A concentration of 7 mM Mg was lethal for all species. The results of ZIMMERMANN suggest that

- 1) Lemnaceae have more need for Ca than for Mg
- 2) Ca and Mg behave as antagonists and are able to detoxicate the effects of each other; especially a high Mg content is toxic if the Ca content is low; if the ratio of Ca/Mg is smaller than 1/20, the fronds of all investigated species die; a reduction of growth rate already takes place at a ratio of 1/10.
- 3) There are differences between species of Lemnaceae in the need of Ca and Mg.

A much more elaborate investigation on Ca and Mg requirements and tolerances of 30 Lemnaceae species is in progress (BEYER 1983 and unpubl. results). Two examples of optimal, maximal, and minimal content of Ca and

Mg in nutrient solutions needed or tolerated for long lasting growth are given in figs. 2.5 and 2.6. There are some differences between species and clones. But they are not as big as could have been expected from the different occurrence of the species in nature. A survey of the maximum and minimum Ca and Mg requirement of each species is given in table 2.11. BOLGIANO (1979) observed optimal growth rate at Ca concentrations of 0.015 mM for L. aequinoctialis and of 0.007 mM for L. minor. In solutions with low Ca concentration he noted marked influence of relatively high amounts of EDTA on growth and appearance of Lemna cultures. He assumes that EDTA removes Ca from the solution.

The frond of L. minor is larger at Ca concentrations higher than 7 mM whereas fronds of S. polyrrhiza and L. gibba are smaller and fronds of L. minuscula remain unaltered at these concentrations. At concentrations below 0.5 mM Ca, the fronds are distinctly smaller in S. polyrrhiza and L. gibba, slightly smaller in L. minuscula and not changed in L. minor compared with a medium concentration. The length/width ratio of the fronds is not varied under different concentrations of Ca and Mg (ZIMMERMANN 1981). The fronds of L. gibba are thickest at 1.5 mM Ca; the Mg concentration did not influence the thickness of the frond. At high concentrations of Ca and Mg, clusters of fronds are formed in all species (incomplete disconnections of the daughter fronds). The variation in root length with concentration of Ca and Mg is not uniform. The roots are small in all species at high concentrations of Ca and Mg. At lower concentrations, the length of the root is dependent on the ratio of Ca/Mg and species specific (ZIMMERMANN 1981). W. arrhiza becomes bigger if the Ca concentration of the Hoagland solution used is doubled. The fronds become smaller if the Mg content is raised. The length/width ratio is highest at the high Ca concentration and lowest at the high Mg concentration (SAROSIEK and WOZAKOWSKA-NATKANIEC 1984). JEFFERIES et al. (1969a) observed a reduction of growth rate of L. minor at Ca concentrations from 2 mM upwards. Different clones behaved differently. However, all clones grew more or less better at Ca concentrations of 0.3 mM than of 1 mM. The discrepancy of these results with those of previously mentioned authors in which much higher Ca concentrations still made an optimal growth possible, cannot be explained. Since the culture conditions are not described, it is difficult to discuss likely reasons (e.g. unbalanced nutrient solution with either a low Mg or K content).

Mg mg/l

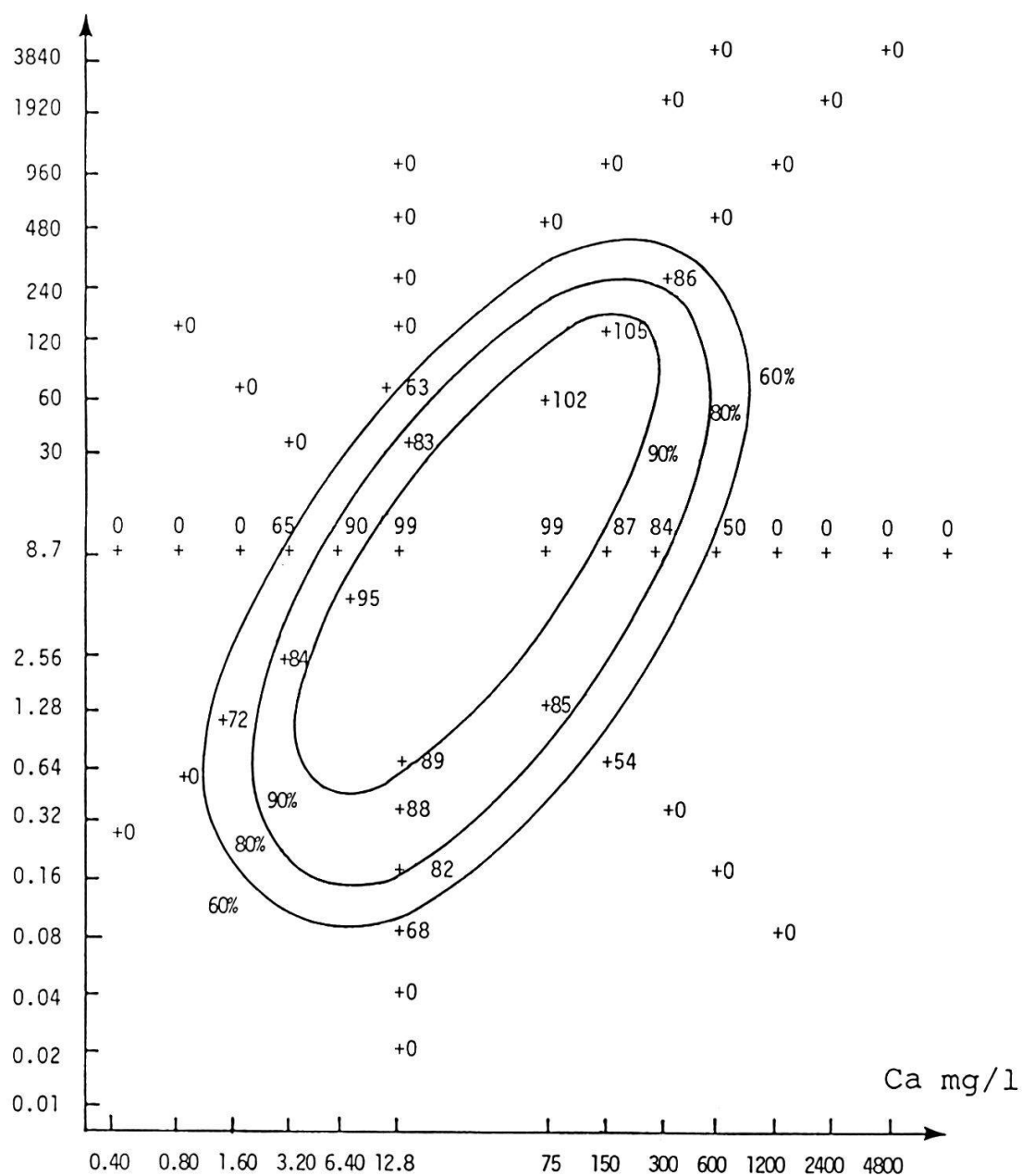


Fig. 2.5. Requirement and tolerance of *Spirodela intermedia* (no. 8410) for Ca and Mg (from BEYER, unpubl. results). Temperature 26.6°C, light intensity 14000 lux, continuous illumination. The inner curve circumscribes conditions for optimal growth rate (>90% of the maximal growth rate), the next two curves connect the points with 80% and 60% of the maximal growth rate.

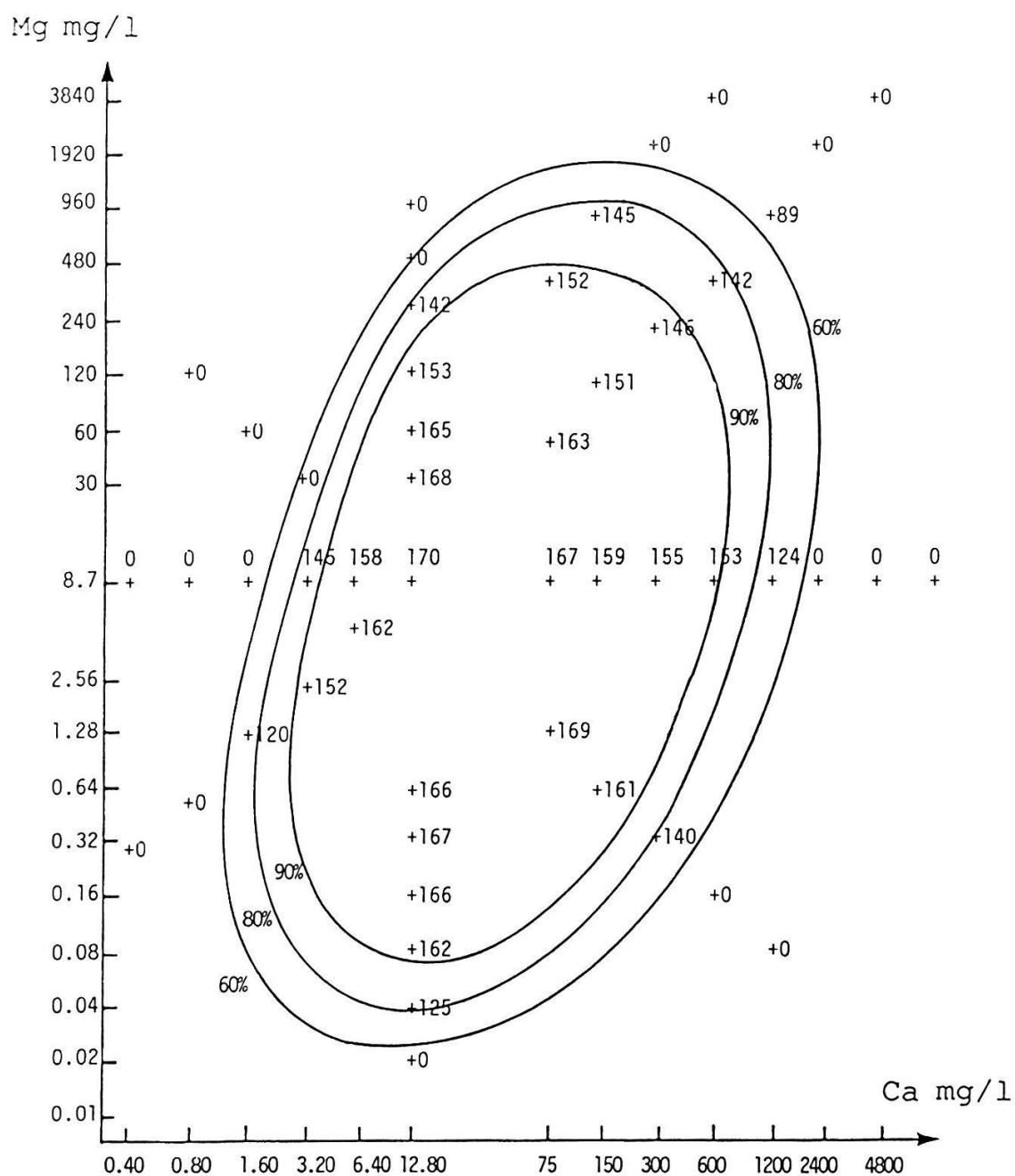


Fig. 2.6. Requirement and tolerance of Wolffia angusta (no. 8713) for Ca and Mg (from BEYER, unpubl. results).  
(Explanations see fig. 2.5)

Table 2.11. Maximum and minimum requirement of Ca and Mg (in mg/l) for long-lasting growth of Lemnaceae species (BEYER, unpubl. results)

Species	no. of clones	Ca		Mg	
		min	max	min	max
S. intermedia	7291	0.8	800	0.2	400
	7342	0.8	800	0.1	400
	7357	0.8	800	0.1	800
	7361	0.4	800	0.1	400
	7820	0.8	800	0.1	400
	8410	0.4	800	0.1	400
S. polyrrhiza	7890	0.8	800	0.1	400
	8403	0.8	800	0.1	400
	7181	0.8	800	0.1	800
S. punctata	7111	0.4	1600	0.05	800
	7449	0.4	800	0.05	800
	7487	0.4	800	0.1	800
L. gibba	7021	0.8	1600	0.1	800
	7262	0.4	800	0.1	400
	8128	0.8	800	0.2	400
L. disperma	7777	0.4	800	0.1	800
	7761	0.8	800	0.1	800
	7223	0.8	800	0.1	400
L. minor	7189	0.8	1600	0.1	800
	7210	0.8	1600	0.1	800
	7567	0.4	800	0.1	800
L. japonica	7427	1.6	1600	0.05	800
L. ecuadoriensis	8896	0.4	1600	0.05	1600
L. obscura	7780	0.8	800	0.1	800
	7720	0.4	800	0.05	800
	7134	0.8	800	0.1	800
L. perpusilla	8017	0.8	800	0.05	400
	8473	0.4	800	0.05	400
	8539	0.4	800	0.05	400
L. aequinoctialis	7122	0.8	800	0.05	800
	7300	0.8	800	0.1	400
	7743	0.8	800	0.05	400

Table 2.11 (continued)

Species	no. of clones	Ca		Mg	
		min	max	min	max
L. valdiviana	7005	0.8	800	0.1	400
	7227	0.4	800	0.05	800
	7546	0.8	800	0.05	800
L. minuscula	6726	0.8	800	0.05	800
	7180	0.4	1600	0.05	800
	7724	0.4	800	0.05	800
W. hyalina	7376	3.2	3200	0.1	800
	7555	0.8	1600	0.05	800
W. neotropica	7225	0.8	800	0.05	800
	7290	0.8	800	0.05	400
	7609	0.8	800	0.05	400
W. microscopica	8359	3.2	800	0.05	200
W. australiana	7631	0.8	1600	0.1	800
W. angusta	7274	0.8	800	0.05	800
	7480	0.8	1600	0.05	800
W. arrhiza	7246	0.8	1600	0.05	800
	8272	0.8	1600	0.05	800
W. globosa	7700	1.6	1600	0.05	800

The content of protein nitrogen, of lipochromes, and lipids of S. polyrrhiza is reduced at high concentrations of Ca (75 or 150 mM) (LECHEVAL-LIER 1977a).

The Ca uptake was studied by STELZ et al. (1975). L. minor is able to absorb maximally 0.216 meq Ca per g dry weight. It behaves as a typical calcicole species. The Ca uptake of L. gibba is hampered by BA and stimulated by ABA (DECOCK and HALL 1981). The Ca and Mg content of the medium has an influence on the K uptake (AYADI et al. 1974). According to JEFFERIES et al. (1969a), an increase of the Ca concentration raises the K uptake of W. arrhiza for a short time. After 15 days the original uptake rate is reached again.

The activity of the malate dehydrogenase is influenced by the Ca concentration of the nutrient solution. Different clones of L. minor showed different growth rates and different reactions of enzyme activities if the Ca concentration was increased. At higher Ca concentrations the enzymes partly agglomerate to aggregates of a higher molecular weight. STEWART and SIMS (from JEFFERIES et al. 1969a) found out that allosteric enzymes of L. minor (such as glucose-6-phosphate dehydrogenase, NADP isocitrate dehydrogenase, and NAD glutamate dehydrogenase) react differently towards varying Ca concentrations. According to NAKASHIMA and TSUDZUKI (1977), Ca is probably necessary in L. gibba to fix the uridine-transporting proteins to the cell membrane. BORNKAMM (1965) and LOETSCH and KINZEL (1971) are of the opinion that Ca plays an important role by precipitating the oxalic acid thereby preventing its accumulation within the cell.

According to NAKASHIMA (1979b), Mg has three different functions in maintaining the RNA synthetic activity of isolated nuclei of L. gibba:

- 1) Mg is a cofactor of RNA polymerase,
- 2) Mg is a stabilizer for the binding of RNA polymerase I to the nuclei,
- 3) Mg is a stabilizer of factors stimulating the RNA synthetic activity.

The exchange of Ca and K ions in L. minor and Ca and Na ions was studied by DEMARTI et al. (1977, 1978). Of the three ions, Ca has by far the highest affinity to the carboxyl groups of the cell wall. Similar results were obtained by MORVAN et al. (1984) who investigated the cell wall selectivity of L. minor for Ca, Mg, K, and Na. Ca was shown to have much higher affinities to the cell wall compared to other ions.

SCHREINEMAKERS (1984) suggests a coupled uptake mechanism of Ca and Zn and of Mg and Mn.

The effect of Ca and Mg on the radiosensitivity of W. arrhiza was studied by SAROSIEK and WOZAKOWSKA-NATKANIEC (1984). If the Mg and Ca content of the Hoagland solution is doubled, W. arrhiza is less sensitive to  $\gamma$ -irradiation. The DL 100 rises from 800 to at least 1300 mC kg<sup>-1</sup>.

**Strontium** (Sr) which is not an essential element for Lemnaceae becomes incorporated into the calcium oxalate raphide crystals in L. minor if added to the nutrient solution (FRANCESCI and SCHUEREN 1986). The growth rate and root length of L. minor and S. polyrrhiza are reduced with rising Sr concentrations up to 100 mg/l (c. 1 mM). S. polyrrhiza is more sensitive to Sr than L. minor (WIEWIORKA and SAROSIEK 1986).

**Barium** (Ba) inhibited growth of L. minor at concentrations of 0.1 mM and



higher. If river water of Illinois river was used in the nutrient solution instead of deionized water, no toxicity of Ba was observed even at 0.5 mM Ba (WANG 1986b).

#### **2.3.3.4.4. Nitrogen (N), phosphorus (P), and sulfur (S)**

##### **2.3.3.4.4.1. Nitrogen (N)**

###### **2.3.3.4.4.1.1. General remarks**

WOLEK (1979) reports that the requirements of nitrogen in Lemnaceae are species specific and dependent on culture conditions. To build up 1 mg dry weight of either S. polyrrhiza or W. arrhiza, 0.05-0.08 mg  $\text{NO}_3\text{-N}$  is needed. EYSTER (1966) measured a minimum amount of  $\text{NO}_3\text{-N}$  for optimal growth of S. polyrrhiza as 0.028 mg / mg dry weight. At high concentrations of N, the nitrogen requirement rose to 0.5 mg / mg dry weight. ALLISON et al. (1948) were not able to demonstrate escaping  $\text{N}_2$  from cultures of L. minor supplied with  $\text{NO}_3^-$  or with asparagine.

###### **2.3.3.4.4.1.2. Sources of nitrogen utilizable for Lemnaceae**

Lemnaceae are able to take up nitrogen in form of nitrate, nitrite, ammonium, urea, or certain amino acids. However, main sources of nitrogen for most species are nitrate and ammonium. The submerged-growing L. trisulca is able to use the following nitrogen sources in order of decreasing effectiveness (decreasing growth rates): urea, aspartic acid, nitrate, glutamic acid, arginine, ammonium, casein hydrolysate. Nitrite supports a relatively high growth rate after an initial lag of 7 days (HOLST and YOPP 1979). Fresh weight and chlorophyll content were highest with casein hydrolysate before nitrite and arginine. According to JOY (1969a) and SIMS et al. (1968), arginine is not a very suitable nitrogen source for L. minor and not at all for S. punctata. Further organic nitrogen sources for Lemnaceae are enumerated in chapter 2.3.3.4.4.1.5.

###### **2.3.3.4.4.1.3. The effect of different nitrogen supply**

EYSTER (1966) observed good growth of S. polyrrhiza between 5 and 50 mM  $\text{NO}_3\text{-N}$ . However at concentrations of 5 mM and lower the fronds looked

yellowish and produced much anthocyanin on the lower surface. For the same species, already 2.4-5 mM  $\text{NH}_4\text{-N}$  were sufficient for good growth. At 1 mM  $\text{NO}_3\text{-N}$  or  $\text{NH}_4\text{-N}$ , a slow growth rate was noted and some turions were formed; on the other hand, the growth rate slowed down at 100 mM  $\text{NO}_3\text{-N}$  or 75 mM  $\text{NH}_4\text{-N}$ . L. aequinoctialis shows a reduced growth rate at 50 mM N, wilting effects at 400 mM and dead fronds at 1200 mM (FERNANDEZ and BALDOS 1981, FERNANDEZ et al. 1983). According to INGEMARSSON et al. (1983, 1984), L. gibba is able to take up  $\text{NO}_3\text{-N}$  from solutions of 0.01 mM upwards.

At low nitrogen concentrations, the growth rate of Lemnaceae is directly proportional to the amount of N added (fig. 2.7). A reduction of the N supply results in a reduced net photosynthesis rate of L. gibba. This effect is due to a reduction in chlorophyll content (observed in L. gibba, L. minor, L. aequinoctialis) (ERICSSON et al. 1982). The respiration rate also decreases concomitantly (INGEMARSSON et al. 1983, 1984). LUE-OEND (1980, 1983) investigated the growth rates and other characteristics of 1 clone of 4 Lemnaceae species (S. polyrrhiza, L. gibba, L.

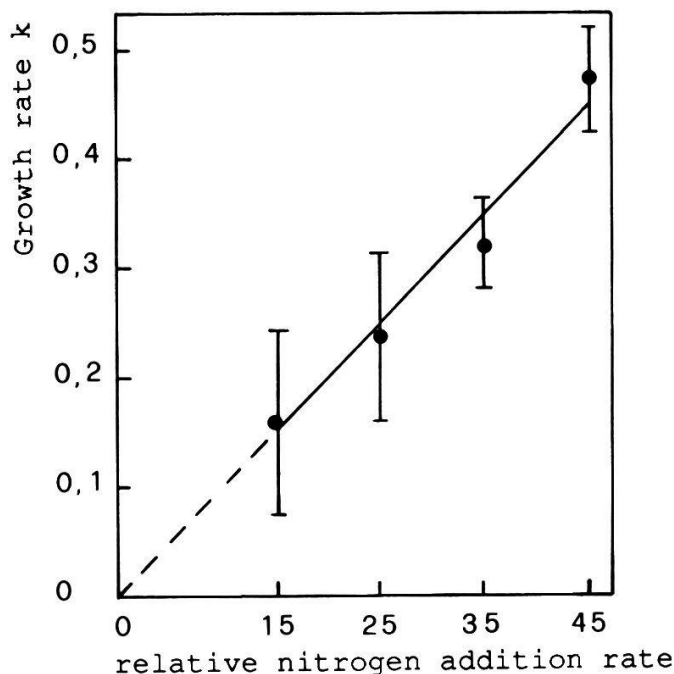


Fig. 2.7. Relation between growth rate and nitrogen supply in Lemna gibba (from ERICSSON et al. 1982)

minor, and L. minuscula) at ten different N concentrations: 0.0003, 0.0016, 0.008, 0.04, 0.2, 1.0, 5.0, 25, 125, and 625 mM. All four species died at the two highest concentrations of N. Best growth (at least 80% of the maximum growth rate) was achieved for: S. polyrrhiza at 0.2, 1.0, 5.0 and 25 mM, L. minor, L. gibba and L. minuscula at 0.04, 0.2, 1.0, 5.0 and 25 mM N (as  $\text{NH}_4\text{NO}_3$ ). Fifty percent of the maximum growth rate is still reached at N concentrations of 0.0016 mM (L. minuscula), 0.08 mM (L. minor, L. gibba), and 0.04 mM (S. polyrrhiza). Below 0.2 mM N, S. polyrrhiza sometimes forms turions.

ERICSSON et al. (1982) confirmed the results of LUEOEND which impress that L. gibba is much less able to utilize low nitrogen concentration than L. minor (and L. aequinoctialis). Table 2.12 shows the different

Table 2.12. Nitrogen requirement and nitrogen tolerance of different species of Lemnaceae: minimum amount of nitrogen (to result in half of the maximum growth rate), optimum amount of nitrogen (to achieve at least 80% of the maximum growth rate), and maximum amount of nitrogen (to reach at least half of the maximum growth rate) from DOCAUER 1983 (1), EYSTER 1966 (2), FERNANDEZ and BALDOS 1981 (3), INGEMARSSON et al. 1984 (4), LUEOEND 1983 (5), MUELLER 1983 (6), STANLEY and MEADWELL 1976a (7), WHITE 1937a (8).

\* at lower N concentrations, turions were formed

Species	requirement of N in mM		
	minimum	optimum	maximum
<u>S. polyrrhiza</u>	0.04 * (5) 0.006 (1)	5-30 (2) 1-25 (5) -10 (6)	120 (2) 100-120 (6)
<u>S. punctata</u>		-20 (6)	80-120 (6)
<u>L. gibba</u>	0.01 (4) 0.008 (5)	0.04-25 (5) -50 (6)	150-200 (6)
<u>L. obscura</u>			60-100 (7)
<u>L. turionifera</u>	0.005 (1)	0.02- 5 (6)	150-200 (6)
<u>L. minor</u>	0.005 (1) 0.008 (5) 0.008 (8)	0.2 -25 (5)	100-150 (6)
<u>L. aequinoctialis</u>			450 (3)
<u>L. minuscula</u>	0.0016 (5)	0.04- 1 (5)	30- 50 (6)
<u>W. borealis</u>	0.01 (1)		
<u>W. columbiana</u>	0.003 (1)	0.01(1)-5(6)	

reactions of Lemnaceae species in relation to the N concentration. The results are only partly comparable. It is known that different clones of one species may behave differently. The lower limit of the optimum nitrogen supply for L. gibba, for instance, varies between 0.008 and 1 mM depending on the clone investigated (DANN 1982, LANDOLT and DANN 1983). Also, a species does not show exactly the same reactions under different culture conditions. For instance WHITE (1937a) was able to demonstrate that increasing light intensities enhance the optimal N concentration for L. minor. Finally, differences can be observed between different N sources (ammonium versus nitrate) (e.g. LUEOEND 1980, EYSTER 1966). From table 2.12 we can summarize the following:

- 1) the minimum need of N for growth of different species decreases in the following sequence: S. polyrrhiza, L. gibba, W. borealis, L. minor, L. turionifera, W. columbiana, L. minuscula;
- 2) the maximum amount of N tolerated for constant growth decreases in the following order: L. aequinoctialis, L. gibba, L. turionifera, L. minor, S. polyrrhiza, S. punctata, L. obscura, L. minuscula.

The maximum frond size is reached at medium concentrations (0.04-1 mM N for S. polyrrhiza, L. gibba, L. minor and L. minuscula) (LUEOEND 1983). DANN (1982) reported greatest size of L. gibba at concentrations of 0.2-25 mM depending on the clone investigated. The greatest dry weight per frond in S. polyrrhiza was achieved at 25 mM N (EYSTER 1966). The N concentration does not significantly influence the length/width ratio of S. polyrrhiza, L. gibba, L. minor and L. minuscula (LUEOEND 1983) nor the gibbosity of L. gibba (DANN 1982, LANDOLT and DANN 1983). The length of the root is characteristic for the carbon/nitrogen ratio, according to WHITE (1937b). The longest roots are found if the rate of photosynthesis is high and the nitrogen supply low. At very low N concentrations, the roots become smaller again. This root reduction is reported by LUEOEND (1980, 1983) at the following concentrations and lower: 0.0003 mM N (for L. minor) and 0.008 mM N (for S. polyrrhiza, L. minuscula and L. gibba). This reduction is probably due to the fact that photosynthesis is already disturbed at very low N concentrations. DANN (1982) found the longest roots in most clones of L. gibba (6 of 10) at 0.04 mM N. In two clones, the maximum showed up at 0.008 mM and in the remaining two clones at 0.2 mM. Many other authors report on the influence of N concentrations on certain characteristics of Lemnaceae (table 2.13). Low N concentrations result in a reduction of growth rate, size of frond,

chlorophyll content, respiration rate, and starch content (WHITE 1936b, WHITE and TEMPELMANN 1937 with L. minor). Long roots, long root cells, reduced photosynthesis rate, reduced respiration have been observed by PIRSON and GOELLNER (1953) with L. minor. In addition, HUMPHREY et al. (1977) reported for L. minor a reduced glycolysis, a great amount of free amino acids and a high rate of protein degradation in solution with low nitrogen supply. These authors suppose a change of enzyme systems at

Table 2.13. The effect of low nitrogen supply on characteristics of Lemnaceae

+ increase, - decrease, 0 no effect,

\* at very low N concentrations the roots are shorter again (6)

a	<i>S. polyrrhiza</i>	1	DANN 1982
b	<i>S. punctata</i>	2	ERIKSSON et al. 1982
c	<i>L. gibba</i>	3	EYSTER 1966
d	<i>L. minor</i>	4	HUMPHREY et al. 1977
g	<i>L. aequinoctialis</i>	5	INGEMARSSON et al. 1984
h	<i>L. minuscule</i>	6	LUEOEND 1983
		7	PIRSON and GOELLNER 1953
		8	THORNSTEINSSON et al. 1985
		9	WHITE 1936b, WHITE and TEMPLEMAN 1937
		10	TANAKA 1986

Characteristics	effect	species	author
area of frond	-	a,d,h	3,6,9
dry weight	-	a	3
length/width ratio	0	a,c,d	6
	+	h	6
gibbosity	0	c	1
length of root	+ *	a,c,d,h	6,7,9
length of root cells	+	d	7
flowering	+	g	10
turion formation	+	a	3,6
nitrogen content	-	c,g	2
starch content	-	d	9
content of free sugars	0	c	8
anthocyanin content	+	a,c	3,6
chlorophyll content	-	c,d,g	2,9
content of free amino acids	+	d	4
respiration rate	-	c,d	5,7,9
photosynthesis rate	-	c,d,g	2,7
glycolysis rate	-	d	4

low N concentrations. The content of free sugars is not affected (THORSTEINSSON et al. 1985). In L. gibba, the ratio root length / dry weight of the fronds is higher under lower N concentration (INGEMARSSON et al. 1984).

The contents of nitrogen and chlorophyll in the fronds of L. gibba, L. minor, and L. aequinoctialis are proportional to the nitrogen supply. With limited nitrogen, the net  $\text{CO}_2$  assimilation rate per frond area is reduced (ERICSSON et al. 1982). The nitrogen content of Lemnaceae, the rate of nitrate uptake as well as the nitrate reductase activities are not dependent on the nitrate concentration in the nutrient solution when medium concentrations of  $\text{NO}_3^-$  are administered (MUELLER 1983). However, the enzyme activity was species specific. Grown on a medium containing 5 mM  $\text{NO}_3^-$ , the species showed the following enzyme activities in  $\mu\text{mol NO}_3^-$  per g dry weight: 40-53 for S. polyrrhiza and S. punctata, 25-45 for L. turionifera and L. minor, and 10-28 for L. gibba. Maximum nitrate reductase activity in L. minor was achieved at 0.25 mM N, half maximum rate at 0.1 mM (STEWART 1972a).

L. minor does not show aldolase activity in N deficient solutions because an accumulation of low-molecular proteins hampers this process (SARAWEK and DAVIES 1977b). If nitrogen is lacking, proteins are transformed more frequently, and newly formed amino acids are utilized (DAVIES and HUMPHREY 1978). N deficiency reduces the life-span of L. minor fronds (BOESZOERMENYI and BOESZOERMENYI 1957), it stimulates turion formation of S. polyrrhiza and results in progressive senescence of the fronds (MALEK and COSSINS 1983a).

The optimal nitrogen supply for turion germination of S. polyrrhiza is 1 mM (as  $\text{KNO}_3$ ), according to SIBASAKI and ODA (1979). No germination could be observed in solutions without nitrate.

In mixed cultures of natural ponds, the optimal supply of the Lemnaceae with nitrogen is reached between 4 and 8 mg per liter (0.3-0.6 mM N). Higher N concentrations in the water do not raise the N content of the fronds which reaches about 6% of the dry weight (see fig. 1.1, p. 14) (REJMANKOVA 1979, 1981).

#### 2.3.3.4.4.1.4. The effect of nitrate and ammonium; uptake mechanisms

ULLRICH (1987) concludes from a literature survey that Lemnaceae take up  $\text{NO}_3^-$  ions by nitrate/ $\text{H}^+$  cotransport, and  $\text{NH}_4^+$  ions by an ammonium

import which is antagonized by the plasmalemma proton extrusion pump. Therefore the uptake of  $\text{NO}_3^-$  ions results in an elevation, the uptake of  $\text{NH}_4^+$  ions in a reduction of the pH of the solution. However, JUNG-NICKEL and IBRAHIM (1983) report that L. minor is not able to use nitrate in a solution if the pH becomes neutral or weakly alkaline. This is not in accordance with the fact that L. minor and other Lemnaceae species are fast-growing in weakly alkaline solutions if supplied only with nitrate as a nitrogen source (e.g. KOPP et al. 1974a). Several publications deal with the different effect of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  as a nitrogen source on morphological characteristics and metabolism of Lemnaceae. LUEOEND (1980, 1983) investigated the effect of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  as sole nitrogen sources for S. polyrrhiza, L. gibba, L. minor, and L. minuscula. The form of nitrate ( $\text{KNO}_3$ ,  $\text{Ca}(\text{NO}_3)_2$ , or  $\text{NaNO}_3$ ) did not produce any difference in the growth rates. A 10-20% higher growth rate was achieved if  $\text{NH}_4\text{Cl}$ ,  $(\text{NH}_4)_2\text{SO}_4$ , or  $\text{NH}_4\text{NO}_3$  were used. The greatest difference was observed in S. polyrrhiza. The roots of all species were distinctly longer in solution with  $\text{NO}_3^-$  than with  $\text{NH}_4^+$ . Investigations of LANDOLT (unpubl.) resulted in the same direction for roots of S. polyrrhiza, L. gibba, L. disperma, L. minor, L. obscura, L. turionifera, and L. aequinoctialis. The projections of W. microscopica but not the appendages of W. hyalina behaved in the same way. Also for S. punctata it is known that roots become longer in solutions with nitrate than with ammonium (FERGUSON and BOLLARD 1969).

According to MUELLER et al. (1977), the difference in growth rate of L. minor cultivated in nitrate or ammonium solutions depends on the  $\text{CO}_2$  concentration of the air. At low concentrations (100 ppm), no difference was observed whereas at high concentrations (9000 ppm) the culture with ammonium showed a slightly higher growth rate. HUBALD and AUGSTEN (1977a) achieved a greater dry weight but only an insignificantly higher growth rate in L. gibba supplied with ammonium than with nitrate. FELLER and ERISMANN (1973) recorded a somewhat higher ash content for L. minor with nitrate. The malate and iron content was enhanced seriously and the nitrogen content lowered (ca. 20% less). If ammonium was the sole nitrogen source, the following compounds were at a higher level in S. punctata (FERGUSON 1966, 1969a, 1970, FERGUSON and BOLLARD 1969): ammonium, arginine, asparagine, and glutamine. Similarly, ERISMANN and KIRK (1969) and MUELLER et al. (1977) observed more glutamine and alanine and less aspartic acid and glutamic acid in L. minor. In cultures with ammonium



as a nitrogen source, L. minor produces more free sugar and shows higher specific activity of glycolate oxidase than in cultures with nitrate (EMES and ERISMANN 1982). If supplied with ammonium, one of the primary assimilation products of L. minor is glutamic acid (60%) (BAUER et al. 1973). The protein content of L. minor is 20-30% higher in ammonium than in nitrate solutions (DICHT et al. 1976). Lowering the temperature from 25°C to 15°C resulted in lower net C fixation and lower oxidation/carboxylation ratio of L. minor if supplied with ammonium as the only nitrogen source. In nitrate solutions, only the net C fixation was reduced. Glyoxylate decarboxylation is stimulated by ammonium added to the solution (FUHRER and ERISMANN 1984). Ammonium and glutamine in the medium both activate the glutamine synthetase in L. minor (RHODES et al. 1975). The NAD dependent L-glutamate dehydrogenase of L. gibba was increased 5 times if the nitrogen source was ammonium or glutamate instead of nitrate (SHEPARD and THURMAN 1973).

The form of the nitrogen has also a different effect on the anthocyanin formation in different species (LANDOLT, unpubl. results). S. polyrrhiza and L. turionifera show a similar degree of pigmentation in solutions with ammonium and nitrate, L. gibba and L. disperma have more anthocyanin in nitrate solutions, and L. obscura is most intensely coloured in ammonium solutions. 0.1 mM  $\text{NH}_4^+$  added to the solution inhibits flowering of L. gibba (KANDELER 1969a) and L. aequinoctialis (HILLMAN and POSNER 1971). Many publications deal with the mode of uptake of nitrate, nitrite, and ammonium. Since the uptake of the different forms of nitrogen results in a change of pH, it would be desirable to keep the pH at a constant level in all investigations. This was not done in most studies. This might explain some of the deviating results. According to FERGUSON and BOLLARD (1969) and GROB et al. (1973), S. punctata takes up  $\text{NH}_4^+$  first, if  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are offered simultaneously. Within the first 10 days 80% of the ammonium but only 20% of the nitrate is used. The preference for ammonium is explained by the fact that the activity of nitrate reductase is inhibited by ammonium. INGEMARSSON et al. (1984) investigated the nitrogen utilization in exponentially growing nitrogen-limited cultures of L. gibba. The successive uptake of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  from the solution supplied daily with  $\text{NH}_4\text{NO}_3$  is shown in fig. 2.8. FELLER and ERISMANN (1971) reported similar results with L. minor. Ammonium was taken up first, whether the plants were cultivated in ammonium or nitrate containing solutions before. PORATH and POLLOCK (1982) corrobor-



ated these results with L. gibba. S. polyrrhiza also prefers ammonium if both ammonium and nitrate are supplied (TATKOWSKA and BUCZEK 1983). According to FERGUSON (1966, 1969a, 1970) and FERGUSON and BOLLARD (1969), nitrite can be absorbed together with ammonium. Arginine, asparagine, and glutamine do not influence the activity of nitrate reductase. The nitrate reductase is also present in the frond when nitrogen is lacking in the solution. However, for maximal activity  $\text{NO}_3^-$  ions are important. Inactivation of nitrate reductase by ammonium was also observed by STEWART et al. (1974) and OREMBAJO and STEWART (1974, 1975a,b). PORATH and AGAMI (1986) noted a correlation between the ammonium (but not the nitrate) content of the water and the occurrence of Lemnaceae in the Negev

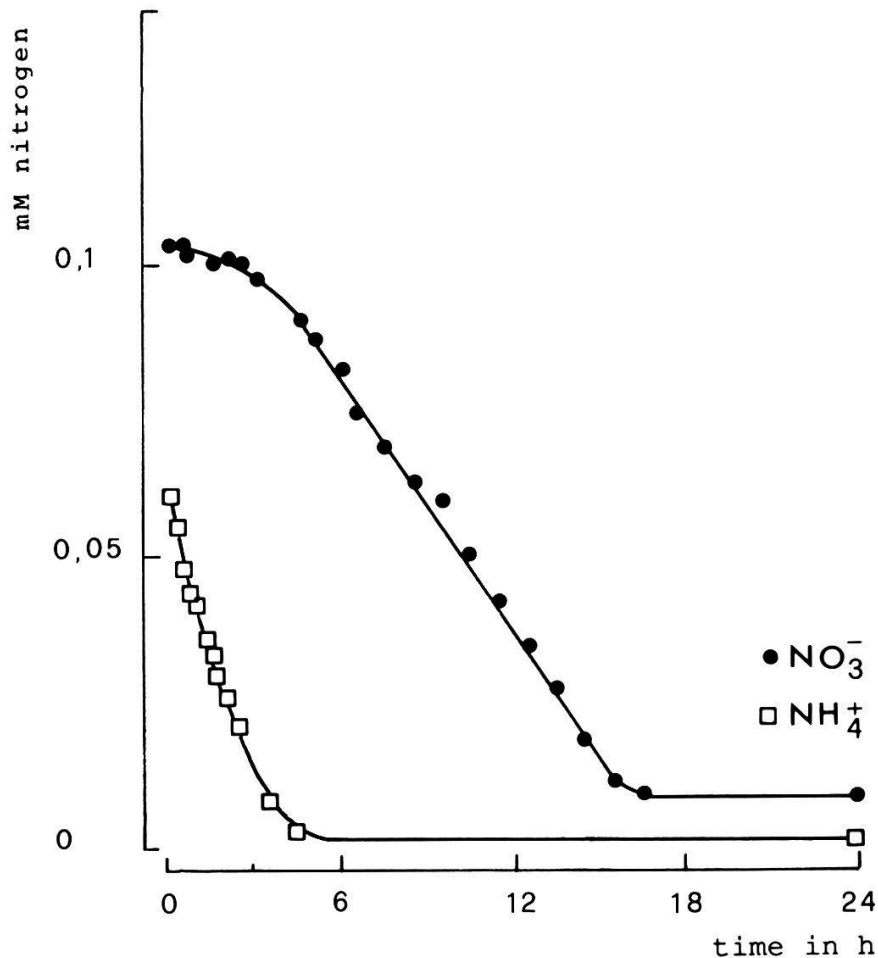


Fig. 2.8. Depletion of ammonium and nitrate in the medium by Lemna gibba adapted to a relative nitrogen addition rate of 0.25 per day (from INGEMARSSON et al. 1984a)

desert. Since nitrogen and phosphorus were the only chemical parameters studied by the authors the results that the nitrate content is of no importance for the growth of Lemnaceae in nature is not conclusive. JOY (1969a) noted, for L. minor, that the level of nitrate and nitrite reductase remains low in solutions with ammonium or with amino acids. STEWART (1972b) reported similar results. Nitrate reductase in S. punctata is only formed if nitrate or nitrite are present in the solution. High levels of nitrate reductase activity can be induced by the addition of ascorbate to the solution (FERGUSON and KNYPL 1974). Whereas in L. minor nitrate is able to stimulate the formation of nitrate as well as nitrite reductase, the effect of nitrite is restricted to the formation of nitrite reductase (JOY 1969a). No nitrate reductase was observed in W. arrhiza either when nitrite as the only nitrogen source was in the solution (SWADER and STOCKING 1971). The nitrate reductase activity of W. microscopica was studied by BAKSHI (1983). GROB et al. (1973) showed that in cultures of S. punctata stimulated by glucose, both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are absorbed simultaneously (20%  $\text{NO}_3^-$  and 40%  $\text{NH}_4^+$  after 3 days). MELZER and MUELLER (1984) studied nitrate reductase activities of 6 Lemnaceae species using an in vivo test. The activity was strongly enhanced in L. gibba with increasing nitrate concentration in the medium while it was inhibited in L. minuscula. The behaviour of S. polyrrhiza, S. punctata, L. minor and L. turionifera was somewhere between (MUELLER 1983). The nitrate reductase activity of L. trisulca is very low even if it is transferred to water rich in nitrate (MELZER 1980). An enhancement of the nitrate concentration from 0.03 to 0.8 mM did not raise the activity of the nitrate reductase. An addition of 0.3 mM ammonium inhibited the nitrate reductase activity to about 50% after 24 hours. No inhibition was observed in solutions with 0.0006 mM ammonium (MELZER and EXLER 1982, MELZER and MUELLER 1984).

The nitrogen uptake in L. gibba was studied in detail by ULLRICH and co-workers. They observed a different behaviour of nitrogen-starved fronds and fronds supplied with sufficient nitrogen. In fronds cultivated on nitrate solution previously, the  $\text{NO}_3^-$  uptake was slow and not inhibited by the addition of ammonium. However, nitrogen-starved fronds absorbed nitrate rapidly and ammonium still faster if given separately. An addition of ammonium to the nitrate solution immediately reduced the nitrate uptake to about one third until the ammonium was consumed. Phosphate uptake was inhibited by ammonium similarly to nitrate uptake, but only in

fronds starved of P and N simultaneously not of P alone. The inhibition of the nitrate uptake seems to be due to a decrease of pH rather than of membrane potential depolarisation, and occurs when this decrease cannot be overcome by the  $H^+$  extrusion pump (ULLRICH et al. 1984a,b). LESCH and ULLRICH (1984) observed two phases of ammonium uptake in L. gibba at a pH of 6.5. At the beginning of uptake, the rate is high and scarcely stimulated by light nor impeded by blockers of energy metabolism. The uptake following later is generally lower, in light much higher than in darkness, and sensitive to blockers. The authors conclude that the ammonium uptake is performed by carrier transport as well as by diffusion (at least at pH 7 and higher). The uptake during the first phase of the carrier transport is limited by the bypass through the plasmalemma, during the second phase by the utilization of ammonium within the metabolism. Nitrate reductase activity in S. punctata is 8 times higher in the light than in darkness. Sucrose, glucose, and fructose increase the activity in the light as well as in darkness; DCMU inhibits it completely in the light. Under these conditions ammonium stimulated the nitrate reductase activity whereas amino acids decreased it (VIJAYARAGHAVAN et al. 1982). Contrary to these findings and to results with green algae which absorbed up to 50 times more nitrate in the light than in darkness, the nitrate uptake is not stimulated significantly in L. gibba by light (ULLRICH 1979). The uptake of nitrate in W. arrhiza was studied by SWADER and LIN (1975). They detected two different uptake mechanisms: one for low concentrations of nitrate (0.5-0.75 mM N) and another for higher concentrations. The  $NO_3^-$  uptake is an active process, 6-methylpurine blocks the nitrate reductase synthesis which results in a reduction of nitrate uptake (TROGISCH and ULLRICH 1984). According to INGEMARSSON et al. (1985), the uptake of nitrate by Lemna is influenced by the intracellular nitrate concentration. As long as there is a potential for  $NO_3^-$  storage in the vacuole, the uptake exceeds the reduction and the nitrate is divided between the readily converted metabolic pool and the storage pool. Once the vacuolar pool is full, the uptake equals assimilation. The nitrate assimilation at low pH is stimulated by Mo (YOSHIMURA 1943).

The complexity of the preferential uptake of ammonium against nitrate is shown in a preliminary experiment with twelve species (LANDOLT unpubl. results). Each species was cultivated in solutions with  $NO_3^-$  or  $NH_4^+$  as sole nitrogen sources, and with  $NH_4NO_3$  at the same N concentra-

Table 2.14. pH values of culture solutions with nitrate, ammonium-nitrate, and ammonium after cultivation of 14 and 40 days. Initial pH 5.5 (LANDOLT unpubl. results)

Species	no. of clone	NO <sub>3</sub> <sup>-</sup>		NH <sub>4</sub> NO <sub>3</sub>		NH <sub>4</sub> <sup>+</sup>	
		14	40	14	40	14	40
S. polyrhiza	7010	7.7	4.6	7.0	4.0	3.7	3.7
L. gibba	7135	7.2	4.8	5.8	4.2	3.5	3.5
L. disperma	7223	7.7	6.7	6.6	4.6	3.6	3.7
	7782	7.4	5.2	6.2	4.5	3.5	3.5
L. minor	6578	7.6	5.0	6.9	4.3	3.4	3.2
L. obscura	7856	7.7	5.4	7.3	4.2	3.6	3.5
L. turionifera	6853	7.4	5.7	6.4	4.3	3.3	3.3
L. aequinoctialis	6746	7.7	5.2	6.9	4.5	3.2	3.3
L. minuscula	6600	7.6	4.4	6.3	3.8	3.2	3.2
W. hyalina	7376	8.1	6.1	6.2	4.4	3.8	3.7
W. microscopica	8359	7.7	6.7	7.3	4.9	4.0	4.1
W. australiana	7211	8.0	4.9	7.5	3.9	3.5	3.4
W. angusta	7476	8.2	6.5	8.2	4.5	3.1	3.2
total		7.2 - 8.2	4.4 - 6.7	5.8 - 8.2	3.8 - 4.9	3.1 - 4.0	3.2 - 4.1

tion. The pH which was originally 5.5 was measured after 14 and 40 days. In the solutions with  $\text{NO}_3^-$  alone, the pH rose first to ca. 8 (table 2.14), in solutions with  $\text{NH}_4^+$  it was lowered down to near 4, and in solutions with  $\text{NH}_4\text{NO}_3$  it behaved somewhere between. If the pH increased in this last solution, it was assumed that preferably  $\text{NO}_3^-$  was used, if it declined,  $\text{NH}_4^+$  was supposed to be taken up first. Only 1 clone of each species was used. The two replicates of each clone did not vary significantly. The correlation of the pH with the form of nitrogen supplied might partly be explained by the uptake of other ions too. But since all species were cultivated under the same conditions the differences might be species specific. According to the pH in the solution after 14 days, the tested species can be divided into three groups (in brackets the pH difference):

- 1) Species with a pH only slightly lower in the  $\text{NH}_4\text{NO}_3$  solution than in the  $\text{NO}_3^-$  solution (pH difference 0.0-0.5). It is supposed that these species use a great deal of  $\text{NO}_3^-$  beside  $\text{NH}_4^+$  from the beginning under the given conditions: W. angusta (0.0), W. microscopica (0.4), L. obscura (0.4), W. australiana (0.5).
- 2) Species with a slightly lower pH in the  $\text{NH}_4\text{NO}_3$  solution than in the nitrate solution (pH difference 0.7-0.8). These species probably take up both ammonium and nitrate to a moderate extent, S. polyrrhiza (0.7), L. minor (0.7), L. aequinoctialis (0.8).
- 3) Species with a distinctly lower pH in the  $\text{NH}_4\text{NO}_3$  solution than in the nitrate solution (pH difference 1.0-1.9). These species seem to absorb ammonium preferentially: L. turionifera (1.0), L. disperma (1.2), L. minuscula (1.3), L. gibba (1.4) and W. hyalina (1.7).

It takes 20 to 30 hours for L. minor to change from ammonium uptake to nitrate uptake (KOPP et al. 1974b).

The  $\text{NO}_3^-$  uptake is not linked with the potassium uptake in L. gibba through a common ion permeating system in the cell membrane.  $\text{NO}_3^-$  and  $\text{K}^+$  uptake are separately controlled by oscillators (KONDO 1982c).

HILLMAN and LAMM (1981) report a peculiar behaviour of L. aequinoctialis (clone No. 6612) towards nitrogen utilization. In solutions with  $\text{KNO}_3^-$  as sole N source they observed from time to time chlorotic fronds which occurred spontaneously. If the chlorotic fronds were transferred to solutions with nitrogen in a reduced form (e.g. aspartic acid, glutamic acid, glutamine,  $\text{NH}_4\text{Cl}$ ,  $\text{NaNO}_2$ ), they became green. The inability of nitrate assimilation can appear in the light or darkness and in different

nutrient solutions. The phenomenon does not seem to be a result of a somatic mutation.

A mutant of L. aequinoctialis incapable of ammonium ion uptake and of photosynthesis was detected by POSNER (1962b) and investigated by MONSELISE et al. (1984).

#### 2.3.3.4.4.1.5. Utilization of organic nitrogen

Lemnaceae are able to utilize many organic nitrogen compounds besides ammonium, nitrate, and nitrite (see also chapter 2.3.3.5.2.2). From more than 160 compounds tested by BOLLARD (1966), the following could be used successfully by S. punctata as sole nitrogen source:

- a) protein amino acids: L-alanine, L-aspartic acid, L-asparagine, L-glutamic acid, L-glutamine, glycine, and 6 other rarer amino acids and substituted amino acids;
- b) peptides: glycyl-L-alanine and 6 further compounds;
- c) amides: L-serine amide;
- d) amines: putrescine;
- e) guanidine derivatives: agmatine,  $\beta$ -guanido propionic acid;
- f) urea and urea derivatives: 7 compounds.

No purines and pyrimidines could be used successfully. The absorption of urea in S. polyrrhiza is much slower than that of nitrate or ammonium (EYSTER 1966). The optimal amount of urea for a constant (but rather slow) growth lies between 30 and 150 mg (corresponding to 15-60 mM N). If the supply with nitrate nitrogen is sufficient, an addition of 15 mM urea reduces the growth rate of S. polyrrhiza. S. punctata is only able to use urea if the pH is below 4.3 (BOLLARD et al. 1968). If urea is used as nitrogen source, the concentrations of many amino acids in the fronds of S. punctata are lower than if ammonium is supplied. Asparagine and glutamine are taken up by S. punctata faster than nitrate, and aspartate and glutamate at about the same rate (FERGUSON 1970). Glutamic acid and asparagine allow only a slow, and glycine a moderate growth of L. gibba if applied as sole nitrogen source. L-arginine and L-aspartic acid together as well as L-asparagine alone (in concentrations of 1 mM N) are able to promote the growth rate in solutions supplied with nitrate (HUBALD and AUGSTEN 1977a). The growth rate of L. minor was found to be higher in nutrient solutions with a mixture of amino acids than with nitrate and ammonium (JOY 1969a). According to the same author,

subsequent better growth was attained with the following mixtures: glutamic acid-aspartic acid-arginine, casamino acids, casamino acids-nitrate. The positive influence of the amino acid mixture might be due to inadequate nutrient or light conditions. Certain (branched-chain) amino acids as leucin, valine, and isoleucine inhibit the growth rate of S. polyrrhiza if used alone (BORSTLAP 1974, VAN MAZIJK 1975). Contrary to the results with Spirodela and L. minor, L. trisulca grows better with urea than with nitrate or ammonium. Also aspartic acid and glutamic acid are easily used as nitrogen sources (HOLST and YOPP 1979). The permeability for urea of roots of L. minor is exceptionally high compared with other plants (MARKLUND 1936).

#### 2.3.3.4.4.2. Phosphorus (P)

Phosphorus is taken up mostly as phosphate. According to FITZGERALD (1971), there is also the possibility for L. minor to use P as pyrophosphate, tripolyphosphate and as P bound in teeth of sharks. A utilization

Table 2.15. Phosphorus requirement and phosphorus tolerance for growth of some Lemnaceae species (minimum amount of phosphorus to result in half of the maximum growth rate, optimum amount of phosphorus to achieve maximum growth rate, maximum amount of phosphorus to achieve still half of the maximum growth rate), from AEBLI 1986 (1), DOCAUER 1983 (2), EYSTER 1966 (3), LUEOEND 1983 (4), FERNANDEZ et al. 1983 (5).

\* forming turions at lower concentrations

Species	supply of P in mM		
	minimum	optimum	maximum
<u>S. polyrrhiza</u>	0.00046 (2) 0.0028 * (4)	0.1 -1.0 (3) 0.014 -0.35 (4)	1.75 (4) 10 (2)
<u>L. gibba</u>	0.00056 (4)	0.0028-1.75 (4)	8.76 (4)
<u>L. minor</u>	0.00045 (2) 0.00011 (4)	0.014 -0.35 (4) 0.01 ->0.2 (1)	1.75 (4)
<u>L. turionifera</u>	0.00035 (2)		
<u>L. aequinoctialis</u>			<80 (5)
<u>L. minuscula</u>	0.00011 (4)	0.0028-0.35 (4)	1.75 (4)
<u>W. borealis</u>	0.00552 (2)		
<u>W. columbiana</u>	0.00148 (2)		



of P from lake mud is possible only to a small amount (less than 1%). P released from pond sediments is either more or less easily available for L. minor than  $\text{KH}_2\text{PO}_4$  depending on the type of sediment (FEKETE et al. 1976).

Minimum, optimum, and maximum P content of the nutrient solution for different Lemnaceae species are put together in table 2.15. The need for phosphorus rises in the following order: L. minuscula, L. turionifera, L. minor, S. polyrrhiza, L. gibba, W. columbiana, W. borealis. Relatively high optimum and maximum values are found in L. gibba and S. polyrrhiza. Interestingly, the requirement of phosphorus for the different species is in no correlation to the nitrogen requirement. The ratio of the half saturation constants for nitrate and for phosphate have been calculated by DOCAUER (1983) as follows: L. turionifera (16.9), S. polyrrhiza (13.9), L. minor (11.8), W. borealis (3.0) and W. columbiana (1.6). The high P requirements of W. borealis and W. columbiana especially if compared with the N requirements are remarkable.

The influence of the low P concentration on characteristics of Lemnaceae is surveyed in table 2.16. Interestingly, some species behave differently. The length/width ratio becomes higher at low P concentrations in L. minuscula and L. gibba, it does not change in S. polyrrhiza and L. minor and it becomes lower in L. turionifera. The root length is greatest at 0.003 mM P in L. gibba, at 0.0005 mM P in L. gibba and L. minuscula, and at 0.00002 mM P in L. minor (LUEOEND 1983). Remarkably, L. minor develops longer roots than L. gibba in solutions with a low phosphorus content whereas L. gibba has longer roots than L. minor in solutions with a low nitrogen content. At high concentration of P (as well as of N), the fronds of Lemnaceae remain together longer and often form clusters (LUEOEND 1983). The stipe between mother and daughter fronds becomes longer and distinctly visible. P and N shortage mostly result in similar reactions of the plants, they have, however, an opposite effect in comparison to K shortage.

The number, size, and dry weight of S. polyrrhiza turions is higher in solution with a low phosphorus content (0.5 mM) than in solution with a high P content (1.5 mM). Turions formed under low phosphorus conditions are extremely dormant (JUNGnickel 1986a). DUDLEY (1983, 1987) was able to encourage turion formation of L. turionifera by additional phosphorus.

According to LINDEMAN (1951, 1952, 1972), P shortage lowers the photo-



synthesis rate of L. minor under light saturation. If phosphate is added, the photosynthesis is normalized after a short time. Dark-red light is able to partly compensate the inhibiting effect of P shortage (LINDEMAN 1973). The lack of phosphate results in a 50-fold increase in phosphatase activity of all cell extracts of S. punctata (REID and BIELESKI 1970b) (compare chapter 2.5.6.3). In L. minor, STROTHER (1984) showed that acid phosphatase activity increases in phosphorus-deficient solutions; alkaline pyrophosphatase activity decreases during senescence;

Table 2.16. The effect of low phosphorus supply on characteristics of Lemnaceae

\* at very low P concentrations the roots are shorter again (5)

+ increase, - decrease, 0 no effect

1 only in solution with sugar

a	<i>S. polyrrhiza</i>	1	BORNKAMM 1965
b	<i>S. punctata</i>	2	EYSTER 1966
c	<i>L. gibba</i>	3	JUNGNICKEL 1978
d	<i>L. minor</i>		AUGSTEN and JUNGNICKEL 1983
e	<i>L. turionifera</i>	4	LINDEMAN 1951, 1952, 1972
f	<i>L. trisulca</i>	5	LUEOEND 1983
g	<i>L. aequinoctialis</i>	6	PIRSON and GOELLNER 1953
h	<i>L. minuscule</i>	7	REID and BIELESKI 1970a,b
i	<i>W. arrhiza</i>	8	THORNSTEINSSON et al. 1985
		9	DOCAUER 1983
		10	STROTHER 1984

Characteristics	effect	species	author
area of frond	-	a,d,h	2,5
dry weight	-	a,d	1,2
length/width ratio	0	a,d	5,9
	-	e	9
	+	c,h	5
gibbosity	0	c	5
length of root	+ *	a,b,c,d,h	5,6,7
length of root cells	+	d	6
turion formation	+	a,i	2,3,5
starch content	+	b,c	7,8
content of free sugars	-	c	8
anthocyanin content	+	a,c,f	2,3,5
protein content	0	d	3
content of glutamine and asparagine	+	b	7
oxalate content	-	d	1
respiration rate	-	d	6
photosynthesis rate	-	b,d	4,6,7
phosphatase activity	+	b,d	7,10

this decrease is enlarged by phosphorus-deficiency. Apparently, a certain amount of phosphorus is necessary for flower induction of L. gibba under long-day conditions (CLELAND and TANAKA 1986). JUNGNICHEL (1978) calculated the P requirement to form a frond of 4 different Lemnaceae species. The following amount of P is needed: 0.00034 mg for S. polyrrhiza, 0.00018 mg for L. gibba, 0.00014 and 0.00004 mg for two different clones of L. minor and 0.00001 mg for W. arrhiza. According to EYSTER (1966), S. polyrrhiza requires a minimum content of 3.3 mg P to form 1 g dry weight. FEKETE and RIEMER (1973) reached the maximal P content (1.9%) in fronds of L. minor with a P concentration of 0.1 mM in the medium. Of 10 species of water plants, L. minor accumulated most P of all vascular plants (measured with  $^{32}\text{P}$ ), but much less than the filamentous alga Oedogonium (VERMAAK et al. 1976).

S. punctata is able to transport  $\text{PO}_4^{3-}$  from older to younger fronds if kept under P starvation (KNYPL 1979b). A mutual exchange of phosphorus between the plants takes place (KNYPL 1980). BIELESKI (1968a,b) studied the uptake and the incorporation of radioactive P in S. polyrrhiza. He analysed more than 50 different chemical P compounds. In P starvation cultures, the growth stops after 14 days, and the portions of the different P compounds change. Old fronds become chlorotic and young ones accumulate anthocyanin and starch; roots become longer and the photosynthesis rate is reduced, but the nitrogen balance does not change. The P uptake under P shortage in L. gibba was investigated by ULLRICH-EBERIUS et al. (1981), ULLRICH-EBERIUS and NOVACKY (1984). The uptake of P is stimulated by light. It performed best at a pH of 6 and slowed down with rising pH. According to EL-SHINAWY and ABDEL-MALIK (1980) the uptake is much faster still at pH 5 than at pH 6. After P starvation, the uptake is better. The P uptake is supposed to be directed by the intracellular P content. The monovalent phosphate uptake proceeds by  $\text{H}^+$  cotransport (see chapters 2.5.2.1 and 2.5.2.2.4).

The optimal supply of P in a mixed culture of Lemnaceae in natural waters is supposed to vary between 0.13 and 0.26 mM. At higher P concentrations (up to 1.3 mM), the P content of the fronds (about 1%) did not rise any longer (see fig. 1.1, p. 14) (REJMANKOVA 1979).

#### 2.3.3.4.4.3 Sulfur (S)

Sulfur is mostly taken up as sulfate. Lemnaceae are also able to absorb

sulfide, thiosulfate, sulfite, cysteine, cystine, and cysteic acid. Other substances containing sulfur investigated (e.g. dithionate, tetrathionate, thiourea, methionine, sulfanil amide, sulfanilic acid) either did not have any effect on growth of S. punctata at concentrations of 1 mM S, or the growth rate was reduced (FRASER 1974).

Best growth of S. polyrrhiza was achieved with 0.5 to 20 mM S (EYSTER 1966). At a sulfur concentration of 0.05 mM, the growth rate was distinctly lower, the fronds bigger, heavier, and more intensely pigmented by anthocyanins. At 0.01 mM the fronds looked chlorotic. From 60 mM S upwards the plants stopped growth, appeared small and partly chlorotic, and clustered together. Sulfur deficiency in the medium stimulates turion formation in S. polyrrhiza (MALEK and COSSINS 1983a). The increase of the sulfate content from 0.014 to 1 mM did not change the growth rate of L. aequinoctialis (DATKO et al. 1980a). This difference in S requirement between S. polyrrhiza and L. aequinoctialis is striking even if we consider the different culture conditions. The sulfur requirement to form one g dry weight of S. polyrrhiza was estimated by EYSTER as 0.1-0.5 mg at minimum. For optimal growth the amount of sulfur needed per g dry weight is 10 mg. DATKO et al. (1978a) calculated the amount of sulfur necessary to build up 1 g protein in L. aequinoctialis as 9-12 mg. The requirement is higher if the molybdenum content of the solution is relatively high (DATKO et al. 1980a). Molybdate and sulfate are taken up by a common mechanism (DATKO and MUDD 1984a).

THOIRON et al. (1969) investigated the sulfate uptake of L. minor under different temperature conditions. The authors postulate the existence of two different systems of sulfate absorption. The uptake of sulfate in L. aequinoctialis was studied by DATKO et al. (1978a,b) within the range of 0.0003 and 1 mM S. No volatile S compounds could be detected. Also, no S was transferred from the fronds back to the solution even at the highest  $\text{SO}_4^{2-}$  concentrations. The content of S-containing amino acids increased only 1 1/2 to 2 times between the lowest and the highest S concentration of the medium, whereas the anorganic sulfate increased to the 30fold. At least 2 components are involved in the uptake of  $\text{SO}_4^{2-}$ , one saturating and one linear (non-saturating). 55% of newly taken up  $\text{SO}_4^{2-}$  enters a compartment with a slow turnover (vacuole?), 45% remains in a compartment (cytoplasm?) in which it is rapidly metabolized to organic components. The saturating system contributes 99% of  $\text{SO}_4^{2-}$  uptake under standard conditions. In nature, the same system shares at least

65-70% (DATKO and MUDD 1984a). The down-regulation of the sulfate uptake of the saturating system by high concentrations of  $\text{SO}_4^{2-}$  or cystine is more than compensated by unregulated uptake via the non-saturating uptake system (DATKO and MUDD 1984b). The kinetics of  $\text{SO}_4^{2-}$  uptake in L. gibba was studied by LASS and ULLRICH-EBERIUS (1984a,b). Further details are given in chapters 2.5.2.1 and 2.5.2.2.4.

The sulfide uptake (and also the uptake of gaseous  $\text{H}_2\text{S}$ ) was investigated by ERISMANN et al. (1967), BRAENDLE and ERISMANN (1968), BRUNOLD and ERISMANN (1970, 1974, 1975, 1976). Two kinds of sulfide uptake could be detected, one of which is dependent on photosynthesis and leads mostly to fixation of S within the chloroplasts. Sulfide in the medium slightly inhibits photosynthesis and reduces the  $\text{SO}_4^{2-}$  uptake. This could be demonstrated by gasing the cultures with  $\text{H}_2\text{S}$ .

TAKEMOTO and NOBLE (1984, 1986) studied the growth response of S. punctata, L. gibba, and L. valdiviana to sulfite enrichment under light-limited and light-saturated conditions. Under low light intensity ( $35 \mu\text{mol m}^{-2}\text{s}^{-1}$  photosynthetic photon flux density), L. gibba and, to a lesser extent, S. punctata showed reduced growth rates at 5 and 10 mM  $\text{K}_2\text{SO}_3$ . Inhibition was less pronounced under high light intensities ( $200 \mu\text{mol m}^{-2}\text{s}^{-1}$  photosynthetic photon flux density). No growth reduction was observed for L. valdiviana. Since rates of growth and photosynthesis of L. valdiviana were higher than of S. punctata and L. gibba, the authors assume that rapid metabolic and mitotic activity are important to resist sulfite action. In a later paper (TAKEMOTO et al. 1986), it was demonstrated that the thiol content in the three investigated Lemnaceae species increased by 30-40% with sulfite treatment under low light intensity.  $\text{H}_2\text{S}$  was emitted by all three species, however, emission rates in L. valdiviana which is sulfite-tolerant were up to 4 times higher than in the other species. The thiol content and the  $\text{H}_2\text{S}$  emission of all species was enhanced at high light intensity. The increase of emission rate of  $\text{H}_2\text{S}$  was greater in L. valdiviana and S. punctata (55-60%) than in L. gibba (17%). It was proposed that thiol production and  $\text{H}_2\text{S}$  emission are important sulfite detoxification processes in duckweeds.

#### 2.3.3.4.5. Carbon (C)

Lemnaceae are able to use carbonate and bicarbonate in the solution as carbon source if  $\text{CO}_2$  is a minimum factor (WOHLER 1966, WETZEL and MANNY

1972). Under normal conditions, Lemnaceae floating on the surface of the water take up most of the C needed via gaseous  $\text{CO}_2$  from the air. Only a small amount of inorganic carbon originates from water soluble carbon, according to BOWKER et al. (1980) who studied L. minor. FILBIN and HOUGH (1985) report that L. minor takes, from a hard water lake (with relatively high pH), on an average 86% carbon from aqueous inorganic carbon. Under laboratory conditions, the C uptake from water was lower (37%). SATAKE and SHIMURA (1983) measured in S. polyrrhiza at low pH (5 and 4) up to 10 times more C uptake from the water than from the air. However, ESHEL and BEER (1986) questioned the results of SATAKE and SHIMURA. These authors did not take into account the different solubility of carbon compounds in water. The dissolved inorganic carbon form varies as a function of pH (fig. 2.9, from ESHEL and BEER 1986). ESHEL and BEER observed under laboratory conditions at a pH of 5.9 about 1% C uptake from the water and at a pH of 7.2 and 8.0 about 4-5% (see also table

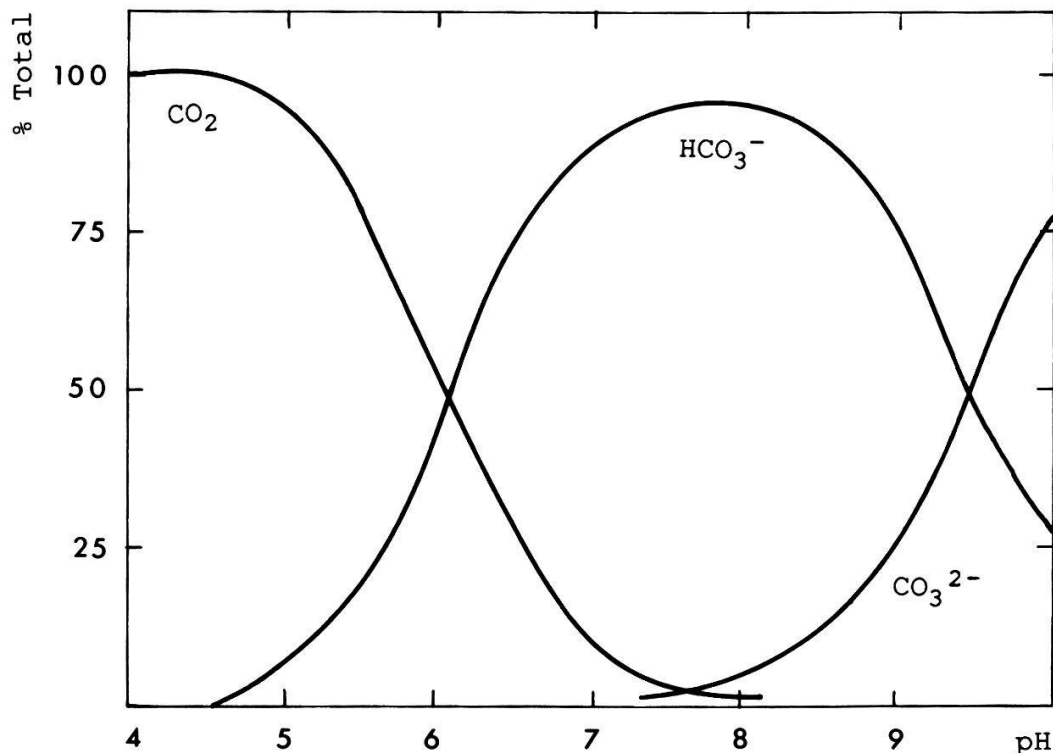


Fig. 2.9. The relative distribution of dissolved inorganic carbon among its various chemical forms as a function of pH (from ESHEL and BEER 1986)

2.6). Certainly, some of these differences are due to different growing conditions. For more details see chapter 2.3.2.2.

LANDOLT (1957) reported that L. trisulca grows in aseptic cultures of Hutner 1/5 solution only if sucrose is added. If distilled water is replaced by tap water, a slow growth without sucrose is possible. BATA (1973) succeeded in growing L. trisulca by adding kinetin. At high pH, the growth took place without kinetin. BATA concluded that L. trisulca is only autotrophic at higher pH. Also LANDOLT (1957) was originally of the opinion, that L. trisulca is heterotrophic for some special agents. The explanation of the "heterotrophy" of L. trisulca is the fact that Hutner solution does not contain any carbonate in contrast to the solution used by BATA (1973) and to tap water. The diffusion of  $\text{CO}_2$  in the water is 10000 times slower than in the air. Submerged species in aseptic cultures do not get enough  $\text{CO}_2$  for good growth. In nature, the  $\text{CO}_2$  in the water is constantly replaced by respiring heterotrophic organisms in addition to the carbonate which is present in the water. A preliminary experiment demonstrated the correctness of the assumption (LANDOLT unpubl. results). If  $\text{NaHCO}_3$  or  $\text{Na}_2\text{CO}_3$  is added to Hutners solution containing no sugar, L. trisulca looks healthy and grows steadily at pH 6 and higher. Also W. gladiata and other Wolffiella species which grow extremely slowly in solutions without sucrose show a more intense green colour and a much faster growth if carbonate is added to the solution. Already STEEMANN NIELSEN (1944, 1947) observed that the  $\text{CO}_2$  content in water (0.015 mM) is not sufficient to enable photosynthesis of the submerged species Myriophyllum spicatum, Ceratophyllum demersum and Elodea canadensis. Therefore, these species are able to utilize bicarbonate. They reach 30% of the maximal growth rate with 0.5 mM  $\text{HCO}_3^-$  and 50% with 1 mM. The bicarbonate remains in solution only at higher pH (6.5 to 9); at lower pH  $\text{CO}_2$  is formed which escapes. Therefore, a relatively high pH is necessary for a good growth of the submerged species mentioned. For L. trisulca and W. gladiata, a high carbonate content of the water or a high content of organic substances is a precondition for the occurrence in nature (see also vol. 1, chapters 4.2.4.8 and 4.2.4.12, LANDOLT 1986). If no bicarbonate is in the water, the photosynthetic rates of submerged species are likely to be limited by the rate of transport processes outside the photosynthetic cells (RAVEN 1981).  $\text{KHCO}_3$  (1 mM) is able to induce flowering in the long-day plant L. gibba under short-day conditions but inhibits flowering under long-day conditions (KANDELER 1964a,b).



#### 2.3.3.4.6. Chlorine (Cl) and other halogens (F, Br, J)

The indispensability of **chlorine** was demonstrated by MARTIN and LAVOLLAY (1958a,b, 1959) and LAVOLLAY (1963a,b, 1965). Chlorine can partly be substituted by bromine. The minimum requirement of Cl rises with higher light intensity. The  $\text{Cl}^-$  ions are not only used for photosynthesis but also for other independent processes. This was concluded from the fact that Cl is also necessary for heterotrophic growth in darkness. The minimum requirement of Cl for best growth is species specific (MARTIN 1963a, 1965). It amounts to 0.015 mM Cl for S. polyrrhiza, 0.001 mM for L. minor and 0.003 mM for L. aequinoctialis. EYSTER (1966) achieved best growth for S. polyrrhiza in Cl concentrations between 0.01 and 1 mM. At 10 mM Cl the growth rate is distinctly lower and at 100 mM the fronds die. At low Cl concentrations (0.001 mM), the fronds are relatively narrow and the older ones have red stripes. At still lower concentrations, they become very small, light green, and form clusters. The greatest dry weight per frond is reached at 0.01 mM Cl. At higher concentrations it reaches almost the same amount.

In an experiment of our own, the positive effect of NaCl addition on growth of Lemnaceae has been confirmed (LANDOLT, unpubl. results). Lemnaceae species are normally kept in our laboratory either on Hoagland agar medium (which does not contain Na and only 0.02 mM Cl) or on 1/5 Hutner agar medium (which contains only 0.02 mM Na if the sodium free complexon 2 (EDTA) is used, and 0.04 mM Cl. If 0.3 mM NaCl is added to either of the 2 solutions, the growth rate of all 4 investigated species (S. polyrrhiza, L. minor, W. Welwitschii and W. microscopica) is at first much faster than in the normal solutions. After two weeks, the species were transferred to the normal solutions again. S. polyrrhiza and L. minor kept the same elevated growth rate whereas W. Welwitschii and W. microscopica slowed down their growth rate significantly. It is not quite sure if this effect is due to the  $\text{Na}^+$  or  $\text{Cl}^-$  content or whether it is a combined effect. It seems more probable, that the Cl content is decisive. This would mean that for long-lasting good growth a Cl content of 0.02 mM is not sufficient (Cl concentration of Hoagland solution). On the other hand, the usual impurifications of the chemicals with Cl are sufficient for a slow growth. Also it seems evident that W. Welwitschii and W. microscopica need more NaCl than S. polyrrhiza and L. minor.

**Iodine** cannot replace chlorine, in contrast to **bromine**. It is more toxic in solutions without chlorine than in solutions with chlorine (in Cl concentrations from  $10^{-4}$  mM upwards). Iodine is probably not a necessary element for L. minor (MARTIN and LAVOLLAY 1959).

The absorption of chlorine in L. minor is 10 to 20 times faster than that of iodine (THELLIER et al. 1967). The authors are of the opinion that the uptake of chlorine is more complicated than the iodine uptake. In the first case, they assume different uptake systems for different Cl concentrations in the solution and for different environmental conditions; in the second case, they observed only one uptake system for all conditions.

**Sodiumfluoride** inhibits the growth rate of S. punctata and L. minor at concentrations of 5 mM; it is lethal at 20 mM (BONG et al. 1980).

#### **2.3.3.4.7. Metallic trace elements**

##### **2.3.3.4.7.1. General remarks**

Of all the natural elements which are not mentioned in the previous chapters of the present volume, a few are essential for the growth of Lemnaceae, others stimulate growth but are not indispensable and still others are indifferent or toxic, according to the concentrations used. The proof of the essentiality of a trace element is rather difficult because the required amounts are small; very often a sufficient quantity of a trace element is available from impurities in the culture vessels, in the chemicals, or in the water. Furthermore, the Lemnaceae are very economical with trace elements, transferring them from mother to daughter fronds. The example of HENSSEN (1954) might illustrate the situation. The author used a nutrient solution without zinc and molybdenum, both elements proved to be essential for Lemnacean growth. She still recorded growth of S. polyrrhiza after a year of cultivation without these elements. However, some deformities could be observed.

The availability of many trace elements is dependent on the pH of the solution. If chelating agents (e.g. EDTA, tartrate, citrate) are added, the uptake of trace elements becomes easier, and the toxicity is not so pronounced (e.g. Fe, Mn, Zn, Cu) (cf. FLY 1935, SIELING 1937, HUTNER et al. 1950, BITCOVER and SIELING 1951). Therefore, the comparison of results within this chapter is difficult. Some of the elements listed in



Table 2.17. Tolerance of Lemna minor towards different micronutrients  
(concentrations in mg per l) (from FRICK 1985b)

Growth	Fe	Mn	B	Cu	Zn	Mo
no inhibition	27.9	54.9	17.3	3.2	6.5	54.4
complete inhibition	100.5	274.5	86.5	6.3	65.3	271.8

the following chapters are not necessary for the growth of Lemnaceae. However, all elements are mentioned, of which the effects on Lemnaceae were studied. In general, Lemnaceae react relatively sensitively to different heavy metals compared to other plants (e.g. Elodea) (BROWN and RATTIGAN 1979), see also chapter 3.6.

FRICK (1985b) tested the tolerance of L. minor to several micronutrients (table 2.17). A combination of the high concentrations of different micronutrients (except Mo) were not inhibitory, even though separate 2-, 3-, and 4-way combinations were.

Some metal ions (e.g. Cu, Ag, W) promote flowering of Lemnaceae. TANAKA et al. (1986a) suggest that this effect is due to the suppression of nitrate assimilation by these ions.

#### 2.3.3.4.7.2. Boron (B) and aluminum (Al)

**Boron** was shown to be essential for S. polyrrhiza (EYSTER 1966), S. punctata (THIMANN and EDMONSON 1949), L. minor (STEINBERG 1946) and W. arrhiza (EICHHORN and AUGSTEN 1974). The minimal amount for good growth of L. minor is 0.005 mM B (STEINBERG 1946), and for S. polyrrhiza 0.1 mM B (EYSTER 1966). At lower concentrations the growth of S. polyrrhiza was slower, and signs of starvation occurred (yellowish colour with darker stripes). Even at a B concentration of 0.0001 mM, a distinct growth rate could be observed although the cultures were kept in boron-free solution for several weeks before. GLANDON and McNABB (1978) did not note any difference in growth rate of L. minor in solutions with 0.001, 0.01, and 0.1 mM B. The B content of the fronds amounted to 0.1, 0.6, and 0.8 mg per g dry weight, respectively. Similar results were obtained by FRICK (1985a). Best growth of W. arrhiza was achieved with 0.008 mM B. At

0.004 mM B, signs of starvation occurred (EICHHORN and AUGSTEN 1974). The toxic limit of B for S. polyrrhiza is 10 mM (EYSTER 1966), for L. minor 5 mM (THELLIER and LE GUIEL 1967a), and for W. arrhiza somewhat higher than 0.02 mM (EICHHORN and AUGSTEN 1974). It is not clear if the different requirements and tolerances are species specific or due to different growth conditions and (partly) due to different concentration intervals between the investigated solutions.

Shortage of boron results for W. arrhiza in a reduction of dry weight, chlorophyll, and protein content as well as in an elevation of the dark respiration rate, of the photosynthesis rate, and of the carbohydrate content. With boron deficiency, the ratio of glycolysis/pentoses shunt pathway is changed in favour of the latter (EICHHORN and AUGSTEN 1974). The protein content of L. minor is reduced, the starch content raised (from 3% to 6.5% of the dry weight) and the fronds clustered if boron is lacking (SCHOLZ 1962). The shortage of boron has a greater effect with nitrate than with ammonium as a nitrogen source. EYSTER (1966) therefore raises the question if boron plays some role in the uptake or assimilation of the nitrate. High light intensities aggravate the boron starvation symptoms, low light intensities moderate them (TANAKA 1966).

In clay suspensions, B concentrations of as low as 0.01 mM inhibit growth of L. minor. However, the toxic effect can be neutralized by addition of Ca (FOX and ALBRECHT 1958). The highest boron content in the frond (4600 ppm B on a dry weight basis) was achieved with 1 ppm B (0.1 mM) and relatively high Ca content. Also BOLGIANO (1979) reports on a more pronounced toxicity of high boron concentrations if the Ca concentration is low. Compared with Ceratophyllum demersum, L. minor takes up much more boron (up to 45 times) (GLANDON and McNABB 1978). The boron uptake is active, according to THELLIER and LE GUIEL (1967a).

There seem to be at least four disparate functions of boron (according to FRICK 1985a): in nucleic acid metabolism, in carbohydrate biosynthesis and transport, in interactions with plant growth substances, and in membrane integrity.

Borate does not considerably influence the absorption of sulfate in L. minor (THELLIER and TROMEUR 1968).

**Aluminum** is not essential for Lemnaceae. It has a toxic effect on S. polyrrhiza and L. minor at a concentration of 1 mM (WIEWIORKA and SAROSIEK 1986).

#### 2.3.3.4.7.3. Molybdenum (Mo) and tungsten (W)

The minimum amount of **molybdenum** required by L. minor is 0.0002 mM (STEINBERG 1946). EYSTER (1966) was not able to demonstrate the necessity of Mo for S. polyrrhiza, probably due to impurities in his chemical substances. However, YOSHIMURA (1943) proved that S. polyrrhiza (as well as different Lemna species) needs at least  $5 \times 10^{-7}$  mM Mo. Ten mM Mo are toxic to S. polyrrhiza (EYSTER 1966). YOSHIMURA (1943) obtained effects of toxicity of Mo already at 1 mM for S. polyrrhiza and Lemna spp. Shortage of Mo resulted in flowering of L. aequinoctialis (YOSHIMURA 1943). This result was corroborated by TANAKA et al. (1979b). Root growth of L. gibba is inhibited at 0.1 mM Mo and completely stopped at >1 mM (LIEBERT 1986a).

Mo stimulates the nitrate assimilation at low pH, enhancing the reduction of nitrate and preventing the accumulation of nitrate in the fronds. If Mo is lacking in the solution, the Lemnaceae cultures die in a medium with  $\text{NH}_4\text{NO}_3$  since they only take up ammonium and in this way lower the pH to a toxic value. According to DATKO et al. (1980a) and DATKO and MUDD (1982), Mo competes with sulfur. Therefore, an addition of higher amount of sulfate or cystine is able to detoxicate the Mo. At low sulfate concentration in the solution, Mo is toxic in concentrations as low as 0.05 mM for L. aequinoctialis. DATKO and MUDD (1980) suppose that Mo inhibits formation of reduced sulfur in L. aequinoctialis.

**Tungsten (W)** is said to have a similar effect on Lemnaceae as Mo (YOSHIMURA 1943). However, tungstate is a competitive inhibitor of molybdate function and was able to induce flowering in L. aequinoctialis similarly to Cu, Hg, Ag, and cyanide ions (TANAKA et al. 1979b, 1986a). It is assumed by these authors that the ions mentioned inhibit nitrate reductase activity and consequently nitrate assimilation, and that this suppression causes day-length-independent flowering.

#### 2.3.3.4.7.4. Iron (Fe)

The fronds of S. polyrrhiza become chlorotic and red underneath below a concentration of 0.001 mM Fe (EYSTER 1966). Also YOSHIMURA (1950) noted chlorotic fronds of S. polyrrhiza under shortage of iron. The fronds accumulated anthocyanins and oxalate and formed turions. Optimal growth of S. polyrrhiza was observed by EYSTER (1966) between 0.01 and 0.1 mM

Fe. Under optimal conditions of Fe, the fronds reach up to 11.5 mm in length. The minimum amount of Fe for good growth of L. minor was determined by STEINBERG (1946) as 0.05 mM. According to ORLANDO and NEILANDS (1982), maximum growth rate of L. gibba and L. minor was obtained with an iron supply of 0.02 mM (added as ferrichrome or ferric EDTA). The maximum iron content was reached at 10 mM in S. polyrrhiza (EYSTER 1966). Already at 1 mM Fe, S. polyrrhiza showed a slow growth and light green fronds. SCHREINEMAKERS (1984) obtained an inhibition of S. polyrrhiza with 0.2 mM Fe and nearly a stop of growth at 0.4 mM.

DOCAUER (1983) measured the Fe content of ponds with different Lemnaceae cover. He concluded a different need of Fe for Lemnaceae species. According to his observations, L. turionifera and L. minor require more iron than S. polyrrhiza and W. columbiana. EYSTER (1966) estimated the amount of Fe required to produce one gram dry weight of S. polyrrhiza as 0.017 mg.

A flower-promoting effect of iron in L. aequinoctialis was reported by NISHIOKA et al. 1986. For further effects of iron on flowering see chapter 2.4.3.1.2.

#### 2.3.3.4.7.5. Manganese (Mn)

The essentiality of manganese for Lemnaceae was already proved by HOPKINS (1931) (for L. minor), MCHARGUE and COLFUE (1932) (for S. polyrrhiza), CLARK (1933) (for S. polyrrhiza), and SAEGER (1933a) (for S. polyrrhiza). S. polyrrhiza needs at least  $10^{-5}$  mM Mn. At this concentration, the fronds were small, rich in anthocyanin and clustered. Shortage of Mn depresses root growth in S. polyrrhiza (SAEGER 1933a) and L. minor (HOPKINS 1931). Maximum growth was attained in S. polyrrhiza between  $10^{-3}$  and 0.06 mM Mn (EYSTER 1966) and between 0.009 and 0.047 mM (CLARK 1933). L. minor requires  $10^{-3}$  mM Mn (STEINBERG 1946), L. minuscula  $10^{-2}$  mM Mn (JUNGNICKEL and GEBHARD 1986) for good growth. Much lower key concentrations were measured by YOSHIMURA (1941) for S. polyrrhiza, L. aequinoctialis and L. valdiviana:  $5 \times 10^{-7}$  mM Mn was the minimal amount for growth and  $10^{-5}$  mM was needed for optimal growth. The maximum concentration to still allow growth for S. polyrrhiza is given by EYSTER (1966) with 0.3 mM Mn. At 3 mM Mn the plants stopped growth, were very small, clumped, and pale green. L. minuscula showed toxic effects at 1 mM Mn (and 50% of the maximum growth rate, JUNGNICKEL and GEBHARD

1986). According to NASU and KUGIMOTO (1981) and NASU (1983), Mn concentrations of 0.02 mM inhibit the growth of L. aequinoctialis to 50% (Bonner-Devirian medium). Reduced growth (50% of the maximum growth rate) was obtained by JUNGNICHEL (1984) at Mn concentrations of 0.1 mM for L. aequinoctialis, at 1 mM for S. polyrrhiza, W. arrhiza, and L. minuscula, the latter species developing the highest tolerance against Mn. The root growth of L. gibba is inhibited at 0.2 mM Mn and stopped completely at 1 mM (LIEBERT 1986a).

EYSTER (1966) estimated the minimal amount of Mn required to form one gram dry weight of S. polyrrhiza as 0.00033 mg. The Mn requirements are higher in darkness than in the light (EYSTER 1966).

Under mixotrophic conditions using nitrate as sole nitrogen source, the minimum Mn concentration for good growth of L. minuscula was 10 times lower ( $10^{-3}$  mM) than in autotrophic cultures. The minimum Mn requirement per frond amounted to 1 pg compared with 50 pg in autotrophic cultures (JUNGNICHEL and GEBHARD 1986).

Mn<sup>++</sup> raises the activity of phenylalanine ammonia-lyase similarly as a reducing agent (GORDON 1977). SCHREINEMAKERS (1984) and SCHREINEMAKERS and DORHOUT (1985) point to the possible existence of a coupled uptake mechanism for Mn and Mg in S. polyrrhiza. For activities of Mn on flowering see chapter 2.4.3.1.2.

#### 2.3.3.4.7.6. Zinc (Zn)

THIMANN and EDMONDSON (1949) proved the indispensability of Zn for S. punctata. The minimal Zn concentration to achieve good growth of L. minor is 0.0006 mM (STEINBERG 1946). HUTCHINSON and CZYRSKA (1975) noted a growth stimulation for L. minor at Zn concentrations of 0.0008 and 0.0016 mM. The lowest Zn concentration to allow a slow growth of S. polyrrhiza was 0.002 mM (EYSTER 1966). Best growth was attained for S. polyrrhiza with 0.02-0.2 mM Zn (EYSTER 1966), for L. minor with 0.002 mM Zn (HUTCHINSON and CZYRSKA 1975), and for L. obscura (named as L. minor) with 0.1 mM (STANLEY and MEADWELL 1976a). With 2 mM Zn the growth rate of S. polyrrhiza is slowed down, and with 20 mM the fronds died (EYSTER 1966). SAROSIEK et al. (1982) observed toxic symptoms in L. minor at concentrations of 0.1 mM (they used tap water as medium). Sixteen mM Zn resulted in the death of L. obscura (STANLEY and MEADWELL 1976a). Fronds of L. aequinoctialis produced brown spots at Zn concentrations of 3 mM

and died at 8 mM (NASU and KUGIMOTO 1981, NASU 1983). VAN DER WERFF and PRUYT 1982) observed no toxic effect of Zn concentrations up to 0.5 mM in S. polyrrhiza and L. gibba. An inhibiting effect of 0.1 mM Zn and higher on the root growth of L. gibba was reported by LIEBERT (1986a). Root growth stopped completely with 1 mM Zn.

According to EYSTER (1966), the minimal zinc requirement for the production of one gram dry weight of S. polyrrhiza amounts to 0.26 mg. Zinc deficiency (in contrast to copper deficiency) lowers the superoxide dismutase activity in L. gibba (VAUGHAN et al. 1982). SCHREINEMAKERS (1984) and SCHREINEMAKERS and DORHOUT (1985) suggest a possible coupled uptake mechanism for Zn and Ca in S. polyrrhiza. The toxic effect of Zn was neutralized by raising the Mg content (MARTIN 1955).

#### 2.3.3.4.7.7. Copper (Cu)

The evidence of the indispensability of copper could not be demonstrated by SAEGER (1937), STEINBERG (1946), THIMANN and EDMONDSON (1949) and EYSTER (1966). EYSTER (1966) showed that many chemicals (e.g.  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ ,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{H}_3\text{BO}_3$ ) and even distilled water contain traces of Cu. Therefore the proof of Cu requirement is extremely difficult. Nevertheless, most authors (e.g. SAEGER 1937) think that copper is an obligatory element for Lemnaceae. THIMANN and EDMONDSON (1949) suppose that a copper containing enzyme participates in the formation of anthocyanin in S. punctata. A growth stimulation in S. polyrrhiza was observed by EYSTER (1966) with 0.001 mM Cu, and by HUTCHINSON and CZYRSKA (1975) in L. minor with 0.0001 mM Cu. The latter authors report on growth inhibition already with 0.0008 mM Cu. Growth reduction was recorded in L. minor with 0.06 mM (FILBIN and HOUGH 1979), and in L. aequinoctialis with 0.0015 mM Cu (NASU and KUGIMOTO 1981, NASU 1983). VAN DER WERFF and PRUIT (1982) did not note any toxicity of Cu concentrations up to 0.5 mM in S. polyrrhiza and L. gibba. Lethal doses are shown in S. polyrrhiza as 0.01 mM (EYSTER 1966), in L. obscura (named as L. minor) as 2 mM (STANLEY and MADEWELL 1976a), and in L. minor as 0.8-1 mM (SAROSIEK and WOZAKOWSKA-NATKANIEC 1980, SAROSIEK et al. 1982), as 0.06 mM (FILBIN and HOUGH 1979) and as 0.005 mM (HUTCHINSON and CZYRSKA 1975). MATTOO et al. (1986a) observed damages of intracellular membranes in S. punctata by copper at concentrations of 0.02 mM and higher. The strikingly different results reflect the fact that the composition of



the nutrient solution, especially the pH and the presence of a chelating agent, has a great influence on the effect of copper and other trace elements. Root growth of L. gibba is inhibited at Cu concentrations of 0.01 mM or higher and is stopped completely at 0.1 mM (LIEBERT 1986a). The effect of high copper concentrations results in the following characteristics (FILBIN and HOUGH 1979): reduction of chlorophyll content, reduction of CO<sub>2</sub> uptake, reduction of sucrose uptake in the dark, and elevation of the respiration rate in the light (dark respiration was not changed).

EDTA in a concentration of 0.03 mM prevented the absorption of Cu (0.005-0.01 mM). If ammonium was added to the solution, the Cu uptake was also greatly suppressed. However, the absorption of Cu was not significantly affected by the initial pH between 3.6 and 5.1 (NASU et al. 1983). A relatively great amount of Cu (0.0005-0.001 mM) in the nutrient solution induces flowering of the short-day plant L. aequinoctialis in both long and short-day regimes, whereas the long-day plant L. gibba loses its ability to flower (HILLMAN 1962, 1965). HILLMAN assumes an effect of Cu on the photoperiodic sensitivity rather than directly on flowering. Also TANAKA and CLELAND (1980) showed that flowering of L. gibba is inhibited by copper under continuous light. This inhibition is largely abolished by salicylic acid (SA) or by EDTA (TANAKA et al. 1982a). Unlike EDTA and ammonium, SA does not affect the absorption of Cu. In L. aequinoctialis, SA multiplied the flowering effect of Cu. The absorption of Cu is not influenced by cadmium, but the flower-inducing activity on L. aequinoctialis is inhibited (NASU et al. 1984). At pH 4.1 0.005 mM Cu results in an unchanged chlorophyll content of L. aequinoctialis, a somewhat reduced growth rate and a substantial induction of long-day flowering. By contrast, at pH 5.1 the same copper concentration causes a reduction in the chlorophyll content and a strong inhibition of growth, but it does not induce long-day flowering (TANAKA et al. 1982b). The flower-inducing activity of Cu is assumed to be the consequence of the suppression of nitrate reductase activity (TANAKA et al. 1986a). KHURANA and MAHESHWARI (1983c) measured a significantly higher copper level in plants of L. aequinoctialis treated with the chelating agent 8-hydroxyquinoline for flowering. Ethylene production in S. punctata is markedly stimulated by CuSO<sub>4</sub> (MATTOO and CONLON 1985). For further details on the flowering effect of copper see chapter 2.4.3.1.3.

The addition of nickel increases the copper uptake in L. minor (HUTCHIN-

SON and CZYRSKA 1975). Coupled uptake mechanisms for copper and iron are assumed by SCHREINEMAKERS (1984) and SCHREINEMAKERS and DORHOUT (1985). RODGERS et al. (1978) showed that copper acts synergistically with zinc and mercury.

CuSO<sub>4</sub> at concentrations of 0.006 and 0.06 mM greatly reduces the growth of Lemnaceae if sprayed as a herbicide. The chlorophyll content of the plants is lowered; the nitrogen content decreases from 4% of the dry weight to 0.3-1% (SINHA and SAHAI 1975).

#### 2.3.3.4.7.8. Cobalt (Co) and nickel (Ni)

EYSTER (1966) was not able to prove any stimulating effect of **cobalt**. Co was toxic to S. polyrrhiza at concentrations of 10 mM. POLAR (1984 in lit.) reported a concentration lethal to a L. minor clone of 0.05 mM Co; another clone showed only a reduction in growth rate of 60% at the same concentration. The uptake of cobalt by L. gibba is enhanced at a low pH (pH was studied between 5 and 9) (EL SHINAWY and ABDEL-MALIK 1980).

For **nickel**, a stimulating effect was reported by GORDON et al. (1978). At a Ni concentration of 0.05 mM, the growth rate of L. aequinoctialis was doubled if urea was used as a sole nitrogen source. The curve of respiration (CO<sub>2</sub> output) shows a double peak daily if Ni is added to the solution whereas without Ni a single peak occurs. According to HUTCHINSON and CZYRSKA (1975), an addition of 0.00017, 0.00085, and 0.0017 mM stimulated the growth of L. minor. At concentrations of 0.005 and 0.008 mM Ni, growth is inhibited, and at 0.017 mM Ni, the fronds die. Ni and Cu have a synergistic effect. If combined they are lethal for L. minor at concentrations at which the single element is not.

#### 2.3.3.4.7.9. Chromium (Cr)

No distinct stimulating effect of chromium is known. However, Lemnaceae are relatively tolerant to Cr. HUFFMANN and ALLAWAY (1973) did not note any influence of Cr at concentrations of 0.0005 mM on the growth rate of Lemna sp. Inhibition of growth was observed by MANGI et al. (1978) in S. polyrrhiza at concentrations of 0.02 mM Cr and in L. minor at 0.00002 mM. Reduced growth of L. minor determined by the frond area, lower chloroplast volume, and decreased content of chlorophyll a and b was measured by BASYNSKI et al. (1981) in solutions with 0.001 to 1 mM Cr; at 10



mM Cr growth was practically stopped. Also CORRADI et al. (1987) observed the loss of grana in chloroplasts of L. minor with 0.02 mM Cr. Chromium affects especially the photosystem II. For L. aequinoctialis, NASU and KUGIMOTO (1981) found growth inhibition at Cr concentrations of 0.02 mM and higher. SHIROISHI and SHIMIZU (1984) observed a slight stimulation of the growth of L. aequinoctialis at concentrations up to 0.002 mM Cr and an inhibition from 0.004 mM upwards. Mortality of L. aequinoctialis was reported by CLARK et al. (1981) at Cr concentrations of 0.005 mM and higher. Concentrations of 0.4 and 1 mM Cr are lethal to L. minor (SAROSIEK and WOZAKOWSKA-NATKANIEC 1980).

STAVES (1980) and STAVES and KNAUS (1985) studied the Cr uptake and the short-time effect of Cr on growth of S. polyrrhiza, S. punctata, and L. gibba using the radiotracer  $^{51}\text{Cr}$ . At Cr levels higher than 0.02 mM growth was greatly inhibited, S. polyrrhiza showing the least tolerance and S. punctata the greatest tolerance towards Cr. At lower concentrations than 0.02 mM no negative effect of Cr has been observed. Between 0.002 mM and 0.4 mM, the uptake of Cr was directly related to ambient Cr concentrations.

#### 2.3.3.4.7.10. Lead (Pb) and cadmium (Cd)

Neither lead nor cadmium stimulate the growth of Lemnaceae.

**Lead** concentrations of 1.5 to 2.5 mM are toxic to L. minor (SAROSIEK and WOZAKOWSKA-NATKANIEC 1980, SAROSIEK et al. 1982). Longest roots were observed with 0.05 mM Pb (longer than at lower or at higher concentrations). Different clones of L. minor showed somewhat different response to Pb. According to FIUSELLO (1973), the chlorophyll content is reduced at 0.1 and 1 mM Pb. The toxicity of Pb is higher if Pb is added as acetate than as nitrate (SCHWEBEL and ANDERSON 1982). S. polyrrhiza (and Eichhornia) have a higher capacity to accumulate Pb than terrestrial species (the Pb given as Pb-EDTA). Under the investigated concentrations of up to 0.5 mM Pb no toxicity was evident.

The uptake of Pb by L. trisulca was studied by EVERARD and DENNY (1985). The absorption is very fast. Within 10 minutes, L. trisulca takes up 0.1 mg Pb per gram dry weight from a solution of 0.005 mM Pb. The uptake is followed by a small net efflux of Pb.

**Cadmium** inhibits growth of L. minor at concentrations higher than 0.0001 mM; it is lethal at 0.005 mM (HUTCHINSON and CZYRSKA 1975). ORNES (1979)

observed growth inhibition of S. polyrrhiza at concentrations of 0.0004 mM. The fronds did not die if temporarily grown with 0.07 mM Cd in contrast to other water plants such as Azolla, Salvinia, and Ceratophyllum. The root growth of L. gibba was reduced at 0.001 mM Cd and stopped at 0.2 mM (LIEBERT 1986a). SETO et al. (1979) report on chlorotic fronds of L. gibba at Cd concentrations of 0.001 and 0.01 mM in a diluted nutrient solution. POLAR and KUECUEKCEZZAR (1986) mention growth inhibition of L. gibba with 0.01 mM Cd. Differently, VAN DER WERFF and PRUIT (1982) did not find any toxicity symptoms in S. polyrrhiza and L. gibba at Cd concentrations of up to 0.5 mM. The toxicity of cadmium is much dependent on the composition of the nutrient solution and on the light regime. It is much more toxic in deionized water than in river water (WANG 1986b). Further results on toxicity and bioaccumulation of Cd are reported by CHARPENTIER et. al. (1987).

In the Hillman M solution (modified Hoagland solution) and in the solution of Bonner-Devirian, 0.0001 mM Cd result in growth inhibition of L. aequinoctialis. In 1/2 Hutner solution, the same effect was achieved at 100 times higher Cd concentrations (NASU and KUGIMOTO 1981, NASU 1983). To prevent the absorption of 0.005 to 0.01 mM Cd, a supply of 0.4 mM EDTA is necessary. Ammonium suppresses Cd absorption only slightly. The absorption of Cd increases with higher initial pH (between 3.6 and 5.1) (NASU and KUGIMOTO 1981, NASU et al. 1983). The uptake of Cd is reduced considerably with increasing concentrations of Cu in the culture solution (NASU et al. 1984). Zn increases the toxicity of Cd (HUTCHINSON and CZYRSKA 1975).

The uptake of Cd by S. polyrrhiza is proportional to the Cd concentration given in the solution. If the Hoagland solution is diluted to 10%, the Cd is absorbed faster than from 50% Hoagland. Afterwards, a release of up to 90% of the Cd absorbed is noted in 10% Hoagland, contrary to the situation in the 50% Hoagland (ORNES 1979). The iron content of L. gibba fronds increases with increasing concentrations of Cd in the solution, the manganese and zinc content decrease (POLAR and KUECUEKCEZZAR 1986).

SUN et al. (1985, paper not seen) report on physiological changes and injury mechanisms in Lemnaceae treated with Cd. SCHREINEMAKERS (1986) studied the Cd absorption and Cd compartmentation in W. gladiata. The Cd concentration in the cytoplasm reached about 20 mM, 240 minutes after adding 0.4 mM Cd to the solution. It amounted to 12 mM at a concentra-

tion of 0.04 mM Cd and to 3 mM at a concentration of 0.001 mM Cd.

#### 2.3.3.4.7.11. Arsenic (As), selenium (Se) and vanadium (V)

No stimulation was observed by the addition of As, Se, or V.

L. obscura (named as L. minor) is able to grow at **arsenic** concentrations of 0.015 mM (as  $\text{NaAsO}_2$ ) or of 0.2 mM (as  $\text{Na}_2\text{AsO}_4$ ) without great damage (STANLEY and MEADWELL 1976a). The growth rate of L. aequinoctialis is inhibited (50%) by 0.01 mM As (NASU and KUGIMOTO 1981, NASU 1983, NASU et al. 1983) and 0.007 to 0.008 mM As reduces the growth rate of L. minor to 50% (SZABADOS et al. 1983).  $\text{NaAsO}_2$  is toxic to Lemnaceae at concentrations of 0.01 mM if sprayed as a herbicide (SINHA and SAHAI 1975).

Arsenic is methylated by S. polyrrhiza, L. minuscula, and W. columbiana, and later, arsoniumphospholipids are formed (BENSON et al. 1981, NISSEN and BENSON 1982). Similarly to other aquatic plants but differently to terrestrial plants, Lemnaceae have evolved mechanisms for rapid detoxification of arsenate, arsenite, and other toxic arsenic substances. According to OERENCIC et al. (1982),  $\text{NaAsO}_3$  does not inhibit chlorophyll synthesis of L. gibba. In this species, however, flowering is inhibited by 0.1 to 0.5 mM disodium arsenate (KANDELER 1968, OOTA 1969a). ULLRICH-EBERIUS and NOVACKY (1984) suggest that the arsenate influx proceeds via the P carrier.

**Selenium** in form of  $\text{H}_2\text{SeO}_3$  is absorbed by L. obscura (named as L. minor) up to a concentration of 0.03 mM without great damage (STANLEY and MEADWELL 1976a). Selenium is a competitor to sulfur. Therefore, it is especially toxic in solutions which lack sulfur (DATKO et al. 1980a). Replacement of the sulfur atom in amino acids by selenium atoms had a toxic effect on L. minor (GULATI et al. 1981). In a solution with equivalent amounts of selenite and selenate, S. punctata takes up three times as much selenite. Colloidal Se is also absorbed. In the soluble cell fraction, Se is present as selenocystine, in the protein as selenomethionine (BUTLER and PETERSON 1967).

**Vanadium** at a concentration of 0.2 mM inhibits growth of L. minor (PIISPANEN and LAEHDESMAEKI 1983). The vanadate/ $\text{H}^+$  cotransport proceeds contrary to arsenate via a carrier which is independent of the P carrier. Vanadate is neither a strong nor a specific inhibitor of the plasmalemma  $\text{H}^+$ -ATPase in vivo for L. gibba as supposed for other plants (ULLRICH-EBERIUS and NOVACKY 1984).

#### 2.3.3.4.7.12. Silver (Ag) and mercury (Hg)

**Silver** inhibits the growth of L. aequinoctialis at a concentration of 0.0001 mM (NASU and KUGIMOTO 1981) and of L. minor at 0.00002 mM (HUTCHINSON and CZYRSKA 1975). The uptake of Ag is not detectable. TAKIMOTO and TANAKA (1973), TAKIMOTO (1981) and NASU and KUGIMOTO (1981) describe a flower-promoting effect of silver in L. aequinoctialis at 0.0001 and 0.01 mM Ag (especially at the low pH of 4.1). This effect is supposed to occur due to the inhibition of the activity of SH groups by Ag.

**Mercury** suppresses growth of Lemnaceae at a concentration of 0.002 mM Hg (CHANG et al. 1977). According to FERNANDEZ et al. (1983), L. aequinoctialis shows a reduced growth rate and brown spots on the frond surface with 0.02 mM Hg; the plants die with 0.03 mM Hg. Hg has a similar effect on flowering of L. aequinoctialis as Cu (HILLMAN 1962, TAKIMOTO 1981, BEPPU and TAKIMOTO 1983).  $\text{HgCl}_2$  at concentrations higher than 0.01 mM depolarizes the membranes of L. gibba markedly and irreversibly. It was concluded that  $\text{Hg}^{++}$  binds irreversibly to SH groups of the  $\text{H}^+$ /sugar and  $\text{H}^+$ /amino acid transport carriers and inhibits these carriers (SCHWEIGER et al. 1984). Further studies on the effect of  $\text{HgCl}_2$  on L. gibba suggest the following sequence of sensitivity of physiological functions: amino-acid and glucose transport and probably  $\text{Na}^+$  and  $\text{K}^+$  proton exchange carriers at the plasmalemma > mechanisms establishing a metabolism-dependent electrical potential at the plasmalemma = passive  $\text{Na}^+/\text{K}^+$  permeabilities at the plasmalemma > respiration and photosynthesis (LOOS and LUETGE 1984).

#### 2.3.3.4.7.13. Germanium (Ge) and silicone (Si)

**Germanium** inhibits growth of L. minor at concentrations of 0.3 mM  $\text{GeO}_3$ . W. arrhiza is able to tolerate somewhat higher concentrations. The inhibition is partly neutralized if silic acid is added to the solution (WERNER 1967). The author supposes that germanic acid disturbs the balance of silic acid. However, it has never been proved that silicone is an essential element for Lemnaceae. LOOMIS (in lit. 1987) tried to substitute germanic acid for boric acid in the nutrient solution. He succeeded in culturing L. minor for 6 months in 0.05 and 0.1 mM  $\text{GeO}_2$ . The plants formed rootless colonies and did not separate normally.

#### **2.3.3.4.7.14. Other minerals**

The uptake and accumulation of further minerals is dealt with in chapter 3.5.3.

#### **2.3.3.5. Organic substances**

##### **2.3.3.5.1. General remarks**

Lemnaceae are able to take up water soluble organic substances besides the inorganic chemical compounds. Four groups of organic substances can be distinguished according to their mode of action in the metabolic processes:

- 1) Energy suppliers and building material: substances which are used as a basis for respiration or which are incorporated in the plant body as reserve substances or as elements of the plant organism.
- 2) Chelating agents: organic substances which form complex compounds with metallic ions enabling a better uptake of the ions and a detoxification of some metallic elements.
- 3) Growth factors: vitamins or hormone-like substances which regulate different metabolic processes; they act in very small doses.
- 4) Toxic substances: chemical compounds which disturb or interrupt certain metabolic processes, or which damage parts of the cells (e.g. pesticides, detergents).

Some of the organic compounds have combined effects. Members of the groups 1) and 3) may also act as chelating agents. Some growth factors behave as toxins in higher doses, and some toxins behave as growth factors in small doses.

The uptake of organic substances in L. aequinoctialis was studied by DATKO and MUDD (1985, 1986). The authors detected 8 distinct uptake and transport systems for organic substances.

##### **2.3.3.5.2. Energy suppliers and building material**

###### **2.3.3.5.2.1. Carbohydrates**

Lemnaceae use carbohydrates as energy suppliers under non-saturating light conditions (cf. HOPKINS 1931, STEINBERG 1941, 1946, GORHAM 1950,

LANDOLT 1957, HILLMAN 1961a, ULLRICH-EBERIUS et al. 1978; cf. also chapter 2.3.5.4). The best effect is realized with glucose, fructose, or sucrose, and to a lesser degree with maltose (YOSHIMURA 1944). Sucrose is changed into invert sugar (glucose + fructose) by autoclaving. The following carbon sources are not usable by Lemnaceae: ethanol, glycerol, mannitol, inuline (YOSHIMURA 1944), starch, lactose, arabinose, ribose, tartrate, succinate, acetate (HILLMAN 1954), citrate, malonic acid, oxalic acid, glucuronic acid, and glutaric acid (CHANG et al. 1977). For L. gibba 0.1% galactose proved to be toxic. The effect is neutralized if glucose is added (DECOCK et al. 1979). According to HUBALD and AUGSTEN (1977a), galactose, ribose, and xylose inhibit the growth of L. gibba. Sorbose has a similar effect on the growth of L. minor as galactose (STROTHER 1981). Five mM sorbose results in complete bleaching of the cultures. The effect can be partly overcome by fructose. It is somehow unclear if glucose or fructose are more favourable to growth of Lemnaceae. The effect is probably dependent on the culture conditions or on the species. THIMANN et al. (1951) achieved better growth in S. punctata with fructose than with glucose. The same was

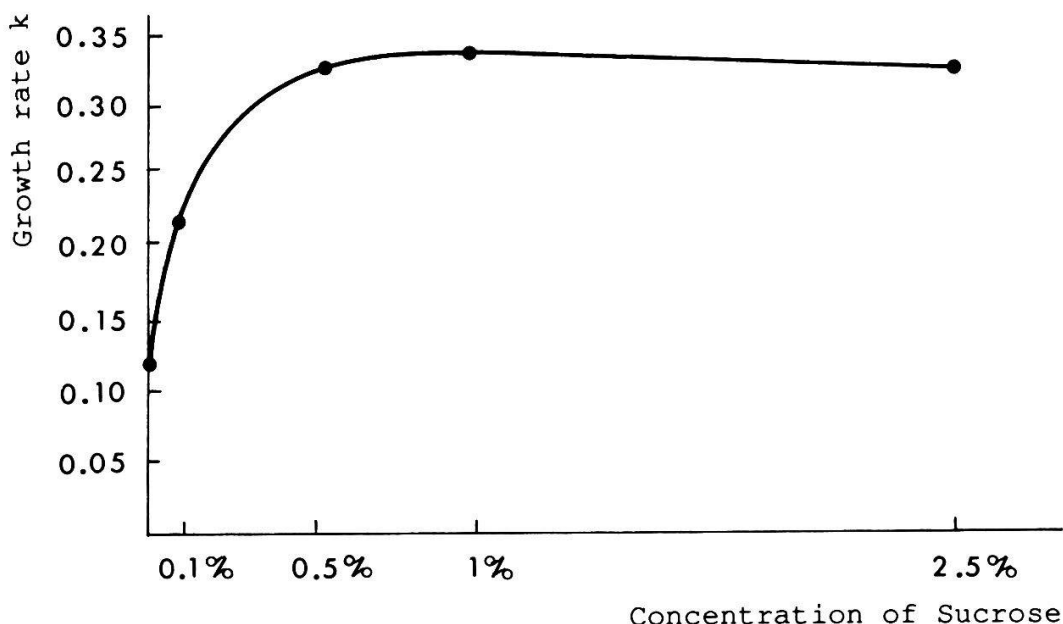


Fig. 2.10. Growth rate of Spirodela polyrrhiza in relation to sucrose concentration at 26°C and light duration of 18 hours per day at 1000 lux (from LANDOLT 1957)

observed by HENSSEN (1954) in S. polyrrhiza. On the other hand, HUBALD and AUGSTEN (1977a) report better growth of L. gibba with glucose than with fructose. DATKO et al. (1980a) mention a similar growth rate in L. aequinoctialis with either glucose or sucrose at the same concentrations.

The best concentration of sucrose for Lemnacean growth is between 0.5 and 2.5% (GORHAM 1950, LANDOLT 1957, ROMBACH 1976) (fig. 2.10). According to DATKO et al. (1980a), the growth rate of L. aequinoctialis still rises slightly between 0.5 and 1% sucrose.

All species of Lemnaceae which are kept under suboptimal light conditions grow faster after adding sucrose. The growth stimulation is limited when the maximum growth rate of the species under optimal light conditions and continuous light is reached. At saturating light intensities, sucrose inhibits growth (LANDOLT 1957). The growth rate of Lemnaceae in solution with sucrose at low light intensities consists additively of the growth rate in darkness (with sucrose) and of the growth

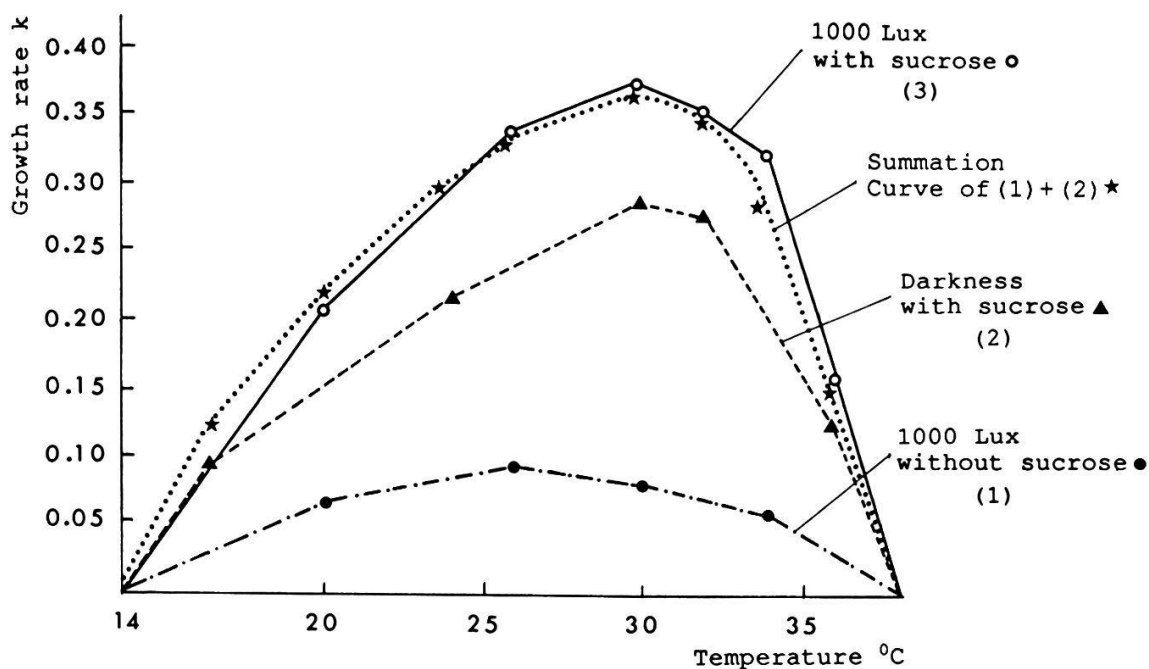


Fig. 2.11. Growth rate of Spirodela polyrrhiza in the dark with 1% sucrose (2), at 1000 lux without sucrose (1) and at 1000 lux with 1% sucrose (3). \*- marks the added values of curve (1) and (2) which corresponds roughly to curve (3) (from LANDOLT 1957)



rate at the same low light intensity in solutions without sucrose (LANDOLT 1957) (fig. 2.11). Species which do not grow on the surface of the water but submerged (L. trisulca, L. valdiviana, Wolffiella species) are dependent upon sugar in the solution if it is not supplemented with a carbon dioxide source (aeration with CO<sub>2</sub>, addition of carbonate or hydrocarbonate) (cf. chapters 2.3.2.2 and 2.3.3.4.5). MONSELISE et al. 1984) investigated a mutant of L. aequinoctialis (6746) incapable of photosynthesis. It was dependent on the supply of sucrose for its growth and showed unusual structures of mitochondria and chloroplasts.

The size of the frond of L. minor decreases with raising of the sucrose concentration from 1 to 3% to about half (ROMBACH 1976). The disconnection of the daughter fronds from the mother fronds is impeded by sucrose; therefore, large colonies develop (e.g. YOSHIMURA 1944, HILLMAN 1954, own observations). The effect of sugars on flowering is presented in chapter 2.4.3.1.4. Fructose and sucrose stimulate the formation of anthocyanin in S. punctata; glucose does this only slightly (THIMANN et al. 1951). GROB and RUFENER (1969) report that 1% glucose in the light changes the chloroplasts of S. punctata. After 25 days, chlorophyll is no longer present; the chloroplasts are filled with starch, the thylakoids have entirely disappeared. Bleached fronds brought into a new glucose-free medium turn green again after a few days. This phenomenon could not be observed in our cultures with sucrose. Glucose is supposed to stimulate the nitrate assimilation (GROB et al. 1973). The content of IAA in S. punctata is enhanced by sucrose (WITZTUM et al. 1978). Sucrose was found to reduce glutamate dehydrogenase activity in L. aequinoctialis; glucose on the other hand increased the activity (DUKE 1975, DUKE and KOUKKARI 1977).

Sucrose uptake was investigated by WOHLER (1966). It amounted to about the same rate in the dark as well as at different light intensities. The uptake rate was different for different species. The following maximum rates were measured in mg sucrose per g dry weight and hour: 10 for S. polyrrhiza, 20 for L. minor, 40 for L. trisulca, and 65 for W. gladiata. For hexoses (glucose and fructose), the absorption is energy-dependent, at least for short times (ULLRICH-EBERIUS et al. 1978). The uptake of hexoses was 1 1/2 to 3 times higher in the light than in the dark. DATKO and MUDD (1985) studied the uptake of aldohexoses (D-glucose, D-galactose, D-mannose) in L. aequinoctialis. Fastest uptake was observed for glucose. Galactose and mannose were absorbed nearly as fast. Fructose,



sucrose, arabinose, ribose, and glycerol showed much lower uptake rates. In nature, sugars might be found in lake water (VALLENTYNE and WHITTAKER 1956, SAUNDERS 1957, HARTMAN 1960, WALSH 1965a,b). Heterotrophic growth in nature is assumed for submerged species such as W. neotropica, W. lingulata, W. oblonga, L. valdiviana. In South America, these species could be observed in the dark under mats of Salvinia, Azolla, and Scirpus cubensis (LANDOLT and ZARZYCKI in prep.).

#### 2.3.3.5.2.2. Amino acids

It is presumed that the addition of amino acids to a well balanced inorganic nutrient solution supplied with a chelating agent does not stimulate growth of Lemnaceae. Some amino acids may inhibit growth because they interfere with the synthesis of other amino acids and metabolic processes. In unfavourable nutrient solutions, especially at low nitrogen concentrations, without a chelating agent or at low light intensities, some amino acids might serve as a nitrogen source, as a chelating agent or merely as an amino acid source. Some of the contradictory results listed below can be explained by not always optimal growth conditions. Mechanisms of uptake of amino acids are described more extensively in chapter 2.5.2.3.1.

If inorganic nitrogen sources are lacking, L. minor is able to use a mixture of glutamic acid, aspartic acid, and arginine as well as casamino acids. Other amino acids such as alanine, valine, methionine, and leucine inhibit growth (JOY 1969a). According to FELLER and ERISMANN (1976), the following amino acids reduce the growth rate of L. minor if added singly to the nutrient solution: arginine, alanine, glycine, asparagine, aspartic acid, glutamine, glutamic acid, ornithine, and citrulline. The inhibiting effect is greater with  $\text{NO}_3^-$  than with  $\text{NH}_4^+$  as nitrogen source. Arginine enlarges the size of the frond of L. minor, promotes the stipes and inhibits the separation of the daughter fronds. Contrary to the results of FELLER and ERISMANN (1976), HUBALD and AUGSTEN (1977a) observed a growth stimulation of L. gibba with the addition of 10 mM L-arginine and L-asparagine. (HUBALD and AUGSTEN used a nutrient solution without EDTA, and a relatively low light intensity of 900 lux). Neither stimulation nor inhibition was found with the following amino acids: glycine, DL-alanine, D-valine, DL-serine, DL-methionine, L-tyrosine, L-histidine, L-proline, and L-tryptophane. NICKELL (1956)

who also worked with a nutrient solution without EDTA reported growth stimulation of L. minor with the amino acids arginine, ornithine, citrulline, and isoleucine. Similar stimulation in S. polyrrhiza with alanine, glutamic acid, and tryptophane was achieved by SMIRNOVA (1975b, 1981, also in solutions without EDTA). The addition of amino acids resulted in a higher dry weight per frond. GULATI et al. (1981) investigated the influence of 55 amino acids not occurring in natural proteins on the growth of L. minor. A replacement of S atoms by Se atoms or certain substitutions by F atoms resulted in toxic effects. Additional methyl and phenyl groups did not change this effect. The authors divide the amino acids studied into 5 groups, according to their toxicity on Lemnaceae.

Alanine, aspartic acid, glycine, and serine inhibit the activity of glutamine synthetase in L. minor, but not that of glutamate dehydrogenase (STEWART and RHODES 1977a). The addition of a single amino acid such as L-valine, L-isoleucine and L-leucine, inhibits the growth of S. polyrrhiza. A combined addition of L-valine and L-isoleucine shows no inhibition, contrary to other combinations (BORSTLAP 1970). Apparently, valine prevents the synthesis of isoleucine, isoleucine that of leucine, and leucine that of valine and isoleucine through blocking of enzymatic processes of synthesis (BORSTLAP 1972, 1977b). The uptake of L-glutamic acid in S. polyrrhiza declined between pH 4.0 and 6.0 and that of L-leucine between pH 4.0 and 8.0 whereas L-lysine uptake was optimal at pH 6 (BORSTLAP et al. 1986). The authors detected two saturable and one non-saturable uptake systems for amino acids. WONG and DENNIS (1973) reported that threonine, homoserine, isoleucine, and to a lesser extent, lysine inhibit growth of L. minor at a concentration of 0.25 mM. Methionine was lethal at the same concentration; so was a combination of lysine and threonine. It is supposed that lysine inhibits the serine hydroxymethyl transferase and in this way the synthesis of methionine (WONG and COSINS 1976). BORSTLAP and VERNOOY-GERRITSEN (1985) studied the influence of leucine and valine, added to the solution, on the leucine and valine synthesis in S. polyrrhiza. If the leucine content increases, leucine synthesis is markedly reduced, valine synthesis only slightly, and isoleucine synthesis apparently not at all. A higher valine content strongly affected the valine synthesis, and the leucine synthesis lesser but not the isoleucine synthesis. The amino acids of the canaline-urea cycle (canavanine, canaline, ureidohomoserine) are toxic to L. minor at a con-

centration of 0.005 mM. Simultaneous application of canavanine and canaline caused an additive reduction of the growth rate. Ureidohomoserine interacted with canaline or canavanine. It increased the additive growth reduction caused by canavanine plus canaline (ROSENTHAL et al. 1975, 1976). Already NICKELL (1956) reported a toxic effect of canavanine on L. minor which could be neutralized by arginine and citrulline. MONSELISE et al. (1986) were able to obtain a partial recovery of plastids in a mutant of L. aequinoctialis which was incapable of ammonium uptake and photosynthesis, by addition of L-methionine and L-cystine.

NAKASHIMA (1964) observed inhibition of flowering of L. gibba by all 20 investigated amino acids (at concentrations between 0.1 and 1 mM). Thirteen of these amino acids did not inhibit growth at the same concentration. The addition of the amino acids glutamic acid, leucine, and serine (at concentrations of 0.001 mM) enhance the flowering percentage of S. polyrrhiza and L. minor under long-day conditions. Valine, tyrosine, and proline stimulated flowering under short-day conditions; cystine and some other amino acids proved to be inhibitory (KRAJNCIC and DEVIDE 1982b). FERGUSON (1970) noted, in S. punctata, a faster uptake of asparagine and glutamine than of nitrate; aspartate and glutamate were taken up at about the same rate as nitrate. NEWTON (1972a) indicates that DL-leucine and uridine are actively transported across the plasmalemma in the roots of L. minor. The uptake of glycine in L. gibba was studied by FISCHER and LUETTGE (1980). The uptake of neutral L-amino acids in L. gibba was shown to be a  $H^+$  cotransport mechanism driven by a proton-electrochemical gradient at the plasmalemma (JUNG and LUETTGE 1980). Similar results were achieved with acidic amino acids (L-alanine, L-serine, L-aspartate) by JUNG et al. (1982). DATKO and MUDD (1985) investigated the uptake of amino acids and other organic compounds in L. aequinoctialis. They identified 8 discrete transport systems two of which for amino acids: a) neutral amino acids (e.g. L-leucine), b) basic amino acids (e.g. L-arginine), c) purine bases (e.g. adenine), d) choline, e) ethanolamine, f) tyramine, g) urea, and h) aldohexoses.

### 2.3.3.5.3. Chelating agents

#### 2.3.3.5.3.1. General remarks

In many older publications, a growth stimulation was reported in cultures supplied with organic substances. BOTTOMLEY (1920b), MOCKERIDGE (1920), and CLARK (1926) observed better growth in Lemnaceae cultures to which extracts of soil, manure, or peat were added. SIELING (1937) prevented chlorosis of L. minor by supplying Fe humate to the nutrient solution. In a similar way, TATKOWSKA and KOBYLANSKA (1978) promoted growth rate, dry weight, chlorophyll, phosphorus and iron content in S. polyrrhiza by means of adding Fe humates to the culture medium. All these effects were achieved in solutions with no other chelating agent. Fe humate is also able to neutralize the toxic effect of the detergent DBSS on S. polyrrhiza diminishing the ratio of P/Fe (GUMINSKI et al. 1978). NICKELL and FINLAY (1954) achieved a growth stimulation of L. minor with addition of antibiotics which might be attributed to chelating properties of these chemicals. Also, a supply with citrate (BITCOVER and SIELING 1951) and kojic acid (YOKOTA and SHIMADA 1958) resulted in higher growth rates of S. polyrrhiza if the solutions were void of a chelating agent. OLSEN supposed, already in 1930, that humus substances promote the availability of Fe to the plants in nutrient solution with a high pH by forming a complex salt. Humic acid otherwise stimulates the production of superoxide radicals generated in the xanthine - xanthine oxidase system. The activity of superoxide dismutase in L. gibba is also enhanced (VAUGHAN and ORD 1982). Oxalate promotes the growth of L. gibba and raises the uptake of Ca, according to DEKOCK et al. (1973). CLARK and ROLLER (1931), SAEGER (1925, 1930), WOLFE (1926), ASHBY (1929b), CLARK (1932), and many other authors proved clearly that good Lemnacean growth is possible in aseptic cultures without addition of organic substances. Today, most authors agree that many of the growth promoting organic substances are effective through formation of chelates with Fe and other metallic ions (e.g. Mn, Zn, and partly also Ca and Mg). If these ions are bound in a complex way, they do not precipitate and are available to the plants. On the other hand, the toxic effect of too high concentrations of metallic ions are moderated if complex salts are formed. Besides citrate and humate, tartrate (in form of Fe tartrate) was frequently used as a chelating agent (e.g. in the Hoagland

solution). However, EDTA is more wide-spread in culture solutions as a chelating agent today. EDDHA and SA which are discussed here under the chelating agents have many effects similar to hormones (e.g. cytokinins) and are used in cultivation of Lemnaceae only for special purposes (e.g. to induce flowering).

EYSTER (1966) compared the effect of EDTA, sesquestrene, tartrate, citrate, and  $\alpha, \alpha, \alpha$ -tris-(hydroxymethyl)-methylamine (tris). Best growth was clearly achieved with EDTA.

#### 2.3.3.5.3.2. Ethylenediaminetetraacetic acid (EDTA)

The optimal concentration of EDTA for growth of S. polyrrhiza was found to be 0.6-2.0 mM if Fe was added in concentrations of 0.05-2.0 mM (fig. 2.12). According to EYSTER (1966), EDTA is destroyed in culture solutions exposed to the light and loses most of its effect after 8 days. Aeration also reduces the activity of EDTA. The destructive action of light on EDTA is independent of the presence or absence of Lemnaceae in the solution. In certain nutrient solutions (e.g. Knop), the activity of

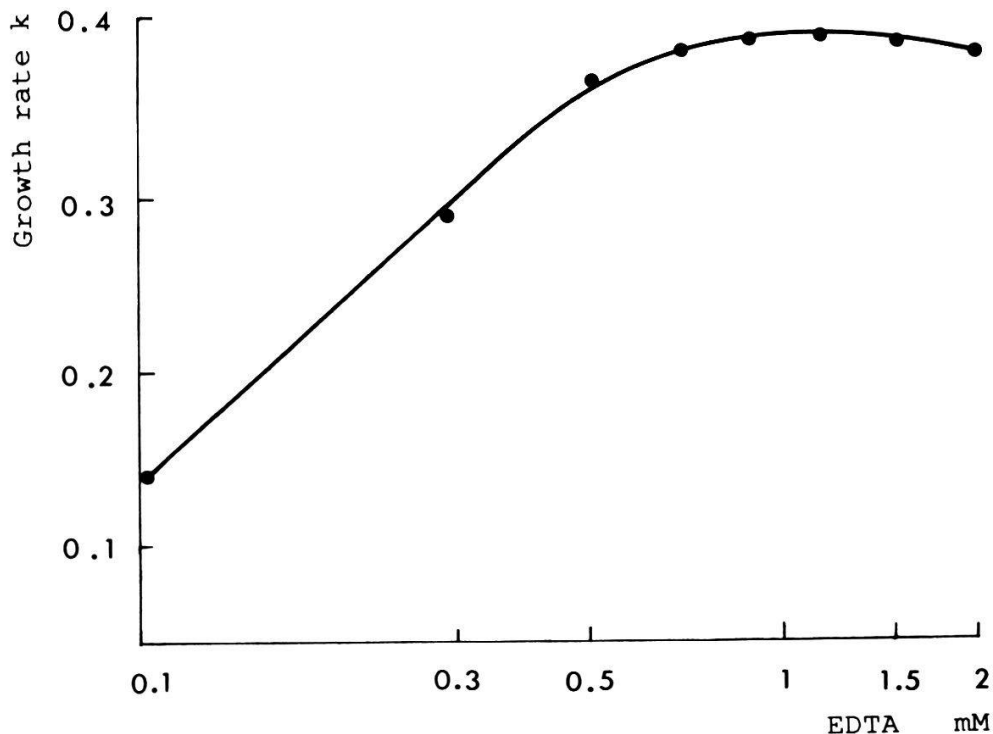


Fig. 2.12. The effect of EDTA on the growth of Spirodela polyrrhiza (from EYSTER 1966)

EDTA is already reduced by autoclaving. It is not clear from our experience why there is no reduction of the growth rate after 8 days cultivation when the EDTA should be destroyed. Under long-day conditions EDTA promotes length of the root, size of the frond and gibbosity of L. gibba, it also intensifies the green colour and stimulates flowering (HILLMAN 1961b). L. gibba which does not flower without EDTA becomes a long-day plant in solutions with EDTA, and the day-length indifferent L. aequinoctialis a short-day plant. This effect is probably due to a preventing action of Cu ions (HILLMAN 1961e, 1962). The flowering effect of old cultures on L. aequinoctialis even under long-day conditions described by LANDOLT (1957) and many other authors may be attributed to the destruction of EDTA by light.

According to BOLGIANO (1979) EDTA removes  $\text{Ca}^{++}$  ions from the solution and therefore, may be toxic to Lemnaceae in solutions with a low Ca content. The author observed an adaptation of the fronds after some time which he ascribes to a possible modification of the enzyme system. It seems more probable that the "adaptation" is due to the destruction of the EDTA after the first few days.

#### 2.3.3.5.3.3. EDDHA, SA, ASA, BEA, 8-HQ

EDDHA and SA have similar effects on L. gibba (PIETERSE and MUELLER 1977) and S. punctata (SCHARFETTER et al. 1978). In L. gibba, both substances initiate gibbosity (10 and 1 ppm, resp.) and flowering under short-day conditions. The stimulation of flowering and the enhancement of the dry weight by 0.01 mM SA was already observed by BHALLA and SABHARVAL (1975). At higher concentrations of SA, flowering and dry weight are reduced. In S. punctata, EDDHA and SA cause a reduction of the size of the frond, a prolongation of the root, a diminution of the number of roots from 2-4 to 1, more intercellular air spaces, more prominent papules, and a delayed separation of daughter fronds; also more chlorophyll and more anthocyanins are formed, and flowering stimulated. The flowers of L. aequinoctialis are induced by SA and aspirin under non-flowering conditions (KHURANA and MAHESHWARI 1978). Also the short-day plant W. microscopica flowers under long-day conditions if either EDDHA (MAHESHWARI and SET 1966a), SA, BEA, aspirin, or salicylaldoxime are added to the solution (KHURANA and MAHESHWARI 1983a). EDDHA, BEA and SA also initiate the flowering of the day-neutral S. polyrrhiza. The fronds

of this species become gibbous and the chlorophyll and anthocyanin content is enhanced (KHURANA and MAHESHWARI 1980, 1986b). LANDOLT (unpubl. results) was able to get the following species to flower in solutions with EDDHA: S. intermedia, S. polyrrhiza, S. punctata, L. gibba, L. disperma, L. minor, L. obscura, L. japonica, L. trisulca, L. valdiviana, W. Welwitschii, W. brasiliensis, W. australiana, and W. columbiana. TAMOT et al. (1987) and TAMOT and MAHESHWARI (1987) were able to induce flowering of W. hyalina under short-day conditions if  $10^{-2}$  mM SA was provided. EDTA, EDDHA, 8-HQ, and zeatin had no effect. The flowering effect of SA only occurs if ammonium, copper, or iron are present in the solution (DEKOCK et al. 1974). More details on the flowering effect of EDDHA and SA can be looked up in chapter 2.4.3.1.2.

SA inhibits the growth rate of L. obscura at concentrations of 0.1 mM or higher. Anthocyanin formation is stimulated at 0.0005 mM and inhibited at 0.05 mM and higher (LEATHER and EINHELLIG 1985). The gibbosity effect of EDDHA and SA on L. gibba is reversed by  $\beta$ -naphthol (PIETERSE 1978b) and by breakdown products of  $GA_3$  (formed during autoclaving) but not by  $GA_3$  itself (PIETERSE 1976, 1982). In S. polyrrhiza (DE LANGE and REVIER 1982) and in S. intermedia (own observations), no gibbosity is induced under the same conditions as for L. gibba. However, this seems to be a question of the right concentration since KHURANA and MAHESHWARI (1980) obtained gibbous S. polyrrhiza with EDDHA. At 0.1 mM SA, the netto photosynthesis is completely inhibited in L. minor (MARTI et al. 1986).

Probably, EDDHA and SA activate the IAA oxidase by delivering of Mn and in this way lower the auxin level of the plants. NAA shows antagonistic effects on EDDHA and SA (SCHARFETTER et al. 1978). CLELAND et al. (1982) suppose that EDDHA is degraded to SA-like fragments within the plants; this explains the similar effect of both substances. SCHARFETTER et al. (1986) assume that EDDHA decreases the endogenous level of free auxins (and, possibly, the endogenous cytokinin level) thus changing the sensitivity to endogenous ethylene. Flowering, production and development of the number of roots, length of the roots, and the size of frond colonies in S. punctata seem to need a certain intermediate ethylene level which can be suppressed by AVG, and AOA or increased by ACC and ethrel.

**Benzoic acid** (BEA) and BEA derivatives have a flowering effect on L. minor and L. aequinoctialis (KAIHARA et al. 1981, TAKIMOTO 1981, WATANABE and TAKIMOTO 1979, WATANABE et al. 1981, 1983, TAKIMOTO and KAIHARA 1986). Two clones of L. aequinoctialis flowered readily on application



of BEA. GA, IAA, ABA, and lutein neutralized the effect. The addition of trans-zeatin on the other hand had a synergistic effect (FUJIOKA et al. 1983b, 1985, 1986d). Cytokinins in general improve the activity of BEA but not of SA. SA is more effective in L. gibba than BEA, but less effective in L. aequinoctialis (CLELAND 1974c, WATANABE and TAKIMOTO 1979). Similar effects of SA, ASA, and BEA on the long-day plant L. gibba are described by CLELAND and TANAKA (1979), CLELAND and BEN-TAL (1982) and FUJIOKA et al. (1985). An addition of BEA intensifies the flower-inducing activity of vitamin K in L. aequinoctialis (KAIHARA and TAKIMOTO 1985a, TAKIMOTO and KAIHARA 1986). Chlorination of BEA suppresses the uptake rate in L. minor (KENNEY-WALLACE and BLACKMAN 1972). KHURANA and MAHESHWARI (1983c, 1984) used **8-hydroxyquinoline** (8-HQ), a well known copper chelating agent, to induce flowering in L. aequinoctialis and W. microscopica (KHURANA et al. 1986) under non-flowering conditions. The endogenous levels of copper and iron are significantly higher in the plants treated with 8-HQ.

SCHARFETTER et al. (1986) showed that the antiauxin PCIB can mimic EDDHA effects in S. punctata to a high degree, causing only one root per frond, large colonies of fronds, good visibility of air chambers and pronounced papillae, but not flowering.

#### 2.3.3.5.4. Vitamins

In cultures with optimal light intensity, Lemnaceae form vitamins endogenously. Therefore, an addition of vitamins has no stimulating effect. CLARK et al. (1938) demonstrated the presence of vitamins A, B<sub>1</sub>, and C in S. polyrrhiza and NAKAMURA (1960) the vitamins A, B<sub>2</sub>, B<sub>6</sub>, and C in W. globosa (named as W. arrhiza). Further references are put together in chapter 1.2.10. YONE and TOMIZAMMA (1952) reported growth promotion of L. aequinoctialis by purified liver fraction which was not due to folic acid nor to vitamin B<sub>12</sub> (this compound stimulates growth only slightly). It must be assumed that this unidentified growth promotion was only possible under the not very favourable conditions applied. However, in darkness, some of the Lemnaceae species need vitamins from the B group (especially B<sub>1</sub>) (cf. GORHAM 1950, ONO 1952a, LANDOLT 1957, HILLMAN 1961a, ROMBACH 1974a,b, 1976, LEUCHTMANN 1979). For more details see chapter 2.3.5.4 on heterotrophic growth. The thiazole component of **thiamine** substitutes in L. minor for the thiamine (B<sub>1</sub>) (ROMBACH 1976).



**Vitamin C** (0.1 mM) promotes flowering of L. aequinoctialis under continuous light (KANDELER 1971a). BARBER and CALDWELL (1976) studied the effect of vitamin C (ascorbic acid) on L. gibba. They observed an influence of vitamin C on the RNA synthesis (2 H-uridine incorporation) and on flowering. Possibly, vitamin C acts as a regulator of the gene activity (especially in relation to flowering). According to FERGUSON and KNYPL (1974), ascorbate can induce high levels of nitrate reductase activity in S. punctata.

A flower inducing activity of **vitamin K** ( $K_1$ ,  $K_3$ ,  $K_5$ ) in L. aequinoctialis was observed by KAIHARA and TAKIMOTO (1985a).  $K_5$  was most efficient in inducing flowering at 0.001 mM. The activity is higher at a pH of 4 than of 5. The effect was intensified by the addition of 0.0005-0.001 mM BEA, but not of BA. Copper as  $CuSO_4$ , and ferricyanide ( $K_3(Fe(CN)_6)$ ) worked synergistically with vitamin  $K_5$ . Dicoumarol and 4-hydroxycoumarin (but not coumarin), known as antagonists of vitamin K, promoted flower-inducing activity of vitamin  $K_5$  and induced flowering in L. aequinoctialis in a similar way to BEA.

**Folic acid** (vitamin  $B_2$  group) shows antagonistic activities against high cytokinin concentrations which inhibit growth of Lemna. It is assumed that folic acid accelerates the photochemical degradation of cytokinins (VAN EYCK 1963, BEZEMER-SYBRANDY et al. 1968, BEZEMER-SYBRANDY 1969). Also ROMBACH (1976) observed a stimulation of the growth rate of L. minor in solutions with 0.001 mM folic acid. FROMM and O'DONNELL (1952) succeeded in enhancing the growth rate of L. minor with PABA, which is an intermediate in the biosynthesis of folic acid. PABA is able to neutralize the inhibiting effect of sulfanil amide but not of ammonium sulfate, sulfaguanidine and sulfathiazole (FROMM and O'DONNELL 1953, 1955).

**Nicotinic acid** (vitamin  $B_3$ ) extracted from culture solutions with flowering L. gibba and L. aequinoctialis showed flower-inducing activity on the same species (FUJIOKA et al. 1986a). An addition of IAA,  $GA_3$ , or ABA inhibits the flower-inducing effect on L. aequinoctialis whereas zeatin leads to a further stimulation of flowering.

The minimum concentration of nicotinic acid to induce flowering of L. aequinoctialis is 10 to 1000 times higher (0.01 mM) than of SA, BEA, and EDDHA (TAKIMOTO and KAIHARA 1986). In L. gibba, the flowering effect of nicotinic acid is inhibited by all four hormones mentioned (FUJIOKA et al. 1986b).

### 2.3.3.5.5. Hormones and hormone-like substances

#### 2.3.3.5.5.1. Auxins, auxin antagonists and effectors of auxin metabolism (BZ, 2,4-D, IAA, IBA, NAA, PAA, PCIB, PPA, TIBA)

**Growth rate.** The auxins regulate mainly the extension of the cells. At low concentrations, there is probably no distinct effect on multiplication of cells and on growth rate (GORHAM 1941 with IAA, IBA, and NAA in L. minor; BLACKMAN and ROBERTSON-CUNINGHAME 1954 with IAA in L. minor; CLARK and FRAHM 1940a,b with IAA, PAA, and PPA in L. minor). ONO (1952a) reported on a stimulation of the growth rate of S. polyrrhiza and Lemna sp. with IAA and NAA at concentrations of  $10^{-4}$  to  $10^{-2}$  mM. However, he had a very incomplete nutrient solution (no Fe, no trace elements), his results are therefore not representative. The same is true for the results of the following authors which did not work with very balanced nutrient solutions either. CLARK and FRAHM (1940a,b) stimulated the growth rate of L. minor with intermittent addition of IAA, PAA, and PPA. According to CHANG et al. (1977, 1978), IAA, IBA, and NAA (0.01-0.025 ppm) improved the growth rate of L. aequinoctialis, but not of S. polyrrhiza and W. globosa (named as W. arrhiza). The growth was only measured for 5 days. Possibly, the increase of fronds was achieved by faster elongation of the cells which makes the daughter fronds visible earlier. TIBA at concentrations of  $10^{-2}$  to  $10^{-1}$  mM inhibited the growth rate of L. gibba (OOTA 1965).

**Size of frond.** The frond is enlarged by BZ (cells larger, but not greater in quantity (HILLMAN 1954, 1955). The lengths of fronds and stalks of L. trisulca are increased by the addition of IAA to the solution (BATA and NESKOVIC 1982a). In S. intermedia the frond size is reduced with increasing concentrations of IAA and with high concentrations of TIBA (1 mg/l), but enlarged with low concentrations (0.1 mg/l) of TIBA (KLICH et al. 1987b).

**Length of roots.** Low concentrations of IAA and NAA ( $10^{-4}$  to  $10^{-3}$  mM) result in longer roots of S. polyrrhiza and Lemna spp., higher concentrations in shorter roots (ONO 1952). Similar results were obtained in L. minor by BLACKMAN and ROBERTSON-CUNNINGHAME (1954) with IAA, by HILLMAN (1954) with IAA and NAA, and by CLARK and FRAHM (1940a, b) with IAA, PAA, and PPA. The roots of L. trisulca become shorter if IAA is added at concentrations of 0.001, 0.01, 0.1, and 1 ppm (BATA and NESKOVIC 1982a).

Root elongation of L. minor is completely inhibited by 3.4 mM BZ (HILLMAN 1955). TILLBERG and ELIASSON (1976) report on a root elongation in L. gibba with  $10^{-6}$  mM IAA and an inhibition with  $10^{-4}$  mM and higher. IBA reduces root length but not root number of S. intermedia with increasing concentrations (KLICH et al. 1987b).

**Stipes of the daughter fronds.** The stipe of L. minor becomes elongated by auxine-like substances: CLARK and FRAHM (1940a, b) with IAA, PAA, and PPA, BLACKMAN and ROBERTSON-CUNNINGHAME (1954) with IAA, and HILLMAN (1954, 1955) with IAA, NAA, BZ, and PCIB (the last only at high concentrations). The stalk of L. trisulca is also elongated by IAA (BATA and NESKOVIC 1982a).

**Formation of accessory nerves.** The addition of IAA, NAA, and TIBA results in the formation of accessory lateral nerves in L. minor (SARGENT 1957).

**Closing of stomata.** TIBA (1 ppm) is able to close the stomata of S. intermedia completely. The closing is coupled with a characteristic lower potassium content and a lower starch content within the stomata cells (KLICH 1986).

**Development of daughter fronds.** If TIBA is added to the culture solution, the daughter fronds of L. minor develop simultaneously on both sides of the mother fronds. It can happen that the first daughter frond appears on the side where normally the second one is formed. Additional IAA and NAA strengthens the effect at medium concentrations of TIBA, but lowers it at low and high concentrations (WANGERMANN and LACEY 1953, SARGENT 1957). According to WITZTUM (1966), TIBA prevents the formation of axillary buds in Lemna.

**Flowering.** The flowering of L. aequinoctialis (GUPTA and MAHESHWARI 1970, TAKIMOTO 1981) and of L. gibba (OTA 1965, TAKIMOTO 1981; FUJIOKA et al. 1983b) is inhibited by auxin-like substances. According to OTA and TSUDZUKI (1971), IAA hampers the flowering of L. gibba at concentrations of  $10^{-3}$  mM and stimulates it at  $10^{-6}$  mM.

**Protein content.** MACIEJEWSKA-POTAPCZYK et al. (1976) observed a 39% increase of the protein content in solutions with  $5 \times 10^{-6}$  mM IAA.

**Uptake of the substances and mode of action.** BLACKMAN and SARGENT (1959) and BLACKMAN et al. (1959) observed a constant absorption of PAA by L. minor whereas 2,4-D and TIBA are released again after the first two hours. PAA and TIBA are more easily taken up at low pH than at high pH. On the other hand, the effect of BZ in L. minor is greater at pH 6.2

than at pH 4.2 (HILLMAN 1954, 1955); the cell elongation effect is not affected by purines, but neutralized by PCIB. The higher the chlorination of PAA, the faster is the uptake (KENNEY-WALLACE and BLACKMAN 1972). The uptake and the hydrolysis of IAA conjugates in L. gibba have been studied by SLOVIN et al. (1984). IAA and IBA substantially increase the inhibitory effect of ABA on growth of L. gibba. NAA and 2,4-D do not show any synergistic effect. PCIB used as an antiauxin did not counteract the synergistic action of IAA and ABA. Apparently, the enhancing effect of IAA on the ABA action is not a typical auxin effect (TILLBERG and ELIASSON 1976). A chelating effect of IAA for Cu and Fe ions has been observed by OOTA and TSUDZUKI (1971).

#### 2.3.3.5.5.2. Cytokinins (adenine, BA, iP, kinetin, purine, thiokinetin, zeatin)

**Growth rate and weight.** Cytokinins (at low concentrations) improve the growth rate and enhance the dry weight of Lemnaceae at low light intensities (e.g. 900 lux) and in darkness but have no marked effect at high light intensities (see also chapter 2.3.5.4). It is assumed that under optimal conditions a sufficient amount of cytokinins is formed by the plants themselves to perform maximal cell multiplication. However under suboptimal conditions, the cytokinin level in the plants is lowered. In this situation, the addition of cytokinin has an accelerating effect on cell division (TREWAWAS 1979). ROMBACH (1976) achieved a stimulation of the growth rate in L. minor to about 170% by kinetin. The stimulation disappears at light intensities above  $200 \mu\text{W cm}^{-2}\text{s}^{-1}$ . TILLBERG et al. (1979) enhanced the growth rate to about 110% and the fresh weight to 120-140% with  $10^{-6}$  mM BA and at light intensities of  $132 \mu\text{W cm}^{-2}\text{s}^{-1}$ . The effects of cytokinins on Lemnaceae are surveyed in table 2.18. The effective concentration is dependent on species, form of cytokinin, and culture conditions (e.g. light intensity, day-length, see OOTA 1966 and AL-SHALAN and KANDELER 1978). In general, concentrations of cytokinins higher than  $10^{-2}$  mM are lethal for Lemnaceae (PERLMAN and SEMAR 1967).

**Size and form of frond.** The fronds of Lemnaceae are generally enlarged (even at high light intensities) when cytokinins are added to the culture solution (ROMBACH 1961, 1976, OOTA 1965, LETHAM 1967, MAHESHWARI and VENKATARAMAN 1966, BEZEMER-SYBRANDY 1969, VENKATARAMAN et al. 1970, SCHWEBEL 1973, LE PABIC 1976a, TILLBERG et al. 1979, KLICH et al.

1987c). In L. minor, the cell is also enlarged by adenine (WANGERMANN and LACEY 1953). In L. trisulca, the frond is not enlarged significantly, but the stalk is elongated (BATA and NESKOVIC 1982a). The fronds of S. punctata become vaulted after the addition of zeatin (KERN and NAEF-ROTH 1975).

Table 2.18. Influence of cytokinins on growth rates of Lemnaceae

Kind of cytokinin	concentration in mM			species	author
	stimulation	inhibition	lethal		
kinetin	$10^{-3}$			<u>S. punctata</u>	13
	$10^{-3}$			<u>L. gibba</u>	14
	$10^{-2}$ - $10^{-3}$			<u>L. minor</u>	8
	$10^{-3}$	$10^{-2}$		<u>L. minor</u>	16
	$10^{-2}$ - $10^{-4}$			<u>L. minor</u>	3
	$10^{-5}$			<u>L. minor</u>	6
		$10^{-2}$		<u>L. minor</u>	7
	$10^{-4}$	$10^{-3}$		<u>L. minor</u>	11
	$10^{-2}$ - $10^{-4}$			<u>L. trisulca</u>	2
	$10^{-3}$ - $10^{-4}$			<u>L. aequinoctialis</u>	4
	$10^{-2}$			<u>W. columbiana</u>	9
	$10^{-3}$ - $10^{-4}$			<u>W. globosa</u>	4
	$10^{-5}$			<u>L. minor</u>	6
	$10^{-2}$ - $10^{-5}$	$10^{-1}$ - $10^{-2}$	1	<u>S. polyrrhiza</u>	12
thiokinetin	$10^{-3}$			<u>S. punctata</u>	13
BA	$10^{-3}$ - $10^{-4}$			<u>L. gibba</u>	5
	$10^{-3}$			<u>L. minor</u>	17,18
	$10^{-2}$ - $10^{-4}$			<u>L. minor</u>	3
	$10^{-3}$ - $10^{-4}$	$10^{-1}$		<u>L. minor</u>	18
	$10^{-3}$			<u>L. minor</u>	1
	$10^{-3}$ - $10^{-4}$			<u>L. aequinoctialis</u>	5
		$10^{-3}$ - $10^{-4}$		<u>L. aequinoctialis</u>	15
	$10^{-5}$ - $10^{-7}$			<u>L. aequinoctialis</u>	10
	$10^{-3}$			<u>L. minor</u>	18
	$10^{-4}$			<u>S. punctata</u>	13
iP					
zeatin					

#### References

- 1 ALBANELL et al. 1985
- 2 BATA and NESKOVIC 1982a
- 3 BEZEMER-SYBRANDY 1969
- 4 CHANG et al. 1977
- 5 CURTIS 1971
- 6 DEYSSON 1959
- 7 ERISMANN and WEGNER 1967
- 8 HILLMAN 1957
- 9 HILLMAN 1960a
- 10 KOUKKARI and DUKE 1973
- 11 KRAINCIC 1982
- 12 LE PABIC 1967a, b
- 13 LETHAM 1967
- 14 OOTA 1965, 1966
- 15 PERLAN and SEMAR 1967
- 16 ROMBACH 1961, 1976
- 17 SCHWEBEL 1973
- 18 TASSERON-DE JONG and VELDSTRA 1971a,b

**Length of roots.** The root growth is inhibited in L. gibba by BA and kinetin (BEZEMER-SYBRANDY 1969), and in L. trisulca by kinetin (BATA and NESKOVIC 1982a). However, BA increases the length of the root of L. gibba at low concentrations ( $10^{-5}$  to  $10^{-4}$  mM). Only at higher concentrations ( $10^{-3}$  to  $10^{-1}$  mM) there is an inhibiting effect (LIEBERT 1980a). In S. intermedia, the number and length of roots are not affected by BA up to a concentration of 0.1 mg/l (KLICH et al. 1987c).

**Development and disconnection of daughter fronds.** Kinetin ( $5 \times 10^{-1}$  mM) and still more effectively BA ( $10^{-2}$  mM) stimulate the development of the second daughter frond neutralizing the inhibition by the first daughter frond (GUERN 1965). BA inhibits the disconnection of daughter fronds of L. minor (SCHWEBEL 1973).

**Turion formation.** Turion formation is completely inhibited at BA concentrations of  $10^{-2}$  mM (JUNGNICKEL and AUGSTEN 1986).

**Turion germination.** Cytokinins (iP, BA) are able to overcome turion dormancy in S. polyrrhiza and stimulate germination (MALEK and COSSINS 1983).

**Flowering.** BA inhibits the flowering of L. gibba but not of L. aequinoctialis (CURTIS 1971). Kinetin at high concentrations impedes flowering of L. gibba, too (similar to IAA); at low concentrations ( $10^{-6}$  mM) the flowering is stimulated. The flowering of L. aequinoctialis is promoted by cytokinins under short-day conditions, but the critical dark period required is not altered (KHURANA and MAHESHWARI 1983b). The flowering of L. aequinoctialis LP<sub>6</sub> a photoperiodically neutral clone is stimulated by cytokinins in the simultaneous presence of a high iron level (0.1 mM) (KHURANA and MAHESHWARI 1986d). The authors assume that cytokinins might stimulate the effect of chelating agents. Cytokinins induce the flowering of the short-day plant W. microscopica (MAHESHWARI and VENKATARAMAN 1966, VENKATARAMAN et al. 1970). Flowering of S. polyrrhiza and L. minor is enhanced under flowering conditions by kinetin at concentrations of 0.025- 0.05 ppm and 0.05-0.1 ppm, respectively; kinetin stimulates apical floral induction as well as the postinductive stages in the floral development. 0.2 ppm of kinetin inhibit flowering of both species (KRAJNCIC 1982). W. arrhiza, too, achieves higher percentage of flowering under flowering conditions (preculture under long-day, culture under short-day conditions) if BA, zeatin, or kinetin is added to the culture solution. Flowering under short-day conditions (without long-day preculture) is induced by BA, zeatin and kinetin; flowering under long-day



conditions is achieved only by application of zeatin (KRAJNCIC 1983). When isolated flower primordia are cultivated in vitro, L. aequinoctialis has a tendency to form more readily the male organs, L. gibba the female ones. Kinetin is able to neutralize this unilateral tendency (HUEGEL 1976c).

**Senescence.** Senescence in L. gibba is delayed by cytokinins (PARK et al. 1987).

**Content of starch and sugars.** The starch content of L. minor fronds rises by 200% 4 days after application of kinetin, the content of soluble sugars by 40% (FANKHAUSER and ERISMANN 1969a). This causes the chloroplasts to burst after 5 days (ERISMANN and WEGNER 1967, ERISMANN and FANKHAUSER 1967). According to FANKHAUSER and ERISMANN (1969a) kinetin does not directly influence the sugar metabolism but rather the protein synthesis. Due to a general growth inhibition coupled with a relatively intact photosynthesis, the carbohydrates are stored. Also BA results in starch accumulation in L. minor. The starch content rises from 10% to 25% of the dry weight in a medium with 0.1 mM BA (TASSERON-DE JONG and VELDSTRA 1971a). KLICH et al. (1987c) did not observe any change in the starch content of the cells of S. intermedia after addition of kinetin or BA.

**Protein metabolism.** Cytokinins enhance the protein content of Lemnaceae (ERISMANN and FANKHAUSER 1967, KRZECZOWSKA and ZIMNA 1972, MACIEJEWSKA-POTAPCZYK et al. 1976, LE PABIC 1976a). VAUGHAN et al. (1983) report of a decrease of protein content of L. gibba in solutions with 4 and  $40 \times 10^{-4}$  mM BA. The protein synthesis is accelerated and the decomposition reduced by cytokinins (TREWAWAS 1972a,b). Kinetin is able to enhance the RNA content of L. minor for a short time (ERISMANN and FANKHAUSER 1967); the synthesis as well as the decomposition of RNA are stimulated (TREWAWAS 1970). MCCOMBS and RALPH (1972a,b) were able to demonstrate protein synthesis of S. punctata in darkness when kinetin was added to the solution. The formation of DNA and RNA was accelerated. According to BEZEMER-SYBRANDY and VELDSTRA (1971a,b), BA is incorporated in nucleotides of L. minor; with alkaline hydrolysates cytokinins are established in the t-RNA. Kinetin inhibits the degradation rates of mRNAs encoding two major chloroplast proteins in L. gibba (TOBIN and TURKALY 1982). A cytokinin-modulated regulation of mRNA levels for the chlorophyll a/b protein in L. gibba is reported by FLORES and TOBIN (1985).

**Lipid composition.** BERUBE et al. (1982) observed distinct modifications in the lipid composition of L. minor after application of BA: greater

proportions of phospholipids as compared with galactolipids, greater  $\alpha$ -linolenic acid content of total neutral lipids. The author supposes that the cell membranes (particularly those of the chloroplasts) and their functions may be altered by high doses of cytokinins. LE PABIC (1980) noted a greater content of total phospholipids after application of  $10^{-3}$  mM BA. The proportion of unsaturated polar lipid fatty acids increased slightly.

**Content of chlorophylls, carotinoids and flavonoids.** The chlorophyll and carotinoid content in S. polyrrhiza is lowered by  $10^{-2}$  mM BA and slightly raised by  $10^{-4}$ - $10^{-3}$  mM BA (LE PABIC 1976a,b). The anthocyanin content of S. polyrrhiza is also reduced at kinetin concentrations of  $5 \times 10^{-1}$  mM and at BA concentrations of  $10^{-2}$  mM. ERISMANN and WEGNER (1967) observed a reduction of the chlorophyll content of L. minor in solutions with kinetin. Cyclic adenosine monophosphate (cAMP) treatment inhibits chloroplast replication of W. arrhiza but results in an increased exponential chloroplast growth rate: 260% of that of the kinetin control treatment; whereas cyclic guanosine monophosphate (cGMP) results in a rate of that of the kinetin treatment (HONDA 1983a,b). The flavonoid content of S. intermedia was enhanced by kinetin ( $10^{-3}$  mM) in the light but not in the dark (McCLURE 1973).

**Other effects and mode of action.** The growth stimulation caused by BA results in a lower content of calcium in S. polyrrhiza; the content is enhanced again by the addition of ABA (DEKOCK and HALL 1981). Cytokinins generally counterbalance the effects of ABA. BA is able to neutralize the growth inhibiting influence of ABA (VAN OVERBEEK and MASON 1968). Kinetin prevents the turion formation of S. polyrrhiza stimulated by ABA (STEWART 1969). Adenine is able to neutralize the growth stimulating as well as the inhibiting effect of cytokinins (GUERN 1965, PERLMAN and SEMAR 1967). According to BEZEMER-SYBRANDY (1969), adenine and adenosine prevent only the uptake of cytokinins but do not succeed in suppressing the growth stimulating effects. BA has no influence on the pectinase activity in L. minor (SCHWEBEL 1973). However, it decreases the activity of superoxide dismutase and the content of zink in L. gibba at concentrations of  $4 \times 10^{-4}$  mM (VAUGHAN et al. 1983). Kinetin has no effect on the photosynthesis of L. minor (ROMBACH 1976). Addition of cytokinins reduces the longevity of L. minor in darkness (TASSERON-DE JONG and VELDSTRA 1971b). According to VAN EYCK and VELDSTRA (1966), the kinetin-type activity on the growth of L. minor seems to be restricted to adenine derivatives.



### 2.3.3.5.5.3. Gibberellins (GA)

Gibberellic acid ( $GA_3$ ) is transformed to allogibberic acid in the nutrient solution after autoclaving. This latter substance is more effective than  $GA_3$  in inhibiting flowering of L. aequinoctialis. In a similar way, 1,3-desoxy allogibberic acid originates from  $GA_7$  (PRYCE 1973a).

**Growth rate and dry weight.** The growth rate of L. gibba (PIETERSE 1974b, 1975a), of L. minor (LOOS 1962), and of L. aequinoctialis (HILLMAN 1960b, PRYCE 1973a, CHANG et al. 1977) is raised by GA, that of S. intermedia lowered (at a concentration of  $10^{-2}$  mM) (McCLURE 1973) and that of L. trisulca remains nearly unchanged but the dry weight per frond is lowered (BATA and NESKOVIC 1982). CCC as a blocker of gibberellin synthesis inhibits growth of L. minor at concentrations of  $10^{-3}$  mM and is lethal at 1 mM (SUPNIEWSKA 1963).

**Size of frond.** The size of the fronds of L. minor (LOOS 1962), of L. aequinoctialis (HILLMAN 1960b, PRYCE 1973b), and of L. trisulca (BATA and NESKOVIC 1982a) is reduced by GA; however, the length of the stalk in L. trisulca is not altered.

**Gibbosity and buoyancy.** The gibbosity of L. gibba fronds is lowered drastically after addition of GA to the nutrient solution (PIETERSE 1975a). Correspondingly, addition of CCC results in thicker fronds of L. minor (SUPNIEWSKA 1963). No effect on gibbosity was observed in S. intermedia at concentrations of 0.1 mg  $GA_3$ /l. But at concentrations of 1 and 3 mg/l, the ability of the frond to float was affected by allowing the entrance of water into the air spaces (KLICH et al. 1985). The percentage of submersed fronds increased up to 100. An indirect action of GA on the epidermal structure affecting water permeability is not excluded by the authors.

**Epidermis and stomata.** GA (1 or 3 mg/l) increases the number of stomata and decreases the number of epidermis cells per unit area in S. intermedia (KLICH et al. 1987d).

**Length of roots.** The roots of L. minor (LOOS 1962) and of L. trisulca (BATA and NESKOVIC 1982a) are shorter in solutions with GA compared with controls.

**Development of daughter fronds and connecting stipes.** GA stimulates the growth of the second daughter frond of S. polyrrhiza and L. trisulca; the growth of the stipes is promoted by GA (GUERN 1965). SUPNIEWSKA (1963) reported on inhibited disconnection of daughter fronds in L. minor by the action of CCC.

**Flowering.** The flowering of L. aequinoctialis is inhibited by GA (HILLMAN 1960b, PRYCE 1973b). GA promotes the formation of male organs in explanted flower primordia of L. gibba and L. aequinoctialis (HUEGEL 1976a,b,c). Flowering of L. gibba is stimulated by GA at concentrations of  $10^{-2}$  mM and prevented at  $10^{-1}$  mM (OOTA and TSUDZUKI 1971). According to PIETERSE (1974b), allogibberic acid inhibits flowering of L. gibba at concentrations higher than  $10^{-3}$  mM (under continuous light). HUEGEL and KANDELER (1974) suppose that GA is produced in the meristem of the long-day plant L. gibba at lower concentrations than in the short-day plant L. aequinoctialis. The short-day plant is believed to contain more GA in relation to the counteracting ethylene. The amount of GA which is taken up from the solution and transported to the meristem is regulated by light through the phytochrome system (HARTUNG and KANDELER 1976). The synthetic compound hexahydrofluorene-9-carboxylic acid has the same effect on flowering of L. aequinoctialis as allogibberic acid (PRYCE 1974, 1978). Chapter 2.4.3.1 deals with further information on flowering.

**Content of chlorophylls, carotinoids, flavonoids, and fatty acids.** The decomposition of chlorophyll in old fronds of L. trisulca is prevented by GA. This results in a prolongation of the green colour of old fronds for 2-3 weeks compared with the fronds of controls (BATA and NESKOVIC 1974). Chlorophyll and carotinoid content is reduced at  $10^{-2}$  mM gibberellic acid concentration (D'HARLINGUE 1976). SUPNIEWSKA (1963) reported higher chlorophyll content in L. minor with the addition of CCC to the solution. GA<sub>3</sub> at concentrations of  $10^{-2}$  mM inhibits the formation of flavonoids of S. intermedia in the light but not in the dark (McCLURE 1973). The total fatty acid content of S. polyrrhiza is reduced, the sterole content increased in 0.1 mM gibberellic acid (D'HARLINGUE 1976).

**GA uptake and transport.** The uptake of gibberellin in L. aequinoctialis and its accumulation and distribution was studied by AL-SHALAN and KANDELER (1979), in L. gibba by KANDELER and HARTUNG (1976). The rate of GA transport within the plant amounts to about  $2.5-3.0 \text{ mm h}^{-1}$  (HARTUNG and KANDELER 1976).

#### 2.3.3.5.5.4. Abscissic acid (ABA)

**Growth rate.** ABA inhibits growth processes. The cell elongation of L. gibba is hampered by ABA concentrations of  $10^{-4}$  mM (CHEN and PARK 1976). At higher concentrations, cell division as well as cell elongation in

L. minor is prevented (NEWTON 1972b, 1977). According to CURTIS (1971), growth reduction of L. gibba and L. aequinoctialis can be observed at ABA concentrations higher than  $10^{-6}$  mM. Evidently, the effect of ABA is dependent on culture conditions. Further growth inhibitions are reported by KLICH et al. (1987a) in S. intermedia, by STEWART (1969) in S. polyrrhiza, VAN STADEN and BORNMANN (1969) in S. punctata, CHEN and PARK (1976), LIEBERT (1977), AL-SHALAN and KANDELER (1978), DEKOCK et al. (1978), TILLBERG et al. (1979), and DEKOCK and HALL (1981) in L. gibba, VAN OVERBEEK and MASON (1968), SCHWEBEL (1973), McLAREN and SMITH (1976), NEWTON (1977), and ALBANELL et al. (1985) in L. minor, CURTIS (1971) in L. aequinoctialis, and PIETERSE (1972) in W. gladiata. Growth promotion has been observed at low ABA concentrations: at  $10^{-7}$  to  $10^{-5}$  mM in S. polyrrhiza (McWHA and JACKSON 1976, SMART and TREWAVAS 1983a), at  $10^{-6}$  mM in S. punctata (VAN STADEN and BORNMANN 1969), and at  $10^{-7}$  to  $10^{-4}$  mM in L. gibba (CHEN and PARK 1976, AL-SHALAN and KANDELER 1978).

**Size of frond, thickness, and colour.** The frond of L. minor is smaller in solutions with ABA concentrations of  $10^{-2}$  mM than in controls, and the colour is more intensely green (NEWTON 1972b, SCHWEBEL 1973). The size of the frond of L. gibba only amounts to 53-65% of the control in solutions with  $10^{-4}$  mM ABA (TILLBERG et al. 1979). The layers of air spaces in L. minor are reduced by ABA (SEVERI and BARONI FORNASIERO 1983a). KLICH et al. (1987a) did not see any change of aerenchymatic tissue of S. intermedia after addition of ABA. However, the frond size became smaller at concentrations of  $10^{-3}$  mM or higher.

**Length of root.** ABA inhibits the growth of the root of L. minor. Both, maximum length and number of the cells are reduced (NEWTON 1972b, 1974a, 1977). In a similar way growth of L. gibba roots is hampered at ABA concentrations of  $10^{-5}$  mM and higher (LIEBERT 1980a). In S. intermedia, the root length is reduced by ABA but the root number is not affected (KLICH et al. 1987a).

**Development and disconnection of daughter fronds.** ABA treatment results in the production of fewer daughter fronds in L. minor (SCHWEBEL 1973) and in L. gibba (KANG and CLELAND 1985). The disconnection of daughter fronds of L. minor is induced by ABA (SCHWEBEL 1973, OSTROW-SCHWEBEL 1979). The area of disconnection in the stipe of L. minor was studied by OSTROW-SCHWEBEL (1979) in detail. Contrary to these results, NEWTON (1972b, 1977) observed a retardation of the disconnection of L. minor in

solutions containing ABA; the fronds remained in clusters. However, his concentrations of ABA were nearly three times stronger (2 ppm) than those of SCHWEBEL (1973).

**Turion formation.** Turion formation of S. polyrrhiza is stimulated by ABA at concentrations of  $10^{-3}$  to  $10^{-5}$  mM (PERRY and BYRNE 1969, STEWART 1969, SAKS et al. 1975, 1980, SMART and TREWAVAS 1983a). The same is true for the formation of resting fronds of L. minor (VAN OVERBEEK et al. 1968, SEVERI and BARONI FORNASIERO 1983a), of L. perpusilla and of L. aequinoctialis (KANDELER and HUEGEL 1974a). PERRY and BYRNE (1969) report a mutant of S. polyrrhiza which showed neither growth inhibition nor turion formation in response to ABA. The ABA induced turions of S. polyrrhiza are characterized by reduced  $Ca^{++}$  exchange across the tonoplast and low vacuolar  $Ca^{++}$  content compared with vegetative fronds. In addition, the turions exhibit a higher plasmalemma flux with a correspondingly high  $Ca^{++}$  concentration in the cytoplasm. The accumulation of  $K^{+}$  and  $Cl^{-}$  is much lower in the cytoplasm of the ABA induced turion than in the vegetative frond (SMART and TREWAVAS 1984b). For further information on turion formation see chapter 2.4.2.

**Flowering.** ABA (at concentrations of 0.1-1  $\mu$ g/l) significantly promoted flowering of the long-day plant L. minor under long-day conditions. At higher concentrations (10-50  $\mu$ g/l), it inhibited or suppressed the floral induction of S. polyrrhiza, L. gibba, L. minor, and W. arrhiza. ABA (0.1-1  $\mu$ g/l) in combination with EDDHA (7 mg/l) had a synergistic effect on flowering of L. minor and S. polyrrhiza (KRAJNCIC 1985). At concentrations of 0.1  $\mu$ g/l, ABA enhances flowering of L. minor (especially the initial stages of apical flower induction), at concentrations of 10  $\mu$ g/l and higher, it inhibits flowering (KRAJNCIC and BAVEC 1986). Increasing concentrations of ABA reduced stamen development in explanted flower primordia of L. gibba and pistil development in such explants of L. aequinoctialis (HUEGEL 1976c). ABA counteracts the flowering effect of BA (FUJIOKA et al. 1983b).

**Senescence.** Senescence of L. gibba is prematurely induced by ABA (PARK et al. 1987).

**Content of starch and sugar.** The starch and sugar content of Lemnaceae is enhanced in solutions with ABA (VAN STADEN and BORNMANN 1969, McLAREN and SMITH 1976, 1977, DEKOCK et al. 1978, TILLBERG et al. 1979, KLICH et al. 1987a). The uptake of glucose and glycine is stimulated by ABA (HARTUNG et al. 1980). SEVERI and BARONI FORNASIERO (1983a) observed plas-

tids with especially large starch grains in L. minor growing on solutions containing ABA. THORNSTEINSSON et al. (1985) recorded increasing amounts of free sugars in L. gibba in solutions with higher ABA content. The effect of ABA was in part counteracted by BA.

**Mineral composition.** ABA changes the mineral composition in the frond of L. gibba; it lowers the P/Fe ratio from 33 to 6 and the K/Ca ratio from 3 to 0.6 (DEKOCK et al. 1978). ABA ( $10^{-3}$  mM) lowers the K content in L. gibba from 60 mg/g dry weight to 20 mg (LIEBERT 1977).

**Chlorophyll and carotinoid content.** ABA reduces the chlorophyll content of L. minor and enhances the carotinoid content (SEVERI and BARONI FORNASIERO 1983a). ALBANELL et al. (1985) observed a higher chlorophyll content in fronds of L. minor at concentrations of  $10^{-3}$  mM ABA.

**Photosynthesis and respiration.** The  $\text{CO}_2$  fixation in L. minor is inhibited by ABA; a direct effect on the transport of electrons of the photosystem II is supposed (BAUER et al. 1976). Also TILLBERG et al. (1981) noted an inhibition of the  $\text{CO}_2$  uptake in L. gibba after addition of ABA to the solution. The activity of photosynthetic enzymes in L. minor is, however, enhanced by the action of ABA, according to SANKHLA and HUBER (1979). Respiration in the dark was stimulated within the first 2 to 7 days. Unlike other phanerogams, Lemna does not close the stomata after ABA application (TILLBERG et al. 1981).

**Other life processes and mode of action.** DEKOCK et al. (1978) observed a rise of enzyme activities of phosphatase, peroxidase, amylase and invertase in L. gibba after application of ABA. The RNA content is, according to this author, higher. On the other hand, NEWTON (1972b, 1974a) reports an inhibition of the RNA synthesis of L. minor in solutions containing ABA. Also TREWAVAS (1970) noted a markedly reduced synthesis rate of ribosomal RNA but unaltered degradative rates in L. minor after adding ABA. According to ANDRES and SMITH (1976), the content of free amino acids in fronds of S. polyrrhiza, L. minor, and L. aequinoctialis is raised by ABA; the same is true for the release of amino acids to the culture solution. The authors assume that ABA increases the permeability of the cell membrane. ABA does not influence the ability of S. polyrrhiza to induce nitrate reductase (STEWART and SMITH 1972). The cellulase activity of L. minor becomes enhanced by  $3 \times 10^{-3}$  mM ABA whereas pectinase activity is not significantly affected (SCHWEBEL 1973). VAUGHAN et al. (1983) recorded an increase of superoxide dismutase and zinc, copper, and protein content in L. gibba at ABA concentrations of 4 to 40x

$10^{-4}$  mM. DNA synthesis in the developing turions of S. polyrrhiza which have been induced by ABA is inhibited by ABA and followed by a repression of protein synthesis while RNA synthesis was not altered (SMART and TREWAVAS 1984a).

The effects of ABA are partly neutralized by BA and other cytokinins but not by gibberellins (VAN OVERBEEK and MASON 1968, VAN STADEN and BORN-MANN 1970b, DEKOCK et al. 1978, ALBANELL et al. 1985). The turion stimulation by ABA is also suspended by kinetin (SAKS et al. 1975).

#### 2.3.3.5.5.5. Ethylene and ethylene releasing substances (ethrel, ACC)

The effect of ethylene on Lemnaceae is partly similar to that of GA and partly to that of ABA. The effect of ethylene is reversed by autoclaved  $GA_3$  (PIETERSE 1976, 1982).

**Growth rate.** At concentrations of  $4 \times 10^{-2}$  mM, ethylene promotes the growth rate of S. polyrrhiza by 50%; at  $4 \times 10^{-1}$  mM it inhibits the growth rate (NEGBI et al. 1972).

**Size of frond and gibbosity.** An enlarging effect on fronds of L. gibba in a mould-infected culture was explained by WETSTEYN et al. (1984) by the assumed production of ethylene by the mould. Gibbosity of L. gibba is caused by ethylene at concentrations of  $10^{-3}$  mM and higher. Similar concentrations could be measured in the field at places where gibbous L. gibba occurred; at places where flat L. gibba was present, the ethylene concentration was significantly lower (ELZENGA et al. 1980). Chloroethanephosphonic acid (ethrel) which was found to release ethylene within the Lemna fronds has the same effect on gibbosity as ethylene (PIETERSE 1976).

**Nerves and disconnection of the daughter fronds.** Ethylene (0.1%) was found to induce the 4th and 5th nerve in L. minor (SARGENT and WANGER-MANN 1959). The disconnection of daughter fronds of S. polyrrhiza is accelerated by ethylene (NEGBI et al. 1972).

**Flowering.** According to PIETERSE (1982), ethylene does not induce or enhance flowering in L. gibba, but SCHARFETTER et al. (1984, 1986) report a role of ethylene in flowering of Lemnaceae. Investigations with ACC, a direct precursor of ethylene, showed a stimulation of flowering in the long-day plant L. gibba at  $10^{-5}$  mM. At  $3 \times 10^{-4}$  mM ACC was flower-inhibiting. The flowering of the long-day plant S. punctata was prevented by  $10^{-6}$  to  $10^{-4}$  mM ACC. In contrast, flowering in the short-day plant L.



aequinoctialis was promoted by  $10^{-4}$  to  $10^{-3}$  mM ACC under long-day conditions (SCHARFETTER et al. 1987). Ethrel promotes the development of female flower parts in explanted flower primordia of L. gibba and L. aequinoctialis (HUEGEL 1976b). For more details on effects of ethylene on flowering of Lemnaceae see chapter 2.4.3.1.

**Senescence.** Ethylene induces premature senescence in L. gibba (PARK et al. 1987).

#### **2.3.3.5.6. Toxic substances and phytochemical substances not classified**

##### **2.3.3.5.6.1. General remarks**

There is an enormous literature on toxic effects of different chemicals on growth and behaviour of Lemnaceae which is not easy to survey. A rather incidental selection is presented here. The reasons for the extensive work are as follows:

- 1) Lemnaceae are suitable test plants for checking the toxicity to organisms of new chemicals (e.g. pesticides, detergents) (see chapter 3.6)
- 2) In some regions it is necessary to keep mass development of Lemnaceae under control (duckweeds are obstacles for shipping, bathing; they may deteriorate the quality of fish ponds etc.).

Some of the investigated substances behave as hormone-like factors at lower concentrations and are therefore dealt with in chapter 2.3.3.5.5. A few substances toxic for Lemnaceae are put together in tables 2.19 and 2.20.

##### **2.3.3.5.6.2. Herbicides**

In general, Lemnaceae are very sensitive to herbicides. They are often used as test organisms to detect the presence of herbicides in water: e.g. L. minor for prometryn and diuron (DECLÉIRE and DE CAT 1977), S. polyrrhiza for ametryn and cyanatryn (SUTANTO et al. 1976) and for simazine, linuron, bromacil, fluorodifen, paraquat, and MCPA (DAMANAKIS 1970, 1972) (see also chapter 3.6).

Heterocyclic compounds (e.g. 6-methylpurin), urea derivatives, and quaternary ammonium compounds belong to the most toxic substances for Lemnaceae. They still affect the growth rate of Lemnaceae at  $10^{-4}$ - $10^{-5}$  mM.

Table 2.19. Effects of some herbicides on Lemnaceae

	Concentration in mM		species	author
	inhibiting	lethal or inhibiting > 50%		
<u>Inorganic compounds</u>				
Ammonium sulfamate		1	d	7
Ammonium thiocyanate		1	d	7
Sodium chlorate		5	d	7
<u>Phenol derivatives</u>				
Nitrofen	$10^{-3}$		c	33
Fluorodifen	$10^{-4}$	$10^{-3}$	a	5
<u>Urea derivatives</u>				
Diuron, DCMU	$10^{-4}$ - $10^{-3}$		c	14
	$10^{-5}$		e	24
	$10^{-5}$	$10^{-3}$	m	18
Fluometuron	$10^{-5}$	$10^{-3}$	m	18
Linuron	$10^{-4}$		a	5
Chlorobromuron		$10^{-4}$	h	35,36
Diphenoxylon		$10^{-4}$	h	35,36
<u>Aliphatic carbonic acids</u>				
2,5-Dimethyldodecanoic acid	$10^{-3}$		d	6
<u>Araliphatic carbonic acids and nitriles</u>				
Chloroflurenol	$10^{-3}$		a	31
2,4-D	$10^{-3}$	$10^{-2}$	d	1,2,7,27,30
MCPA	$10^{-3}$		c	22
	$10^{-3}$		g	34
	$10^{-4}$		a	4,5
<u>Amines</u>				
Ethalfuralin ( $10^{-5}$ mM stimulating)	$10^{-4}$	$10^{-3}$	d	19,20
<u>Carbonic acid amides</u>				
Alachlor		$10^{-4}$	d	10
<u>Quarternary ammonium compounds</u>				
Diquat	$10^{-5}$	$10^{-4}$	a	0,4,5
Paraquat	$10^{-5}$	$10^{-4}$	a	0,4,5
<u>Heterocyclic compounds</u>				
Fluridone	$10^{-4}$	$10^{-3}$	d	20
Lenacil		$10^{-4}$	h	35,36
Bromacil	$10^{-4}$	$10^{-3}$	a	5
Simazine	$10^{-4}$		a	5
		$10^{-2}$	d,k	15
Atrazine ( $10^{-4}$ mM stimulating)	$10^{-3}$ - $10^{-2}$	$10^{-1}$ -1	a,c,m	16,17,18
CPS ( $10^{-3}$ , $10^{-4}$ mM stimulating)	$10^{-1}$		a,c	26
CTS	$10^{-2}$		a	26
	$10^{-4}$		c	26
Ametryn	$10^{-5}$	$10^{-3}$	m	18
Terbutryn	$10^{-5}$	$10^{-4}$	d	20
Prometryn	$10^{-5}$	$10^{-3}$	m	18
2,4-Dichloropyrimidine		$10^{-2}$	d	21
Thiouracil, TU		$10^{-2}$	d	21



Table 2.19 (continued)

	Concentration in mM		species	author
	inhibiting	lethal or inhibiting 50%		
Purin	10 <sup>-8</sup>	10 <sup>-2</sup>	e	23
6-Chloropurin		10 <sup>-2</sup>	e	23
6-Methylpurin, MP		10 <sup>-5</sup>	e	23
		10 <sup>-3</sup>	c	32
<u>Organic compounds containing phosphorus</u>				
Glyphosate, Phosphonomethylglycine		10 <sup>-4</sup> -10 <sup>-2</sup>	c	11
SC-0224	10 <sup>-2</sup>		d	9,25
9-Amine-9-fluorenephosphonic acid	10 <sup>-2</sup>		c	6a
9-Aminofluoren-9-ylphosphine oxides	10 <sup>-2</sup>		b	3
<u>Organic compounds containing arsenic</u>			b	8,13
MSMA	10 <sup>-1</sup>		■	18

#### Species

a	<i>Spirodela polyrrhiza</i>	e	<i>L. aequinoctialis</i>	i	<i>W. columbiana</i>
b	<i>S. punctata</i>	f	<i>Wolffiella gladiata</i>	k	<i>Wolffia</i> sp.
c	<i>Lemna gibba</i>	g	<i>Wolffia brasiliensis</i>	l	<i>Lemna</i> sp.
d	<i>L. minor</i>	h	<i>W. arrhiza</i>	m	duckweeds

#### References

- |                                 |                                  |
|---------------------------------|----------------------------------|
| 0 BLACKBURN and WELDON 1965     | 18 LIU and CEDENO-MALDONADO 1979 |
| 1 BLACKMAN 1952                 | 19 LOCKHART and BLOUW 1979       |
| 2 BLACKMAN and                  | 20 LOCKHART et al. 1983          |
| ROBERTSON-CUNNINGHAME 1954      | 21 NICKELL 1962                  |
| 3 CZERWINSKI et al. 1982        | 22 OOTA 1966                     |
| 4 DAMANAKIS 1970                | 23 PERLMAN and SEMAR 1967        |
| 5 DAMANAKIS 1972                | 24 POSNER et al. 1977            |
| 6a FOY and COOLEY 1987          | 25 PRASAD 1984                   |
| 6 ENTZEROOTH et al. 1985        | 26 RASOL and RENDIC 1977         |
| 7 FROMM 1951                    | 27 SHAMBHU et al. 1976           |
| 8 GANCARZ et al. 1985           | 28 SIMON and BLACKMAN 1953       |
| 9 HARTMAN and MARTIN 1984       | 29 STEPHENSON and KANE 1984      |
| 10 HARTMAN and MARTIN 1985      | 30 SUPNIEWSKA 1963               |
| 11 HOAGLAND and PAUL 1978b      | 31 TREICHEL 1974a                |
| 12 JAWORSKI 1972                | 32 TROGISCH and ULLRICH 1984     |
| 13 KAFARSKI et al. 1985         | 33 WEJNAR and TAIS 1983          |
| 14 KANDELER 1969b               | 34 WORTHLEY and SCHOTT 1971      |
| 15 KARELS and LEMBI 1973        | 35 ZAWADZKI 1974                 |
| 16 KRSNIK-RASOL and RENDIC 1975 | 36 ZAWADZKI and REJMAN 1978      |
| 17 KRSNIK-RASOL and RENDIC 1977 |                                  |

The following classification follows the monographic treatment of herbicides by WEGLER (1977).

#### **Inorganic compounds**

Ammonium sulfamate, ammonium thiocyanate, and sodium chlorate were tested by FROMM (1951) on L. minor. They proved to be moderately toxic (lethal at c. 1 mM). Copper sulfate, sodium arsenite, ammonium thiocyanate, and ammonium 10% were used as foliar spray solutions on S. polyrrhiza and showed lethal effects at the same concentrations (SAHAI et al. 1980).

#### **Phenol derivatives**

Of 36 tested **nitrophenols** 12 impeded the formation of chloroplasts in Lemnaceae (PRICE and WAIN 1976). Whereas phenol only inhibits L. minor at concentrations of 5 mM, 2,4-dinitrophenol does already at  $10^{-2}$  mM (BLACKMAN et al. 1954). The biological effect of substituted phenols was enhanced if alkyl side chains were lengthened or the number of substituted chlorine atoms or alkyl groups in the ring were increased. The substitution on the para position was more toxic than substitution on the ortho position (BLACKMAN et al. 1954, SIMON and BLACKMAN 1953).

**Nitrofen** at concentrations of 0.1 ppm (c.  $5 \times 10^{-4}$  mM) inhibits growth rate and root growth of L. gibba and lowers the content of pigments, chlorophylls a and b,  $\beta$ -carotene, lutein, violaxanthin, and neoxanthin (WEJNAR and TAIS 1983). It destabilizes the chloroplast structure: formation of cisterns within the stroma, widening of the thylakoid interiors, and disappearance of the grana structure (at nitrofen concentrations of  $2 \times 10^{-4}$ ,  $5 \times 10^{-4}$ ,  $5 \times 10^{-3}$  mM) (WEJNAR and MICHEL 1983).

**Pentachlorophenol (PCP)** inhibits the photosynthetic  $O_2$  production of L. minor and decreases its chlorophyll content. There is no pronounced effect on  $O_2$  consumption during respiration in the dark. The influence of the toxin on many enzymes was studied (HUBER et al. 1982). Of 11 aquatic animal and plant species tested, L. minor was the least sensitive to PCP with no apparent effects up to concentrations as high as 1.4 mg/l (HEDTKE et al. 1986). Another study with pentachlorophenol was made by YUI and KOIKE (1955) and YUI (1958).

#### **Carbonic acid and thiocarbonic acid derivatives**

**CIPC** in concentrations up to 10 mg/l has no effect on the photosynthesis of S. polyrrhiza (BIELECKI and SKRABKA 1976).

#### **Urea derivatives**

**Diuron (DCMU)**, a photosynthetic inhibitor inhibits growth and stimulates

flowering in L. gibba (KANDELER 1969b). This effect is not due to altered sizes of ATP pools (GOWER and POSNER 1979). DCMU also inhibits growth of L. aequinoctialis at concentrations of  $10^{-5}$  mM and enables flowering under long-day conditions. A mutant type of strain 6746 is stimulated by concentrations up to  $10^{-3}$  mM DCMU (POSNER et al. 1977). The degradation of the 32 kDa protein in S. punctata is inhibited by DCMU (MATTOO et al. 1986b). Diuron and fluometuron stimulate the activity of the nitrate reductase in S. polyrrhiza at concentrations of about  $10^{-5}$  mM. The activity is lowered at  $10^{-3}$  mM (diuron) or  $10^{-2}$  mM (fluometuron) (LIU and CEDENO-MALDONADO 1979).

#### **Araliphatic carbonic acids and nitriles**

**Morphactins** as chloroflurenol lower growth rate, length of root, rate of respiration, and photosynthesis of L. gibba. They enhance the content of starch and chlorophyll as well as the level of ATP and the activity of amylase and phosphorylase (TREICHLER 1974a,b). KAFARSKI et al. (1985) investigated the toxicity of phosphonic analogues of morphactins in S. punctata. The glycylglycyl and threonyl peptides showed great activity in reducing dry weight.

**2,4-D** has a relatively low activity against Lemnaceae. The activity is dependent on the pH of the solution: at 4.6 the effect on L. minor is ten times greater than at 6.1 (BLACKMAN and ROBERTSON-CUNINGHAME 1953). FERNANDEZ et al. (1972) report a similar pH dependence of the 2,4-D effect on S. intermedia (compare chapter 2.5.2.3.2). At higher light intensities, the effect of low concentrations of 2,4-D is greater than at lower ones. IAA partly neutralizes the inhibiting influence of 2,4-D on L. minor. At high concentrations of 2,4-D, the light intensity is of no significance for the toxic effect. However, a pretreatment with low light intensity enhances the effect (BLACKMAN and ROBERTSON-CUNNINGHAME 1954). 2,4-D is absorbed by S. intermedia mainly by the roots, according to FERNANDEZ et al. (1972). If polystyrene is used as a carrier substance, 2,4-D shows a long-time effect in water. To suppress growth of L. minor, it is necessary that the concentration of 2,4-D does not sink below  $10^{-1}$  mM (SHAMBHU et al. 1976).

2,4-D and CIPC have no effect on the photosynthesis of S. polyrrhiza at concentrations up to 10 mg/l (BIELECKI and SKRABKA 1976). TITOVA (1978a) reports the accumulation of 2,4-D in Lemnaceae. TCDD, a very poisonous impurity of the herbicide 2,4,5-T is accumulated 1000 to 5000 times by L. minor (ISENSE and JONES 1975a) (see also chapter 3.5.3).

2,4,5-T and 2,4-D have been investigated by SAHAI et al. (1980) applied as a foliar spray on S. polyrrhiza at concentrations of 100 ppm and higher. In a similar way, KHARE (1977, 1979) spread MCPA, 2,4-D, and 2,4,5-T on the surface of S. polyrrhiza fronds. Necrosis showed up at concentrations of 1 to  $10^{-1}$  mM. Control of duckweeds in fish ponds was accomplished successfully by spraying 0.7 g/ha endothall and 0.09 g/ha tryad which together proved very toxic to Lemnaceae. The herbicide **mecoprop** killed S. punctata within 10 to 20 hours in sublethal doses of  $10^{-1}$  mM if  $0.5-1.5 \times 10^{-2}$  mM of derivatives of 9-amino-9-fluorene phosphonic acid were added simultaneously (CZERWINSKI et al. 1982).

#### **Quarternary ammonium compounds**

**Paraquat** is very toxic to S. polyrrhiza (DAMANAKIS 1970, 1972). A similar effect on Lemnaceae is caused by **diquat** (FUNDERBURN and LAWRENCE 1963, BIRMINGHAM and COLMAN 1983). Diquat and paraquat give rise to chlorosis in S. polyrrhiza, L. minor, W. gladiata, and W. columbiana; most sensitive is S. polyrrhiza, least W. columbiana. After seven days treatment with 0.01 ppm diquat (in brackets paraquat) the following percentage of chlorosis can be observed: 95% (80%) in S. polyrrhiza, 85% (70%) in L. minor, 75% (65%) in W. gladiata and 5% (5%) in W. arrhiza. The activity of the herbicides rises with light intensity; it is highest in red light and lowest in green light (BLACKBURN and WELDON 1965). Diquat at 1.1-3.4 kg/ha gave the best long-term control of S. polyrrhiza in Florida (better than glyphosate or endothal) (THAYER and HALLER 1985).

**Orellanine**, a toxic substance produced by the basidiomycete Cortinarius speciosissimus has a similar structure as diquat and paraquat. It destroys the photosynthetic pigments and the chloroplasts of L. minor in the same way as the two herbicides (HOILAND 1983). Photosynthetic activity of L. minor is efficiently inhibited by orellanine at a concentration of 0.4 mM (RICHARD et. al. 1987).

The change in cell membrane permeability of L. minor was the criterion used by O'BRIEN and PRENDEVILLE (1978, 1979) to detect the presence of paraquat and diquat in water. The minimum herbicide concentration that could be detected ranged from 0.000017 to 0.00018 ppm (ca.  $10^{-7}$  to  $10^{-6}$  mM). Diquat remains toxic to Lemnaceae for many weeks after treatment if it is absorbed by clay minerals of the aquatic sediment (BIRMINGHAM and COLMAN 1983). A mixture of diquat and cutrine plus was most effective against Lemnaceae for application over wide areas (HARGROVE 1976).

### Heterocyclic compounds

NICKELL (1962) investigated the effect of 87 **pyrimidines** on L. minor, 7 of which (including 2,4-dichloropyrimidine and thiouracil) proved to be strongly inhibiting at concentrations of  $10^{-2}$  mM. Eight compounds had a stimulating effect at the same concentration. Naturally occurring cytosins did not show any effect on L. minor. UMEMURA and OOTA (1965a) studied the effect of FDU, FU, TU, and other antimetabolites and of corresponding metabolites (thymidine, orotic acid, uracil, guanine, methionine) on growth and flowering of L. gibba. All antimetabolites reduced flowering. RIMON and GALUN (1967) also investigated the impact of pyrimidine base analogues on growth of S. punctata. FDU stopped cell division at  $4 \times 10^{-4}$  mM and higher. This effect could be multiplied by the addition of  $4 \times 10^{-2}$  mM thymidine. Neither uridine nor uracil had an antidotal effect. FU at  $10^{-2}$  mM affected cell division, frond elongation, and differentiation. No counteraction by thymidine or uracil was visible. TU inhibited differentiation of frond tissue rather specifically. Uracil and uridine counteracted the effect. **Atrazine** at sublethal doses raises the content of water, chlorophyll, soluble protein, nitrogen, and DNA of L. minor and lowers its photosynthesis. At low concentrations, however, the photosynthesis is stimulated (BEAUMONT et al. 1976, 1978), and the total lipid content enhanced (GRENIER et al. 1979). Phosphatidyl glycerol content is increased and the content of phosphatidyl ethanolamine and of phosphatidyl inositol decrease in the presence of atrazine (GRENIER et al. 1982). Also, sublethal doses of atrazine stimulate the desaturation of fatty acids and the lipid metabolism of the chloroplasts (increase of the number of grana per chloroplast) (GRENIER and BEAUMONT 1983). MATTOO et al. (1984b) noted a higher linolenic/linoleic acid ratio in the thylakoid membrane lipids of S. punctata under sublethal concentrations of atrazine. Fourteen days after application of atrazine, the chlorophyll content is greatly reduced and fronds become chlorotic (GUNKEL 1983). Atrazine inhibits the 32 kDa protein degradation in S. punctata (MATTOO et al. 1986b). GRESSEL (1982a,b) reports on the possible **triazine** interaction with a 32000  $M_r$  thylakoid protein in S. punctata. Triazine inhibits photosynthesis by binding to thylakoids. **Simazine** inhibits the photosynthesis rate of L. minor at concentrations of 1 ppm (SUTTON et al. 1969). The substance was used as a foliar spray solution (at concentrations of 100 ppm and higher) on S. polyrrhiza by SAHAI et al. (1980). **Ametryn** and **prometryn** stimulate the activity of the

nitrate reductase in S. polyrrhiza at concentrations of about  $10^{-8}$  mM. The activity decreases at concentrations of  $10^{-3}$  mM. BAHADIR and PFISTER (1985) report a sufficient checking of L. minor in a simulated flow system by 20 ppb (c.  $10^{-4}$  mM) **terbutryn**. Terbutryn at concentrations of  $10^{-3}$  mM is destroyed faster in ponds with Lemnaceae and Typha sp. than in ponds without water plants, the half-life period being 21 days instead of 30 days (MUIR et al. 1981). In channels sprayed with terbutryn or **cyanatryn**, Lemnaceae are able to thrive due to lack of competition (MURPHY et al. 1981). Of all herbicides investigated, **6-methylpurin** was most toxic. PERLMAN and SEMAR (1967) observed an inhibiting effect on L. aequinoctialis at concentrations as low as  $10^{-8}$  mM. At concentrations of  $10^{-3}$  mM it completely inhibits the growth rate of L. gibba, and reduces chlorophyll and protein content and the activity of nitrate reductase (TROGISCH and ULLRICH 1984).

#### **Organic compounds containing phosphorus**

**Glyphosate** is not very effective against L. minor and W. arrhiza according to KARELS and LEMBI (1973). They had to apply about 1 mM to get toxic effects. However, HARTMAN and MARTIN (1984) noted that glyphosate is lethal to L. minor at concentrations of about  $10^{-4}$  mM. If suspended bentonite clay was added to the solution, glyphosate was not toxic at the same concentration due to unavailability of the herbicide bound to clay particles. N-phosphonomethylglycine appears to inhibit the aromatic amino acid biosynthetic pathway in L. gibba (JAWORSKI 1972).

**Glyphosine**, a plant growth regulator which causes chlorosis in maize and increases sucrose levels in sugar cane, inhibits the growth rate of L. gibba at 1 mM and stops it at 10 mM. In solution with  $5 \times 10^{-1}$  mM glyphosine, newly formed fronds are white or pale yellow and contain less than 0.4% of the chlorophyll of a green frond (SLOVIN and TOBIN 1980, 1982). Lemnaceae often concentrate herbicides within their fronds.

#### **Further organic compounds used as herbicides**

Hydrothol-47 and TD-1929 show a toxic effect on Wolffia sp. but not on Lemna sp. at concentrations of 1-3 ppm (c.  $10^{-2}$  mM) (KARELS and LEMBI 1973).

**Cyanoacrylate** herbicides are potent inhibitors of the photosynthetic electron flow in isolated chloroplast systems. They are highly toxic to L. minor and other water plants (HUPPATZ and PHILLIPS 1981).

SAHAI et al. (1980) tested benzene, kerosene, and xylene as a foliar spray on S. polyrrhiza. Toxic effects showed up at c. 1 mM and higher.

Table 2.20. Effects of some algicides, fungicides, insecticides, and other toxic substances on Lemnaceae

Substances	concentrations in mM		species	author
	inhibiting	lethal or inhibiting > 50%		
<u>Algicides</u>				
HOE 2977		$10^{-2}$	d	2
PH 40:62		$10^{-3}$	d	10
QAC	$5 \times 10^{-3}$	$5 \times 10^{-2}$	b	15
<u>Fungicides</u>				
CuSO <sub>4</sub>		$10^{-2}$ - $10^{-1}$	c	1
2,4,6-TCP	$10^{-4}$	$10^{-2}$	c	1
<u>Insecticides</u>				
Matacil		$10^{-2}$	d	5
Methyl parathion	$> 10^{-2}$		d	11
DDT	$10^{-1}$		f	16
<u>Detergents</u>				
DBST, ENF ( $10^{-4}$ mM stimulating)		$10^{-2}$	a	13
"Super Surf"		$10^{-1}$	a	7
<u>Bleaching substances</u>				
Phenol, Catechol	$10^{-2}$	$10^{-2}$ - $10^{-1}$	e	6
	$10^{-2}$		d	12
Guaiacol	$10^{-2}$ - $10^{-1}$	$10^{-1}$ -1	e	6
9,10 Epoxystearic acid	$10^{-1}$ -1	1	e	6
<u>Disinfectants</u>				
o-Cresol		1	c	1
CS	$10^{-2}$	$10^{-1}$	b,e,f	8
<u>Other toxic substances</u>				
Patulin		$10^{-2}$	c	9
Aflatoxin	$10^{-5}$		d	3
Hydroquinone	$10^{-2}$		d	12
Glycolate, Glyoxylate (0.5-3 mM stimulating)	5		c	14
DMMP, DMMM	$10^{-2}$	$10^{-1}$	b	4

#### Species

a *Spirodela polyrrhiza*      c *Lemna gibba*      e *L. aequinoctialis*  
b *S. punctata*      d *L. minor*      f *Wolffia brasiliensis*

#### References

- |                                |                                  |
|--------------------------------|----------------------------------|
| 1 DAVIS 1981                   | 9 SLOVIN et al. 1984             |
| 2 HARTZ et al. 1972            | 10 SPENCER-JONES 1981            |
| 3 JACQUET and BOUTIBONNES 1970 | 11 STEPHENSON and KANE 1984      |
| 4 KNYPL and OSWIECIMSKA 1986   | 12 STOM and ROTH 1981            |
| 5 PRASAD 1981                  | 13 TATKOWSKA and TOPOROWSKA 1978 |
| 6 ROWE et al. 1982             | 14 TILLBERG 1980                 |
| 7 SAHAI et al. 1977a,b         | 15 WALKER and EVANS 1978         |
| 8 SCHOTT and WORTHLEY 1973     | 16 WORTHLEY and SCHOTT 1971      |



The herbicides aresine, atrataf, tafazine, tafapon, and amitrole were spread on the frond surface of S. polyrrhiza by KHARE (1977, 1979). They produced necrosis at concentrations of 1 to  $10^{-1}$  mM.

#### 2.3.3.5.6.3. Algicides and fungicides

The algicides investigated so far (2-dichloroacetamido-3-chloro-1,4-naphtaquinon; quaternary ammonium compounds; PH 40:62) mostly have a stronger effect on Lemnaceae than on water plants rooted in the soil (HARTZ et al. 1972, WALKER and EVANS 1978, SPENCER-JONES 1981). Fungicides such as 2,4,6-TCP are rather toxic to L. gibba (DAVIS 1981). Cyanobacterin, a natural algicide controlled growth of Lemna if added to the medium (GLEASON and CASE 1986; see also KIRPENKO 1986).

#### 2.3.3.5.6.4. Insecticides and other pesticides

Insecticides spread in nature are often concentrated in Lemnaceae. S. polyrrhiza and L. minor are able to accumulate up to 2 mg DDT per kg fresh weight and up to 1.2 mg HCCH (VROCHINSKIY et al. 19701). The toxin becomes dissolved in the lipids of the cell membrane and is excreted on the inner side of the cell. The dead fronds store DDT at the bottom of the water (VROCHINSKIY et al. 1971). Of 10 pesticides studied, DDT and 2,2',4,4'5,5' hexachlorobiphenyl become most enriched in L. minor. The concentration of the chemicals was more than 1000 times higher than in the water (LOCKHART et al. 1983). Polychlorinated biphenyls (PCB) only slightly soluble in water, are greatly accumulated by Lemnaceae. Twenty-one days after application, the plants contained 0.023 mg/g dry weight, 70 times more than Scirpus, and 6 times more than Eichhornia (ENGLANDE and KAIGATE 1981). Five ppm arochlor 1242 a polychlorinated biphenyl produces a characteristic chlorosis pattern in S. punctata; chlorophyll content is reduced to 56% and content of nucleic acid to 73% (desoxy-ribonucleic acid content remains unchanged) (MAHANTY and McWHA 1976).

**Fenitrothion** is concentrated 6 times by L. minor in an aquatic ecosystem but is decomposed within a short time (MOODY et al. 1978). MALIS and MUIR (1984) measured a 700fold concentration (up to 23 ppm) of fenitrothion in Lemnaceae (dry weight) five days after application. The amount is reduced to about 10% 77 days later. Dowanol TPM (tripropylene glycol-methylether) is in use as an adjuvant in fenitrothion spray formula-

tions. At  $5 \times 10^{-3}$  mM it has a specific deleterious effect on the photosynthetic activity of L. minor (WEINBERGER and CAUX 1985). The insecticide **matacil** containing nonylphenol proved to be lethal to L. minor at concentrations of  $10^{-2}$  mM. Probably the membranes of chloroplasts are destroyed causing the chlorophyll to emerge (PRASAD 1981). The other components of matacil (aminocarb, diluent oil) are not very effective (WEINBERGER and IYENGAR 1983).

**Carbofuran** does not show any toxicity to L. minor in concentrations up to 10 mg/l (HARTMAN and MARTIN 1985). The evidence of alkylbenzene sulfonate and ammonium carbamate in water was established by WARD et al. (1981) using S. polyrrhiza and L. minor as test organisms.

**Permethrine** is also readily absorbed by Lemnaceae, but is not persistent (RAWN et al. 1982).

**Carbaryl** and 3,5-xylol methylcarbamate (**XMC**) is accumulated up to 4000-fold in Lemnaceae (on a dry weight basis) compared to 100-500fold in snails, fish and crustaceae (KANAZAWA et al. 1975).

The fluorinated derivatives of SA which are used as **molluscicides** damage L. minor whereas other higher plants are not affected (WILLOMITZER et al. 1972).

**Sodium fluoroacetate**, a pesticide against rabbits and opossum inhibits the growth of S. polyrrhiza, S. punctata, and L. minor at concentrations of  $10^{-2}$  mM (BONG et al. 1980).

Dinitro-ortho-cresol (**DNOC**) reduces the chlorophyll and carotinoid content in L. minor at a concentration of 10 ppm (KRIVOKAPIC and ERIC 1984).

#### 2.3.3.5.6.5. Detergents

A stimulating effect of the detergents **DBST** and **ENF** on Lemnaceae at low concentrations is explained by TATKOWSKA and TOPOROWSKA (1978) with a chelating action. At higher concentrations ( $10^{-2}$  to  $10^{-1}$  mM), the detergents damage the fronds. An inhibition of nitrite reductase activity and consequently an accumulation of  $\text{NO}_2^-$  and a lowering of the protein content in S. polyrrhiza by 5 ppm DBST and ENF was observed. DBST also lowered the nitrate reductase activity and the dry weight of the fronds (BUCZEK 1984b). Sodium humate counteracts the effect of DBST in S. polyrrhiza (GUMINSKI et al. 1978).

GODZIK (1964) investigated the effect of the detergents **dibutyl-naphtha-**

**lene sodiumsulfonate** and alkylbenzene sodiumsulfonate on the  $O_2$  uptake of L. minor. "**Super Surf**" which contains alkylbenzene sulfonic acid shows a lethal effect on S. polyrrhiza at concentrations of 50 ppm and higher. At lower concentrations, a pronounced inhibition is coupled with a lower chlorophyll content (SAHAI et al. 1977a,b). L. gibba still grows relatively well at concentrations of 15 ppm tetrapropylene benzolsulfonate (AGAMI et al. 1976). KICKUTH and TITTIZER (1974) investigated the effect of **p-toluol sulfonic acid** on different water and marsh plants. At concentrations of 1 mM, the substance inhibits L. minor. Compared to other water plants, L. minor shows a medium tolerance towards the detergent. The effect is believed to be caused mainly by a lowering of the pH in the solution. OERENCIK et al. (1982) observed a slightly lower chlorophyll content in L. gibba growing in waters polluted by detergents. POLAR (pers. comm. 1987) detected different tolerances to detergents between different clones of L. minor.

#### 2.3.3.5.6.6. Further toxins and other plant active substances

All substances tested on Lemnaceae and not incorporated in one of the chapters from 2.3.3.5.3 to 2.3.3.5.6 are placed here. Sometimes it is difficult to classify a substance or group of substances correctly. Therefore, not all readers may agree with the placing of the different compounds. Sometimes, especially if the author worked with many different substances, a single substance may occur at more than one place. The effect of many toxic substances was tested on Lemnaceae: chlorate, bromate, jodate (HESSENLAND and FROMM 1932, HESSENLAND et al. 1933), methoxone (KAR 1947), substituted acryl acids (FROMM 1948, 1949, 1955), maleic hydrazide (BERTOSSO 1950), sulfonamides (FROMM and O'DONNELL 1951, FROMM and PACE 1957), methylcoumarines (CIFFERRI and CIFFERI 1947). 2,4-Dinitrophenol, sodium **azide**, sodium arsenite (at concentrations of 10 ppm) reduced the chlorophyll content of L. gibba only slightly but did not inhibit chlorophyll synthesis, according to OERENCIK et al. (1982). A six hour application of 1 mM sodium azide changes the phase of potassium uptake rhythm but leaves the potassium uptake itself intact (KONDO 1983a). 0.1 mM  $NaN_3$  prohibits the  $CO_2$  uptake in light. HILLMAN (1954) studied the effect of cyanide, azide, phenylthiourea, 2,4-dinitrophenol, and iodineacetate on the respiration of L. minor. He observed a pronounced inhibition by all substances. Flowering of L. gibba under short-

day condition is induced by  $2 \times 10^{-3}$  mM  $K_3Fe(CN)_6$  (TANAKA and CLELAND 1978, 1980). **Cyanide** induces flowering in L. aequinoctialis (TAKIMOTO 1981). It is supposed that the release of HCN from ferricyanide and ferrocyanide is responsible for the flower induction of these agents (TANAKA et al. 1983). Cyanide depolarizes the electrical membrane potential of L. gibba to the energy independent diffusion potential in the dark (ULLRICH-EBERIUS et al. 1983). A 6 hour pulse of 3 mM cyanide or 0.01 mM DCMU shifted the potassium uptake rhythm in L. gibba and considerably reduced the  $CO_2$  uptake in the light and the  $CO_2$  release in the dark. In addition, the potassium uptake activity was greatly inhibited (KONDO 1983a).

**Nalidixic acid** and other inhibitors of the protein and nucleic acid synthesis (e.g. chloramphenicol, cycloheximide, tetracycline, streptomycin, kanomycin, fluorodeoxyuridine, azaguanine, azauracil, aminotriazole) have a bleaching effect on S. polyrrhiza and prevent chlorophyll synthesis (FRICK 1972, FRICK and JONES 1975). SCHER and AARONSON (1958) report the reversible bleaching effect of **streptomycin** on S. punctata. Streptomycin was found to be effective in mutagenesis of L. gibba tissue culture (SLOVIN and COHEN 1986). NICKELL and FINLAY (1954) studied the effect of different antibiotics (including penicillin G and oxytetracycline), they noted a stimulating effect on L. minor. HILLMAN (1961a) supposes that the stimulation is caused by a chelating effect due to the unbalanced composition of the nutrient solution. Some **mycotoxins** are much more toxic. **Orellanine** which has a similar structure as diquat and paraquat has already been mentioned (HOILAND 1983). **Patulin** is toxic to L. gibba at concentrations of  $10^{-2}$  mM (SLOVIN et al. 1984). The aflatoxin **flavacoumarin** inhibits the chlorophyll synthesis at  $10^{-2}$  mM (JACQUET et al. 1971). **Fusicoccin** increases the membrane potential of L. gibba. The uptake of glucose and glycine was significantly enhanced (BOECHER et al. 1980). The toxin hyperpolarizes the membrane of energy deficient L. gibba considerably (LUETTGE et al. 1981). **Tentoxin**, a toxin from Alternaria alternata, caused an inhibition of photoautotrophic growth of L. gibba and a decrease of transmembrane potential in the light (BOECHER and NOVACKY 1981).

ROWE et al. (1982) report the effect of **bleaching substances** (phenols, catechols, guaiacols, p-cymenes on L. aequinoctialis. The higher chlorinated a substance the more toxic it was.

**Leucocyanidin**, procyanidin, propelargonidin, proanthocyanidin, **epicate-**

**chin**, epigallocatechin promote the growth rate of L. aequinoctialis, increase the RNA and protein content; the soluble nitrogen content is lowered and the DNA content not influenced by these substances (RAO and RAO 1984). Chlorophyll a and b content is enhanced considerably after addition of 0.1 and 0.5 ppm of the first four substances (RAO and RAO 1985a). According to LEATHER and EINHELLIG (1985) and EINHELLIG et al. (1985), the allelochemicals catechin, esculetin, chlorogenic acid, scopoletin, and vanillic acid inhibit growth of L. minor in concentrations of 1 mM, 0.5 mM, 0.25 mM, 0.5 mM, and 0.5 mM, respectively. Catechin stimulated growth of L. minor at concentrations of 0.05 and 0.1 mM.

**Tannic acid** induces flowering of L. aequinoctialis under non-inductive photoperiods (KHURANA and MAHESHWARI 1986a). **Mangiferin**, a phenolic growth inhibitor (xanthone group) inhibits the growth of L. aequinoctialis at concentrations of 0.05 ppm (RAO and RAO 1985b).

**p-Coumaric acid** and **p-anisic acid** are inhibiting growth of L. minor at 1 mM and 0.1 mM, resp. (EINHELLIG et al. 1985). Uptake and toxicity of coumarin and ethyl-N-phenylcarbamate in S. polyrrhiza are influenced by the pH (GREENHAM 1986). Sodium **phytate** at 0.1 mM slightly inhibits the growth rate of L. minor and results in longer roots. The root elongation is much more pronounced if distilled water is used instead of Hoagland solution (BOCCHI 1981, 1982). **Methionine sulfoximine** reduces the photosynthesis of L. gibba (JOHANSSON and LARSSON 1985).

Halogenated analogues of IAA proved to be very toxic to L. gibba (BALDI et al. 1984).

The antifreeze **ethylene glycol** and the di(2-ethylhexyl) **phthalate** which is used in vacuum pumps are not very toxic to Lemnaceae. A lethal effect was observed at concentrations of 10 M and higher (DAVIS 1981). Polyethylene glycol reduces growth rate, and the content of protein, liquid chlorophyll and carotenoid in S. polyrrhiza (LECHEVALLIER 1977b). **Croton aldehyde**, a waste product of chemical factories, inhibits respiration activity of L. minor (GODZIK 1964). **Caprolactam** as a monomer for Nylon-6 is scarcely toxic to L. minor. It reduces the growth rate at 1% concentration and inhibits it completely at 10% (LOEWENGART 1984).

STOM and ROTH (1981) investigated the influence of the following substances (in brackets the concentrations at which the growth rate was inhibited to 50% (in mM): phenol (1.8), resorcinol (1.5), dimethyl hydroquinone (0.75), dimethyl pyrocatechol (10), p-benzoquinone (0.2), hydroquinone (0.07), pyrocatechol (0.12). It was assumed that most of

these substances affect cell proteins unspecifically. CS, diazinon, malathion, dieldrin, aldrin, and sevin were tested by WORTHLEY and SCHOTT (1971) and found moderately toxic to W. brasiliensis (inhibition of growth at concentrations of  $10^{-2}$  to  $10^{-1}$  mM). Of three different species of Lemnaceae tested with CS, L. aequinoctialis proved to be most sensitive (more so than S. punctata and W. brasiliensis) (SCHOTT and WORTHLEY 1973). Ethanol inhibits growth rate and anthocyanin production of L. obscura at concentrations of 0.13% and higher (LEATHER and EINHELLIG 1985). 50% growth inhibition of L. aequinoctialis was achieved with the following concentrations of lower fatty acids: 0.2 mM of propionic or butyric acid, and 3 mM of acetic acid (TACHIMOTO et al. 1986).

Puffs of tobacco smoke and some constituents of it (dimethylnitrosamine, methylbenzo(a)pyrene) inhibit both growth and flowering of L. gibba. Tobacco smoke condensate, polynuclear aromatic hydrocarbon fraction of tobacco smoke and benzo(a)anthracene have a marked inhibitory effect on flowering but little influence on growth rate (SABHARWAL and BHALLA 1973). The aqueous extracts of fuel oil, crude oil and especially raw coal distillate reduce the growth rate of L. gibba, L. minor and L. aequinoctialis (KING and COOLEY 1985).

SHIMOMURA et al. (1981) tested the influence of 108 crude drugs and of 10 species of medicinal plants on the growth of L. aequinoctialis and other plants. The following drugs had a stimulatory effect on L. aequinoctialis at concentrations of 2 o/oo: Achyranthis radix, Angelicae dahuricae radix, Asiascari radix, Pruni cortex, Aucubae japonicae foliae, Casuarina (stem). The following drugs showed marked inhibiting effect at the same concentration: Chamomillae flos, Alismatis rhizoma, Astragali radix, Cinnamomi cortex, Coptidis rhizoma (was lethal), Corydalis tuber, Galla rhois (was lethal), Glycyrrhizae radix, Ipecacuanhae radix, Myrica cortex, Phellodendri cortex, Polygalae radix, Rhei rhizoma (was lethal), Senegae radix, Ginseng radix, Granati cortex, Lonicerae flos, Magnoliae cortex, Menthae herba, Pyrethri flos, Schizonepetae herba, Scutellariae radix, Sinapis semen, Araliae cordatae foliae, Chloranthi glabri foliae, Heracleum lanatum var. nipponicum (root).

**Cell wall fragments** of different plants, e.g. from sycamore (Acer pseudoplatanus) and soybean (Glycine max) proved to be inhibitory on flowering of L. gibba but stimulated the growth rate (McNEIL et al. 1984, GOLLIN et al. 1984). The authors assume that plant cell wall fragments participate in the control of growth and development in plants.

## 2.3.4. Influence of temperature

### 2.3.4.1. Dependence on temperature of characteristics and metabolism

The growth rate of Lemnaceae shows a clear relationship to the temperature with minimum, optimum, and maximum temperature (fig. 2.11). The cardinal points of the growth rate curve are partly dependent on other factors, especially light intensity (fig. 2.13) (see also chapter 2.3.5.1.1.2). The growth rate rises up to 25°C to 31°C depending on the species (see chapter 2.3.4.3 and fig. 2.14), and falls down rather abruptly at higher temperatures. However, the dry weight per frond is much lower at optimum temperatures (fig. 2.15; ASHBY and OXLEY 1935). LANDOLT (1950) also observed a much lower dry weight per frond in L. minor at higher temperatures (20-32°C) than at lower ones (14°C). The area

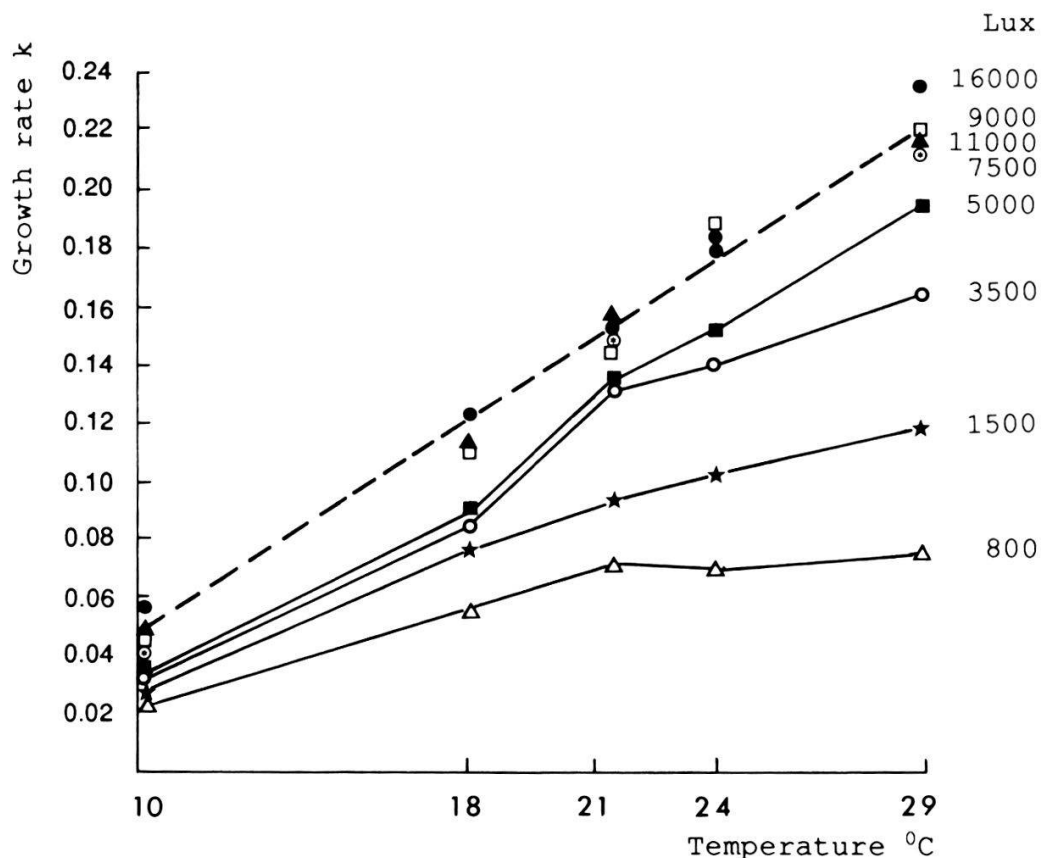


Fig. 2.13. Growth rates of Lemna minor at different temperatures and different light intensities (from ASHBY and OXLEY 1935)



per dry weight in L. minor rises from 12.5°C to 27.5°C to the threefold value (HODGSON 1970). The area itself is greatest at 20°C (5.7 mm<sup>2</sup>) and smaller at 10°C (4.4 mm<sup>2</sup>), 26°C (4.0 mm<sup>2</sup>) and 32°C (2.6 mm<sup>2</sup>). In solutions with sugar, the maximal area is somewhat shifted to higher temperatures (LANDOLT 1957). The frond length of W. microscopica is greater (1.1 mm) at 22°C and short-day conditions than at 27°C and long-day conditions (0.8 mm) (RIMON and GALUN 1968b).

The growth rate of Lemnaceae is the result of many temperature-dependent chemical processes with intricate interactions: nutrient uptake, nutrient assimilation and transport, photosynthesis, respiration, and many other processes which are each composed of a series of enzymatic activities. Physical processes as diffusion of elements, water flow (transpiration) and permeability are also important temperature-dependent events. Photosynthesis and respiration, two dominating metabolic processes, do not have the same temperature curves. The respiration rate of

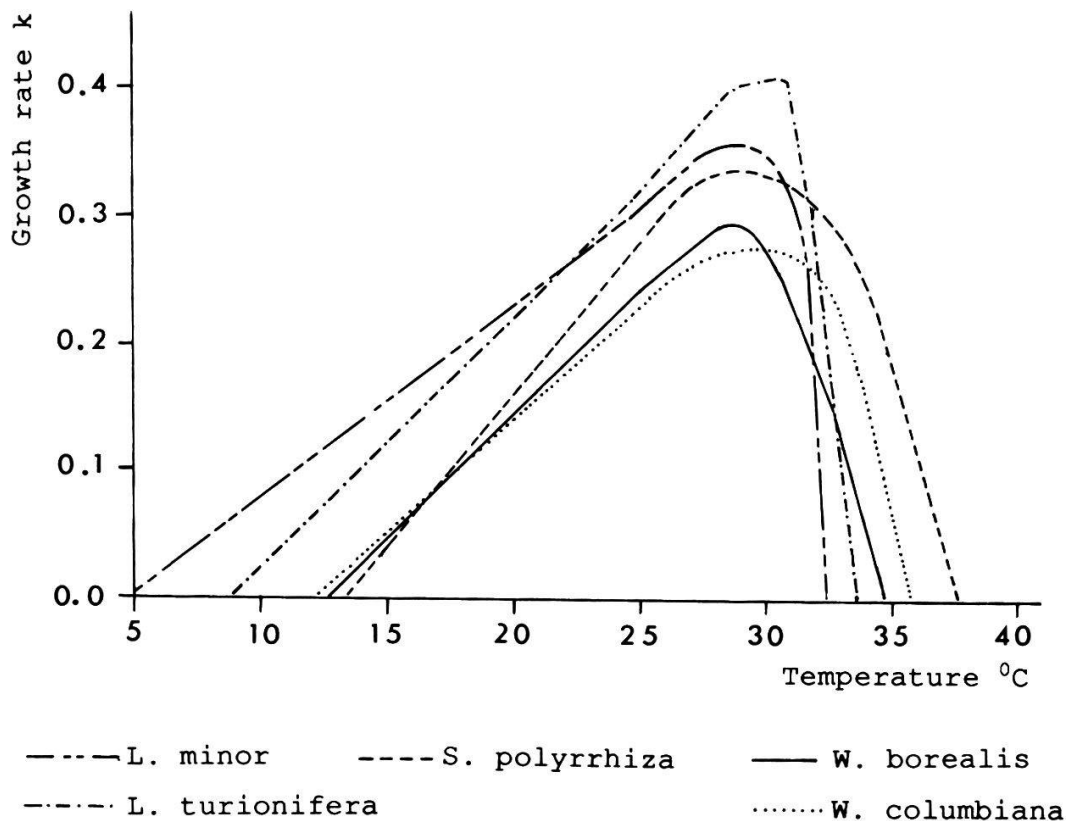


Fig. 2.14. Growth rate of different Lemnaceae species in relation to temperature (from DOCAUER 1983)

S. polyrrhiza rises to 45°C whereas the photosynthesis rate reaches its peak at 30°C (CZOPEK 1967). HODGSON (1970) noted that as a result the rate of net assimilation in L. minor only slightly rises from 12.5°C to 17.5°C and falls to 2/3 of the maximum value at 27.5°C. However, the growth rate is higher at 27.5°C than at 22.5°C. According to ASHBY and OXLEY (1935), the net assimilation rate (mg CO<sub>2</sub> per area) for the same species is higher at 21°C than at 18°C and 24°C (fig. 2.16). The relation between net assimilation rate and growth rate is shown in fig. 2.17. From a certain assimilation rate upwards, ranging between c. 1 and 6 mg CO<sub>2</sub> per dm<sup>2</sup> and hour (higher values at higher temperatures), starch is formed in the frond (ASHBY and OXLEY 1935).

WEDGE and BURRIS (1979, 1982) measured the photosynthetic O<sub>2</sub> evolution and the <sup>14</sup>CO<sub>2</sub> fixation. The maximum O<sub>2</sub> evolution occurred in L. minor at

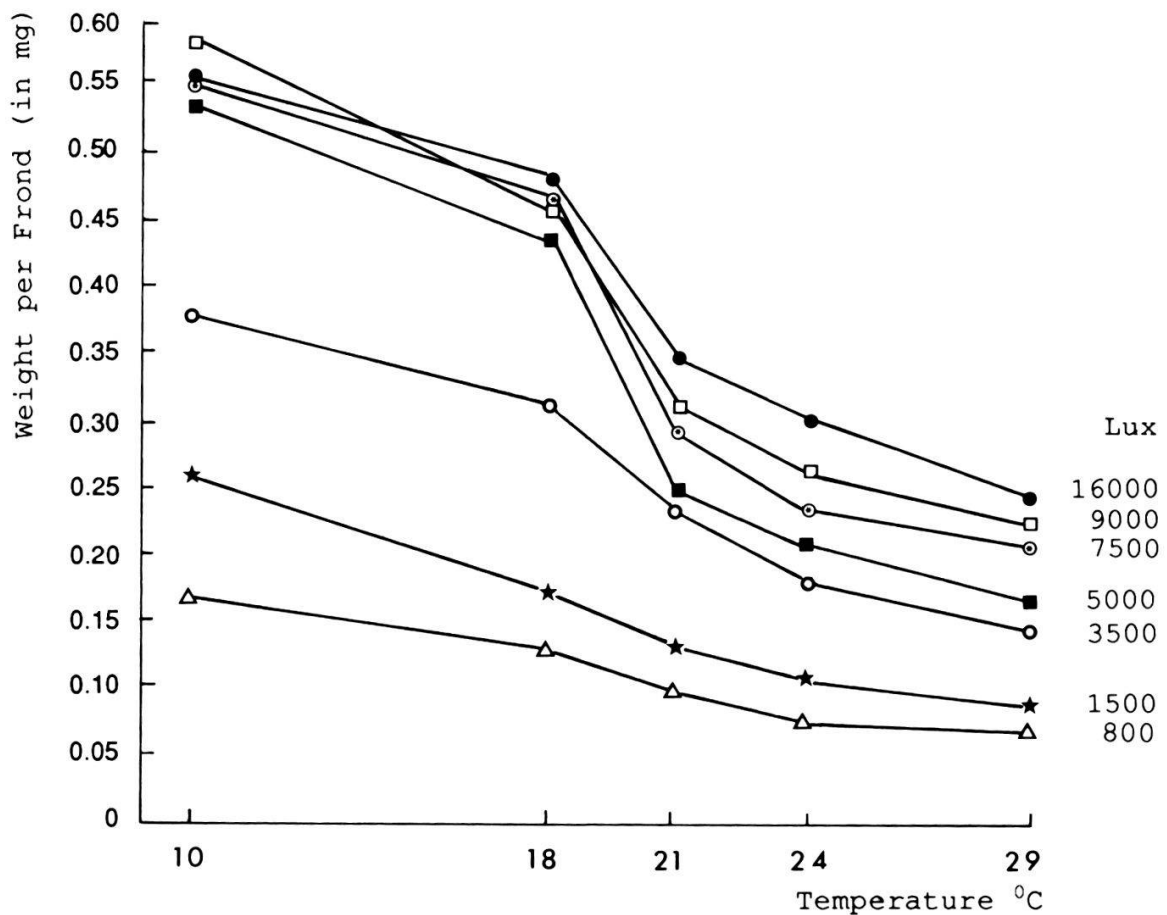


Fig. 2.15. Mean dry weight per frond of Lemna minor at different temperatures and different light intensities (from ASHBY and OXLEY 1935)

30°C and in S. punctata at 35°C. The maximum CO<sub>2</sub> fixation was achieved in L. minor between 20 and 30°C and in S. punctata at 30°C. FUHRER and ERISMANN (1984) observed, in L. minor cultivated in a solution with nitrate, a lower C net fixation and a lower oxidation/carboxylation ratio at 15°C compared with 25°C. If ammonium replaces nitrate, only net fixation is reduced at the lower temperature. It seems that the photosynthesis rate (and the growth rate) varies not only with light intensity but also with the method used and with the time of adaptation of the fronds to the experimental conditions.

Certain temperatures induce or keep physiological processes going. Temperature plays an important role for flower induction (see chapter 2.4.3.1.6). Low night temperatures are known to induce turion formation in S. polyrrhiza (JACOBS 1947). The relation between constant temperature and turion formation in S. polyrrhiza and L. turionifera is presented in fig. 2.18. The turion germination of the same species is also

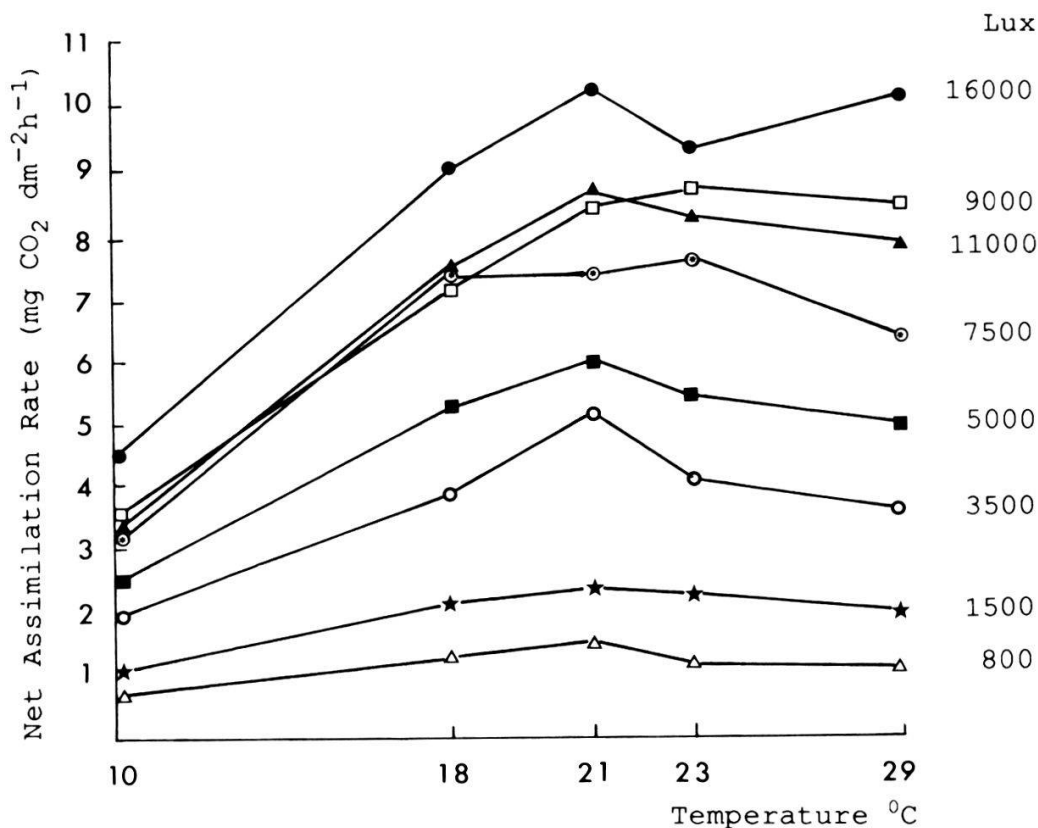


Fig. 2.16. Mean net assimilation rates of Lemna minor in mg CO<sub>2</sub> dm<sup>-2</sup> h<sup>-1</sup> plotted against temperature (from ASHBY and OXLEY 1935)

dependent on temperature (fig. 2.19) (DOCAUER 1983). Chilling was found to be effective in breaking turion dormancy (JACOBS 1947, HENSSEN 1954, PERRY 1968, NEWTON et al. 1978) as well as seed dormancy in L. perpusilla (KANDELER 1975). Germination of seeds of L. gibba takes place faster at higher temperatures than at lower temperatures, continuously from 17°C to 33°C (REJMANKOVA 1976). The germination of turions and seeds is also dealt with in chapters 2.4.2.2. and 2.4.2.3.

The roots of S. polyrrhiza are thicker and stiffer at 23°C than at 28°C (DAVIDSON and SIMON 1981a,b).

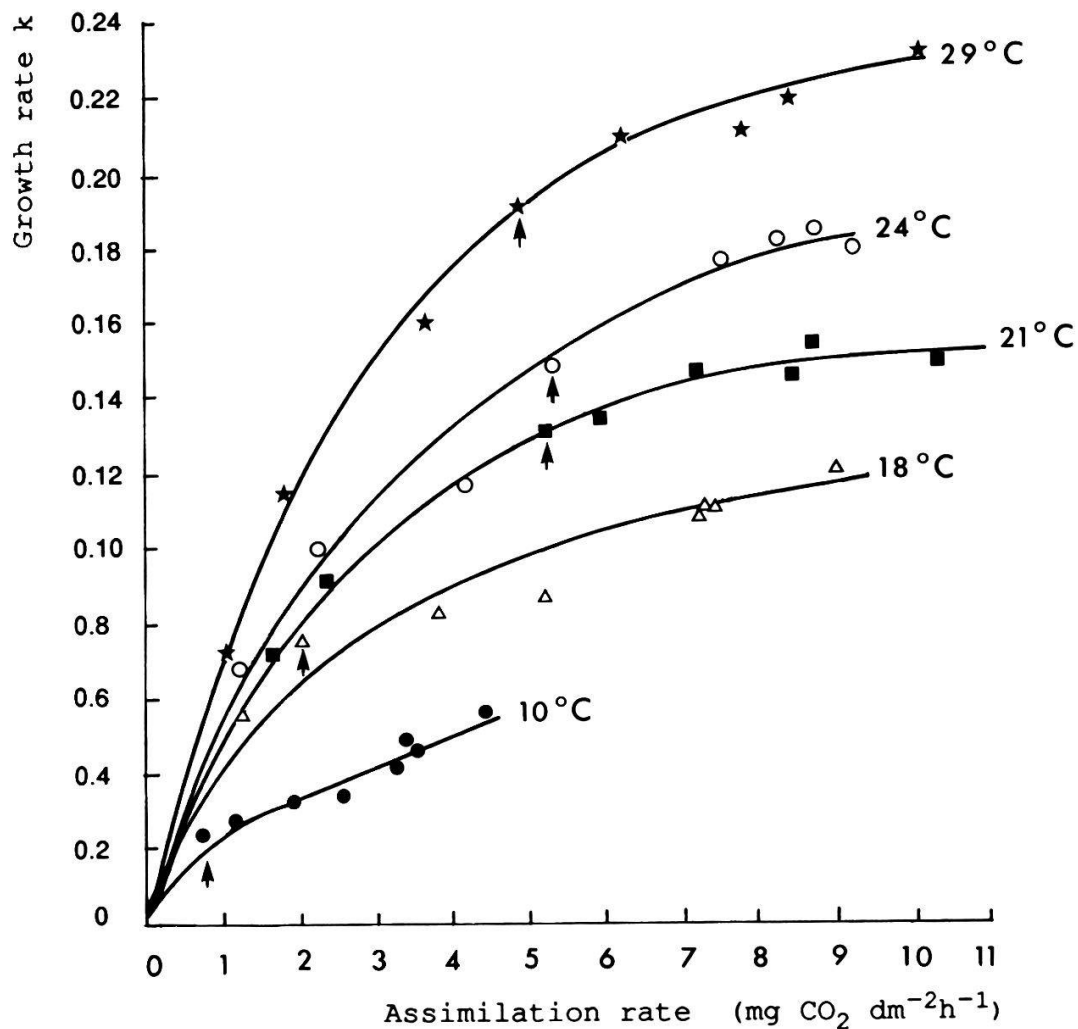


Fig. 2.17. Relation between growth rate and assimilation rate of Lemna minor at different temperatures. The arrows indicate the assimilation levels at which starch appears in the fronds (from ASHBY and OXLEY 1935)

#### 2.3.4.2. Influence of constant and varying temperatures

For the growth rate of Lemnaceae, it is not important, whether temperatures between 15°C and 25°C at medium light intensities are kept constant or whether they vary around an average value. LANDOLT (1957) did not find very pronounced differences between growth rates of several Lemnaceae species kept at a constant temperature of 22°C or alternating at 26°C (16 hours) and 14°C (8 hours). Under the alternating conditions the growth rate was slightly higher in L. turionifera and slightly lower in L. gibba, L. minor, and L. minuscula. Also SHARITZ and LUVALL (1978)

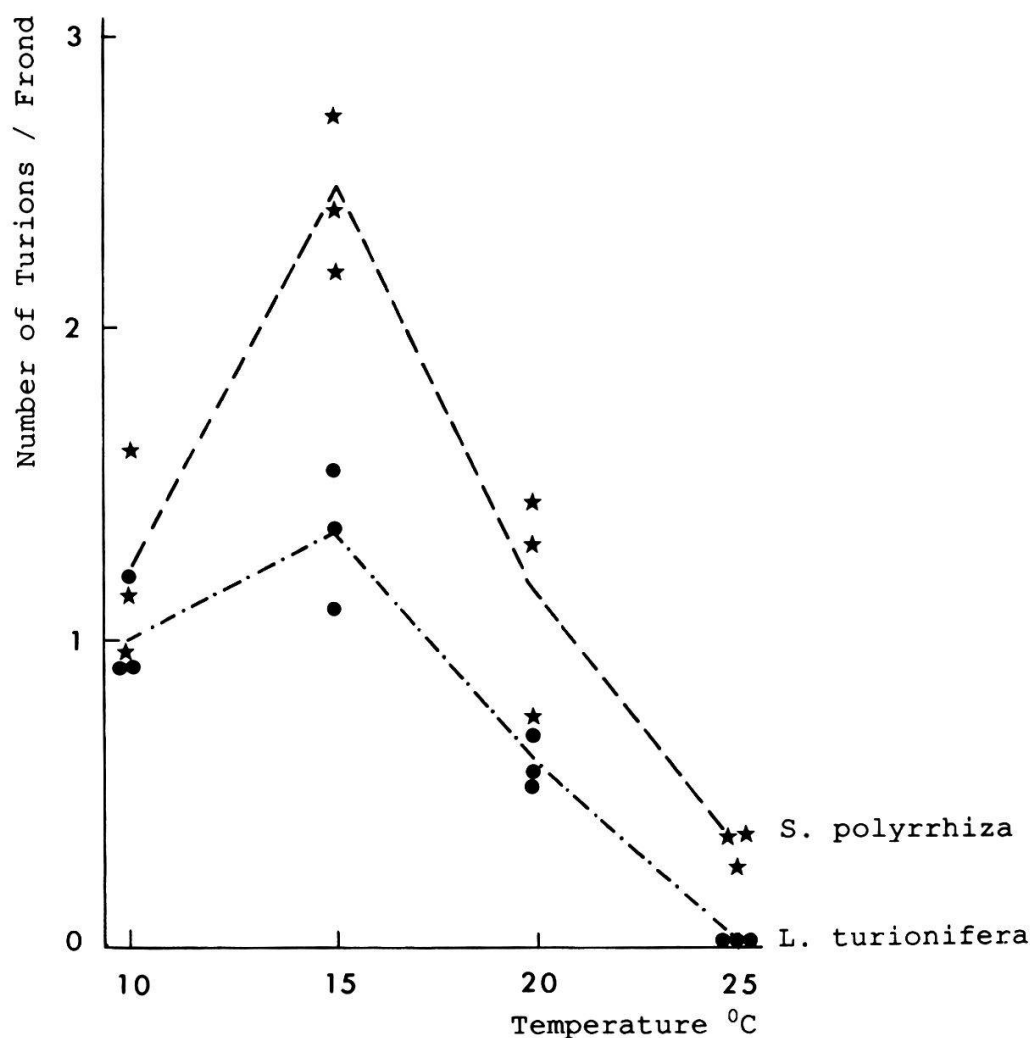


Fig. 2.18. Turion formation of Spirodela polyrrhiza and Lemna turionifera in relation to temperature (from DOCAUER 1983)

did not find different growth rates in S. punctata at constant temperatures of 17.5°C and at regularly or acyclicly changing temperatures between 25°C and 10°C with the same average. However, it is possible that in nature the daily change of temperature has some influence if the extreme temperature values lie near the minimum or maximum temperature of the species. At low temperatures, the alternation might have a positive effect assuming that no resting stage is induced. It is also conceivable that varying temperatures might enable a better gas exchange in the culture. FERNANDEZ and MUJICA (1973) observed a greater number of stomata per number of epidermis cells and in addition bigger stomata in S. intermedia cultivated at the same day temperatures but at cooler night temperatures than at constant temperature. It has never been studied if varying temperatures have a positive or negative effect on flowering of Lemnaceae.

Some differences in values of growth rates at the same temperatures indicated by different authors might be due to the difficulty of measuring

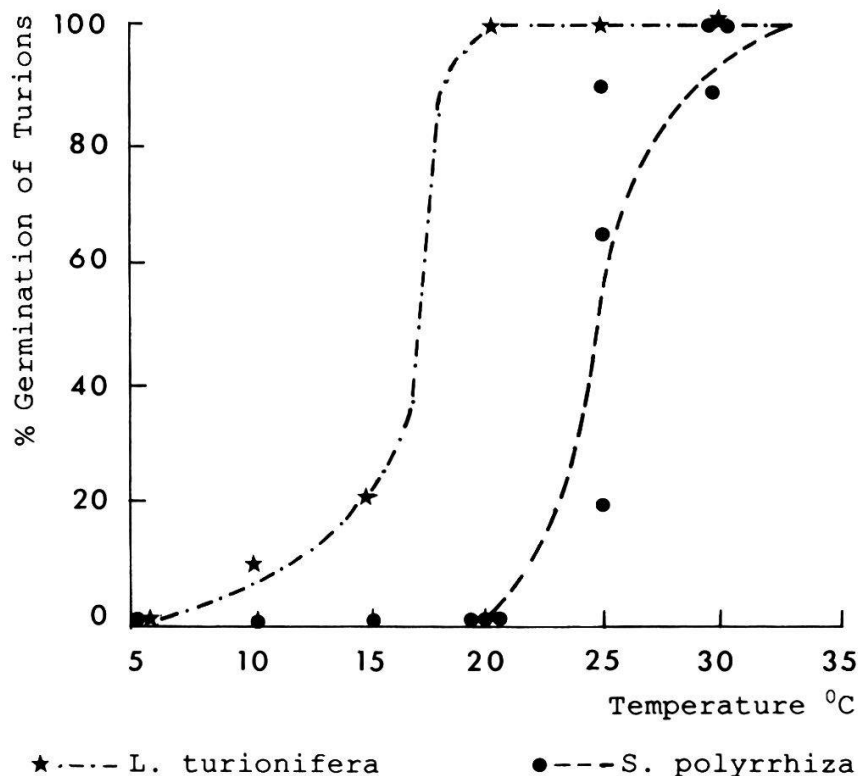


Fig. 2.19. Germination of turions in relation to temperature  
(from DOCAUER 1983)

the physiologically active temperature in Lemnaceae. The temperature in cultures with different day-lengths can only remain constant if the solution is temperature controlled. In experiments with air controlled temperatures, the temperature in the solution is warmed up during daytime by 1 to 4°C, according to the light intensity and the light source used. In addition, the temperature of the frond is again 1 to 3°C higher than the temperature of the surrounding water during irradiation. CASPERSON (1956) measured, at 5000 lux, in open cultures of S. polyrrhiza and at an air and water temperature of 18°C a 2.7°C higher temperature at the upper surface and a 1.4°C higher temperature at the lower surface of the frond. For thin or partly submerged fronds, the difference is probably negligible.

#### 2.3.4.3. Optimum temperature

LANDOLT (1957) noted for all tested species the optimum temperature for growth rate in solutions without sugar and at light intensities between 1000 and 9000 lux within the temperature range of 20°C to 30°C. In solution with sucrose, the optimum temperature was somewhat higher. For S. polyrrhiza, JACOBS (1947) reported an optimum temperature of 25°C in solution without sucrose. ASHBY and OXLEY (1935) found an optimum temperature of 29°C for L. minor at light intensities between 800 and 16000 lux (fig. 2.13). It is possible that the lower optimum temperatures of the first authors who worked in contrast to ASHBY and OXLEY with closed and not aerated culture vessels are due to a shortage of CO<sub>2</sub> at higher temperatures. Differences in growth rates for different species and clones in solution with sucrose and at 2500 lux were presented by LANDOLT (1957). The growth rates were measured at 21°C, 26°C, and 30°C. Three categories could be distinguished:

- 1) Clones with a rather low optimum (growth rate best at 26°C, and higher at 21°C than at 30°C): S. punctata (1 clone), L. trisulca (2 clones), L. gibba (4 clones), L. minuscula (5 clones)
- 2) Clones with a medium optimum (growth rate best at 26°C, and higher at 30°C than at 21°C): L. minor (7 clones), L. turionifera (3 clones), L. minuscula (4 clones), W. arrhiza (1 clone), W. columbiana (1 clone).
- 3) Clones with a high optimum (growth rate best at 30°C): S. polyrrhiza (3 clones), L. aequinoctialis (3 clones), W. globosa (2 clones).



BOSS et al. (1963b) measured optimum temperatures for all 6 species tested (S. polyrrhiza, S. punctata, L. gibba, L. minor, L. aequinoctialis, L. minuscula) at about a temperature between 27°C and 29°C. The optimum temperature for L. gibba was noted by REJMANKOVA et al. (1986) with 28°C. DOCAUER (1983) gives optimum temperatures of 29°C for L. minor and W. borealis, and 31°C for S. polyrrhiza and W. columbiana (fig. 2.14).

In general, species with a wide-spread tropical distribution show a high optimum temperature, whereas species with main distribution in cooler and more oceanic areas have a lower optimum temperature.

#### 2.3.4.4. Minimum temperature

##### 2.3.4.4.1. Minimum temperature for survival

Even tropical species are able to tolerate temperatures of 0°C or slightly lower for short periods. GODZIEMBA-CZYŻ (1970) reported that fronds and turions of W. arrhiza can survive temperatures of -2°C for 8 to 10 days, and of 1°C for 10 to 20 days. Turions of S. polyrrhiza died if exposed to -12°C for 2 days, but remained alive at -8°C for 24 hours and at -4°C for at least 3 months (JACOBS 1947). At about +4°C they survive for at least 4 years (KRONBERGER, personal comm.). Turions of the mainly tropical species W. globosa were able to survive temperatures of 5°C in the dark for 2 months, which means that this temperature might approach the minimum temperature for turion germination but not for frond growth which is much higher (see below) (LANDOLT 1957).

To stand cold seasons, Lemnaceae species often sink into deeper layers of water where the temperatures never fall much below freezing point. Fronds of L. minor can stay alive even when enclosed in ice for a longer time (KRONBERGER, personal comm.). Some of the Lemnaceae species can survive cold winters in form of seeds (e.g. L. gibba, L. perpusilla, L. aequinoctialis), others in form of true turions (e.g. S. polyrrhiza, L. turionifera, many Wolffia species) or in form of resting fronds (e.g. L. gibba, L. perpusilla, L. aequinoctialis, W. gladiata), and still others sink as whole frond groups to deeper layers (e.g. L. minor, L. trisulca). For more details, see volume 1, chapters 2.5 and 4.4.2 (LANDOLT 1986). In this way, Lemnaceae can survive in regions with winter temperatures far below the minimum temperature for growth.

#### 2.3.4.4.2. Minimum temperature for growth

Minimum temperatures which just enable a very slow permanent growth rate were studied by LANDOLT (1957) for several species in dark cultures. 5 categories of behaviour towards low temperature could be distinguished:

- 1) Minimum temperature distinctly lower than 8°C (the growth rate at 8°C amounts to at least 10% of the maximum growth rate): L. gibba (6 clones), L. minor (3 clones), L. trisulca (3 clones).
- 2) Minimum temperature around 8°C (growth is visible at 8°C but much slower than 10% of the maximum growth rate): S. punctata (2 clones), L. minor (5 clones), L. turionifera (5 clones), L. trisulca (1 clone), L. minuscula (8 clones).
- 3) Minimum temperature between 8°C and 13.5°C (not growing at 8°C; growth rate at 13.5°C at least 10% of the maximum growth rate): S. polyrrhiza (1 clone), L. turionifera (1 clone), L. aequinoctialis (1 clone), L. valdiviana (1 clone), L. minuscula (2 clones), W. gladiata (1 clone).
- 4) Minimum temperature between 13.5°C and 16.5°C (not growing at 13.5°C; growth rate at 16.5°C at least 10% of the maximum growth rate): S. polyrrhiza (2 clones), L. aequinoctialis (4 clones), W. arrhiza (1 clone), W. columbiana (1 clone), W. globosa (1 clone).
- 5) Minimum temperature between 16.5°C and 20°C (not growing at 16.5°C; growth rate at 20°C at least 10% of the maximum growth rate): S. polyrrhiza (1 clone), L. aequinoctialis (1 clone).

DOCAUER (1983) estimated the minimum temperatures for a clone of: L. minor at 5°C, L. turionifera at 9°C, S. polyrrhiza, W. borealis, and W. columbiana at 12.5-13.5°C. This is more or less in accordance with the results of LANDOLT (1957).

The results explain why the species with high minimum temperature are found in nature only in regions with very warm growth periods whereas species with lower minimum temperatures are also occurring in regions with cool summers.

#### 2.3.4.5. Maximum temperature

##### 2.3.4.5.1. Short-term maximum temperature

The turions of S. polyrrhiza are able to stand 24 hours at 50°C and one week at 45°C (JACOBS 1947). STANLEY and MEADWELL (1976b) studied the short-term tolerances of L. obscura (named as L. minor) at temperatures between 40°C and 60°C. The temperature of 50% inhibition of growth rate and the temperature of 50% lethality are very near together. Temperatures above 60°C are not tolerated at all, even not for very short periods. The 50% lethality is achieved at 55°C after 30 seconds, at 50°C after 5 minutes and at 42°C after 2 hours. The heat tolerances are somewhat enhanced by the addition of calcium (up to at least 3 mM). Also the nitrogen content has an influence on the temperature sensitivity. The optimal nitrogen concentration for growth at 0.7 mM enabled the highest tolerance. In the light, the fronds survived for a longer time at 45°C than in darkness, but at 55°C, there was scarcely a difference.

##### 2.3.4.5.2. Long-term maximum temperature

Long-term temperature limits are not the consequence of plasma damages by temperature but probably due to an unbalance of metabolic processes at this temperature. Thus, the photosynthesis rate is lowered in S. polyrrhiza at temperatures above 30°C whereas the respiration rate is at this temperature still rising (CZOPEK 1967) resulting in a negative net assimilation rate and finally in starvation. Also other processes must be involved in the long-term effect at high temperatures because the fronds die in solution with sucrose nearly at the same temperature as without sucrose irrespective of cultivation in the light or in the dark. For S. punctata it is known that it loses the ability of synthesizing chlorophyll and evolving oxygen at temperatures around 37°C. This effect was only observed in the light but not in the dark (PORATH and BEN-SHAUL 1971). SCHER and AARONSON (1958) noted a reversible bleaching in S. punctata already at 34°C. STEPHENSON et al. (1980) also report of a growth stop of S. punctata at 35°C.

Long-term maximum temperatures were established by LANDOLT (1957). 5 categories can be distinguished:

- 1) Maximum temperature below 30°C: L. minor (1 clone), L. trisulca (3 clones), L. minuscula (1 clone).
- 2) Maximum temperature between 30°C and 32°C: L. gibba (2 clones), L. minor (5 clones), L. turionifera (2 clones), L. trisulca (1 clone), L. minuscula (8 clones), W. arrhiza (1 clone), W. gladiata (1 clone).
- 3) Maximum temperature between 32°C and 33°C: L. gibba (2 clones), L. turionifera (2 clones), L. minuscula (1 clone), L. valdiviana (1 clone), W. columbiana (1 clone).
- 4) Maximum temperature between 33°C and 34°C: S. punctata (1 clone), L. gibba (1 clone), L. minor (1 clone), L. aequinoctialis (3 clones), W. globosa (1 clone).
- 5) Maximum temperature higher than 34°C: S. polyrrhiza (3 clones).

DOCAUER (1983) obtained similar results for the upper temperature limit of the following species (only one clone measured): 32.5°C for L. minor, 34°C for L. turionifera, 35°C for W. borealis, 36°C for W. columbiana, and 38°C for S. polyrrhiza. DOCAUER measured the temperature at which the species cease growth, LANDOLT the temperature at which the species still grows slowly. This might explain the higher temperatures of DOCAUER. Though different clones of the same species do not behave exactly in the same way, some species tolerate higher temperatures on the average (e.g. S. polyrrhiza) and others only lower temperatures (e.g. L. trisulca). In general, species with higher tolerance are distributed in warmer or more continental areas whereas species with a low tolerance are to be found in rather cool areas. However the differences are not so pronounced as one would suppose from the distribution of the species in different climate zones.

### 2.3.5. Influence of light and other radiations

#### 2.3.5.1. Light (including UV radiation)

##### 2.3.5.1.1. General remarks

The solar radiation within the visible range is absorbed by the Lemna-ceae through different pigment systems. It is used for energy supply as well as for regulation of different life processes.

The measurements of light energy are not always comparable. In the older literature, the light intensity was measured by photo cells in lux or foot-candles (English speaking countries). In the present work the foot-candle was transferred to lux approximately by the factor 10. In more recent publications, the intensity is either described in energy units (Joule or erg) (mostly restricted to the visible range of the radiation spectrum), or in units of the photon flux density which is expressed in Einstein or mmol per squaremeter and second. 1 erg corresponds to  $10^{-7}$  Joule (J). 1 Einstein is identical with  $1 \text{ mmol m}^{-2} \text{ s}^{-1}$ . The energy content of a unit of photon flux density ( $1 \text{ mol m}^{-2} \text{ s}^{-1}$ ) is different at different wavelengths. It amounts to  $800 \text{ kJ m}^{-2} \text{ s}^{-1}$  at 150 nm and  $170 \text{ kJ}$  at 700 nm. Just to give an approximate idea of the correspondence of the different measurement systems, the following example may be given. A light intensity of 20000 lux might correspond to a total energy content of  $100 \text{ J m}^{-2} \text{ s}^{-1}$  (white fluorescent light) and roughly to about  $1 \text{ mmol m}^{-2} \text{ s}^{-1}$  at a wavelength of c. 500 nm. However, this relation is dependent on the type of light source and may vary greatly.

##### 2.3.5.1.2. Light as source of energy in photosynthesis

Relatively high light intensities are necessary for photosynthesis, in contrast to other light-dependent metabolic processes. The chlorophyll system is responsible for the energy transfer. The maximum solar energy conversion efficiency in S. punctata was measured with 4.9% of the photosynthetically active radiation (PAR) (including UV light) (MESTAYER et al. 1984). The response of Lemnaceae to light intensity is dependent on species, clone, temperature, as well as on nutrient and  $\text{CO}_2$  supply. Also, the light quality is important (EICHHORN 1983; fig. 2.20).

#### 2.3.5.1.2.1. Light saturation, minimal and maximal light intensity

At low temperatures, the light saturation is lower than at high temperatures (see chapter 2.3.5.1.2.2). Light saturation is generally lower for growth rate than for photosynthesis rate. Therefore, the dry weight still rises at intensities higher than light saturation for growth rate. In our own experiments (LANDOLT 1957 and unpubl.) the saturation for growth rate was reached at intensities between 5000 and 15000 lux depending on species and clones (see chapter 2.3.5.1.2.4) (continuous light). BOSS et al. (1963b) observed light saturation for growth rate (but only 12 hours day-length) in L. aequinoctialis (2 clones), S. polyrrhiza (1 clone), S. punctata (1 clone), L. obscura (named as L. minor, 1 clone) and L. minuscula (1 clone) at c. 17000 lux (27-29°C). L. gibba reached saturation at still higher light intensities. MESTAYER et al. (1984) noted c.  $50 \text{ J m}^{-2} \text{ s}^{-1}$  at light saturation for growth rate of S. punctata. DOCAUER (1983) measured the half saturation constants  $K_L$

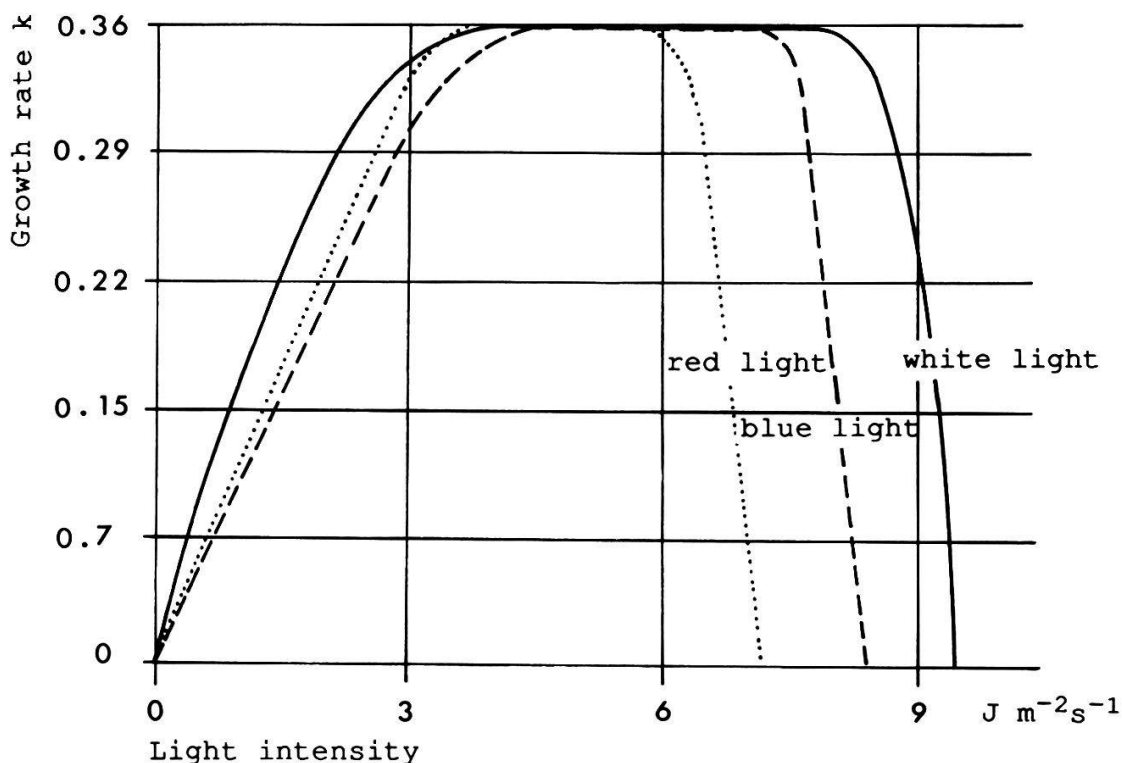


Fig. 2.20. Relation between light intensity and growth rate in Wolffia arrhiza chemostat cultures (from EICHHORN 1983)

for growth rate at 28°C of one clone each of 5 species (see chapter 2.3.5.1.2.4) between  $0.024 \text{ mmol m}^{-2} \text{ s}^{-1}$  (S. polyrrhiza) and  $0.086 \text{ mmol m}^{-2} \text{ s}^{-1}$  (W. borealis). The light saturation is reached at roughly four times higher light intensities than  $K_L$ . In the following references, the light saturation for photosynthesis was determined: CZOPEK (1967)  $91 \text{ J m}^{-2} \text{ s}^{-1}$  for normal fronds and  $32 \text{ J m}^{-2} \text{ s}^{-1}$  for turions of S. polyrrhiza; GAPONENKO and STAZHETSKII (1969)  $20 \text{ J m}^{-2} \text{ s}^{-1}$  or 5000 lux for S. polyrrhiza; LINDEMAN (1951, 1972)  $100 \text{ J m}^{-2} \text{ s}^{-1}$  for L. minor (enrichment of the  $\text{CO}_2$  content of the air); INGEMARSSON et al. (1984)  $1.1 \text{ mmol m}^{-2} \text{ s}^{-1}$  for L. gibba; ANDERSEN (1983)  $0.5\text{-}0.7 \text{ mmol m}^{-2} \text{ s}^{-1}$  for L. gibba; WEDGE and BURRIS (1982)  $0.3\text{-}0.6 \text{ mmol m}^{-2} \text{ s}^{-1}$  for L. minor and  $0.6\text{-}1.2 \text{ mmol m}^{-2} \text{ s}^{-1}$  for S. punctata; FILBIN and HOUGH (1985) c.  $0.35 \text{ mmol m}^{-2} \text{ s}^{-1}$  for L. minor.

Long-term compensation point for L. minor is near 2-3% of full sunlight (WILKINSON 1963). The compensation point of normal fronds of S. poly-

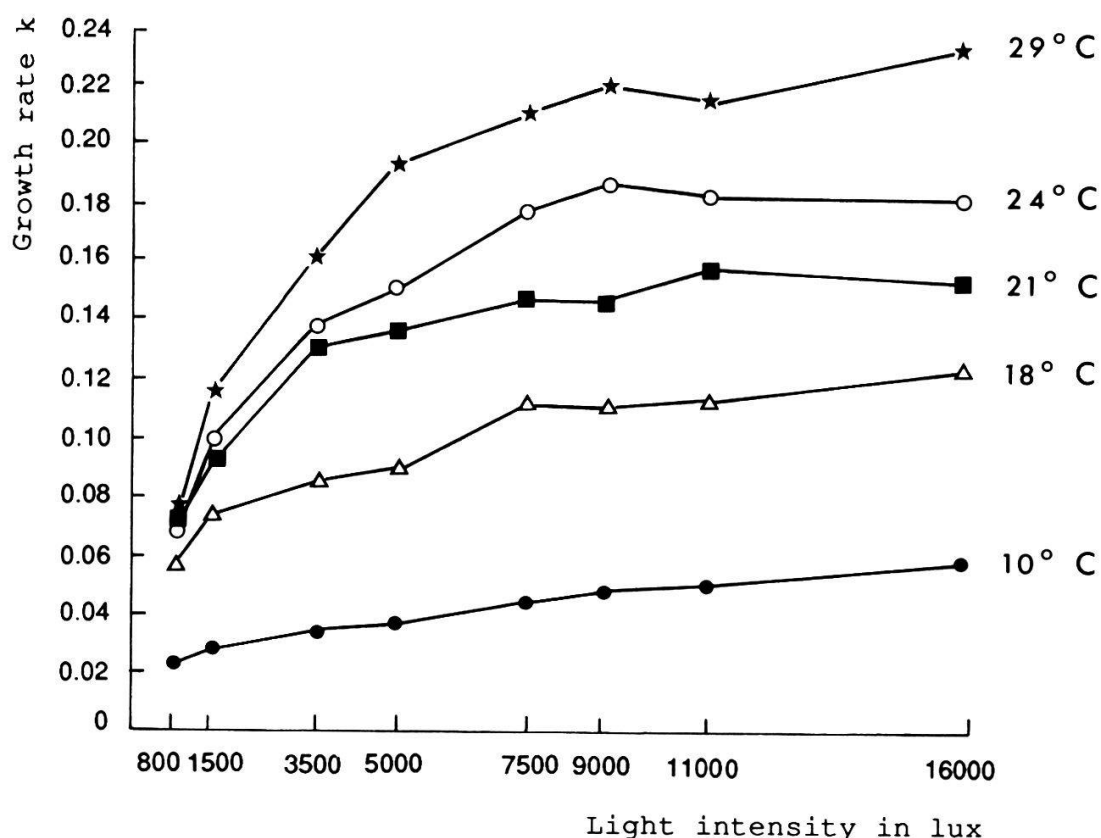


Fig. 2.21. Growth rates of Lemna minor at different light intensities and different temperatures (from ASHBY and OXLEY 1935)



rrhiza was noted by CZOPEK (1967) at  $15 \text{ J m}^{-2} \text{ s}^{-1}$  and for turions at  $11\text{--}13 \text{ J m}^{-2} \text{ s}^{-1}$ . ASHBY and OXLEY (1935) measured a distinct photosynthesis rate for L. minor already at 500 lux (lower intensities not investigated). ULLRICH-EBERIUS et al. (1978) state that photosynthetic  $\text{O}_2$  evolution in L. gibba commences at 100 lux. An occurrence of L. trisulca (together with other water plants) was found by PIP and SIMMONS (1986) in Shoal Lake (Canada) at depths of 12–14 m. At these depths plants received an estimated 0.5–1% of surface light. According to INGEMARSSON et al. (1984a), the light compensation point measured as minimal needed photon flux density lies for L. gibba at c.  $0.05 \text{ mmol m}^{-2} \text{ s}^{-1}$ . It is not affected by nitrogen limitation.

The relation between light intensity and growth rate in Wolffia arrhiza chemostat cultures is shown in fig. 2.20. At low light intensities (see also figs. 2.22 and 2.23) the rates of growth and photosynthesis rise proportionally to the light intensity. At higher intensities, they

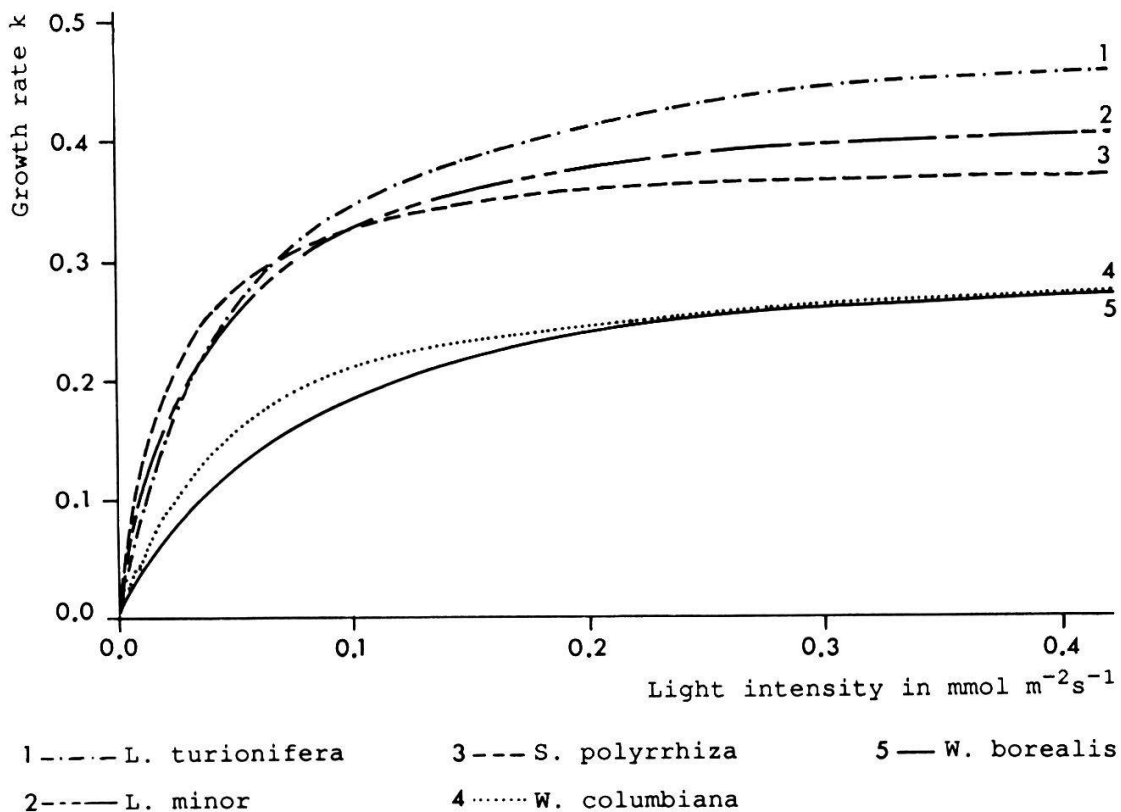


Fig. 2.22. Growth rate of different species in relation to light intensity (from DOCAUER 1983)

approach an optimum asymptotically (ASHBY and OXLEY 1935, WHITE 1937a, MIYATA 1971b, DOCAUER 1983) (figs. 2.21, 2.22). The dry weight increases linearly with the light intensity up to 5000 to 9000 lux depending on temperature (ASHBY and OXLEY 1935, DOCAUER 1983; figs. 2.23 and 2.24). The size of the frond is also mostly higher at high light intensities than at lower ones (KRZECZOWSKA et al. 1975, ROMBACH 1976 for L. minor, ANDERSEN 1983 for L. gibba). ASHBY (1929a), HICKS (1934) and WHITE (1936c) did not find a clear relation between light intensity and area of the frond. The following characteristics of L. minor become greater with increasing light intensity: root length, respiration rate per area (contrary to the respiration rate per dry weight), contents of protein, chlorophyll, and carotenoid (WHITE and TEMPLEMAN 1937, WHITE 1937b,

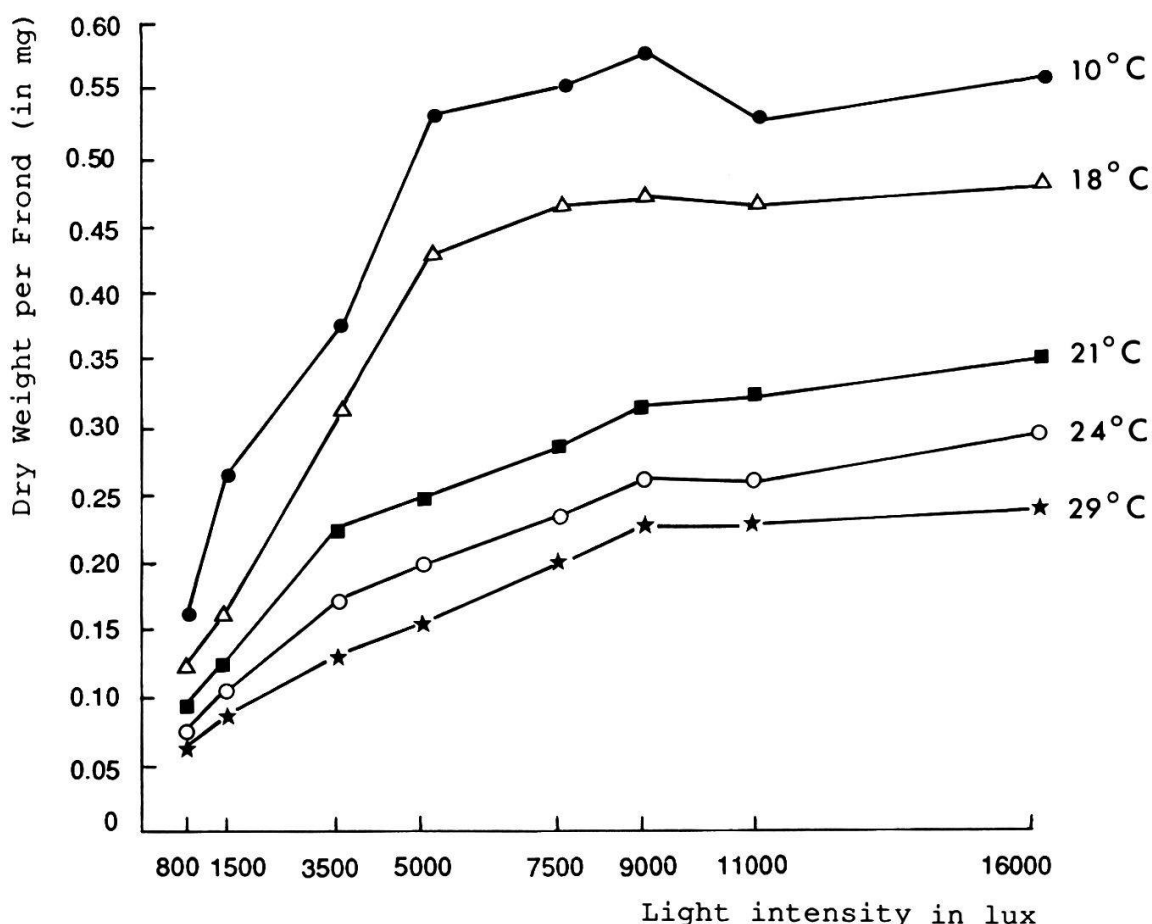


Fig. 2.23. Mean dry weight per frond of Lemna minor at different light intensities and different temperatures (from ASHBY and OXLEY 1935)

1939, BORNKAMM 1970b, ROMBACH 1976). L. gibba also has longer roots at higher light intensities (ANDERSEN 1983). The nitrate uptake of W. arrhiza rises with increasing light intensities up to  $87 \text{ J m}^{-2} \text{ s}^{-1}$  (SWADER et al. 1975). It decreases again above  $174 \text{ J m}^{-2} \text{ s}^{-1}$ . At low light intensities, L. trisulca forms only one daughter frond on one side, consequently spiral colonies develop (GOEBEL 1921). HILLMAN (1961a) supposes that the low sugar content in the fronds regulates the limited production of daughter fronds. In nutrient solutions with sucrose, L. trisulca is able to form daughter fronds on both sides even in darkness.

It is not quite clear if there is a maximum light intensity above which Lemnaceae fronds are damaged. Data in the literature are controversial. WHITE (1936c) reports that L. minor shows injuries at light intensities above 15000 lux. Fig. 2.20 demonstrates that W. arrhiza does not grow at light intensities above  $6 \text{ to } 9 \text{ J m}^{-2} \text{ s}^{-1}$ . This value is extremely low (at least 10 times lower than in other publications) and is probably caused

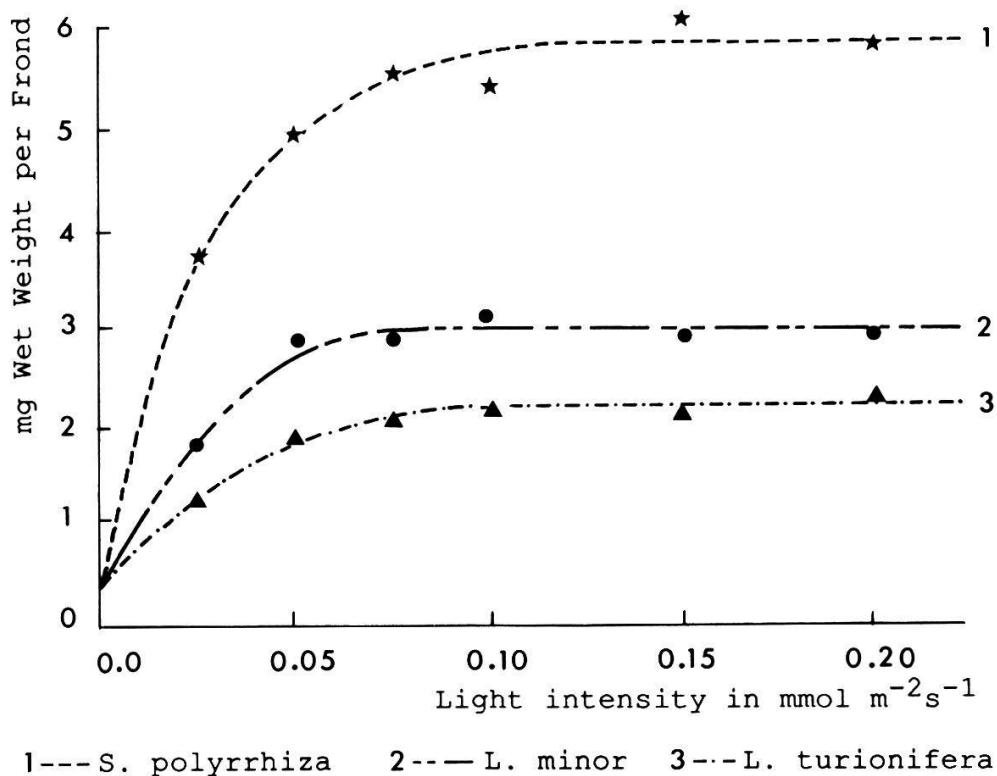


Fig. 2.24. Dry weight per frond in relation to light intensity (from DOCAUER 1983)

by the submerged state of plants in chemostat culture. MESTAYER et al. (1984) measured lower growth rates at levels of PAR above  $80 \text{ J m}^{-2} \text{ s}^{-1}$  than between 50 and 80. According to ZURZYCKI (1957a) photosynthesis of L. trisulca does not function any longer at intensities above  $500 \text{ J m}^{-2} \text{ s}^{-1}$ . Photosynthetic  $\text{O}_2$  evolution of S. punctata and L. minor was photoinhibited above a photon flux density of  $1.2 \text{ mmol m}^{-2} \text{ s}^{-1}$  (WEDGE and BURRIS 1982). OEGREN et al. (1984) and OEGREN and OEQUIST (1984a,b) observed photoinhibition of photosynthesis in L. gibba at 0.65 and  $1.75 \text{ mmol m}^{-2} \text{ s}^{-1}$ , subsequently isolated chloroplasts showed pronounced reduction in the capacity of whole chain electron transport. LANDOLT (1957 and unpubl.) did not see any damage in fronds of all investigated species cultivated at light intensities up to 33000 lux (higher intensities could not be achieved). In nature, much higher light intensities are measured on sunny days (up to 120000 lux). Possibly the relatively low upper limits of light intensities recorded are caused by the experimental conditions. As in other phanerogams, Lemnaceae form different fronds under shady and sunny conditions (LINDEMAN 1973, 1979). Therefore any measurements at high light intensities to determine a long lasting photoinhibition can only be made after having exposed the cultures to the new conditions for at least five weeks.

#### 2.3.5.1.2.2. Light effect in relation to temperature

Figs. 2.25 and 2.21 show the relationship between growth rate and light intensity at different temperatures. According to LANDOLT (1957), light saturation was not reached in the two clones of L. gibba at 9000 lux if temperatures surpassed  $20^\circ\text{C}$ . At  $18^\circ\text{C}$  the maximum growth rate was achieved at 9000 lux; at  $10^\circ\text{C}$  2500 lux and at  $5^\circ\text{C}$  even 1000 lux were sufficient for maximum growth rate. Further studies revealed that all investigated species and clones reached their maximum growth rate at lower temperatures at a much lower light intensity.

OEGREN et al. (1984) and OEGREN and OEQUIST (1984a,b) demonstrated the dependence on temperature of photoinhibition. L. gibba was exposed for two hours to various combinations of photon flux densities and temperatures. The extent of photoinhibition was assayed by measuring the net  $\text{CO}_2$  uptake before and after photoinhibitory treatment. At a constant photon flux density of  $0.65 \text{ mmol m}^{-2} \text{ s}^{-1}$  the extent of photoinhibition increased with decreasing temperature. About 60% photoinhibition of the

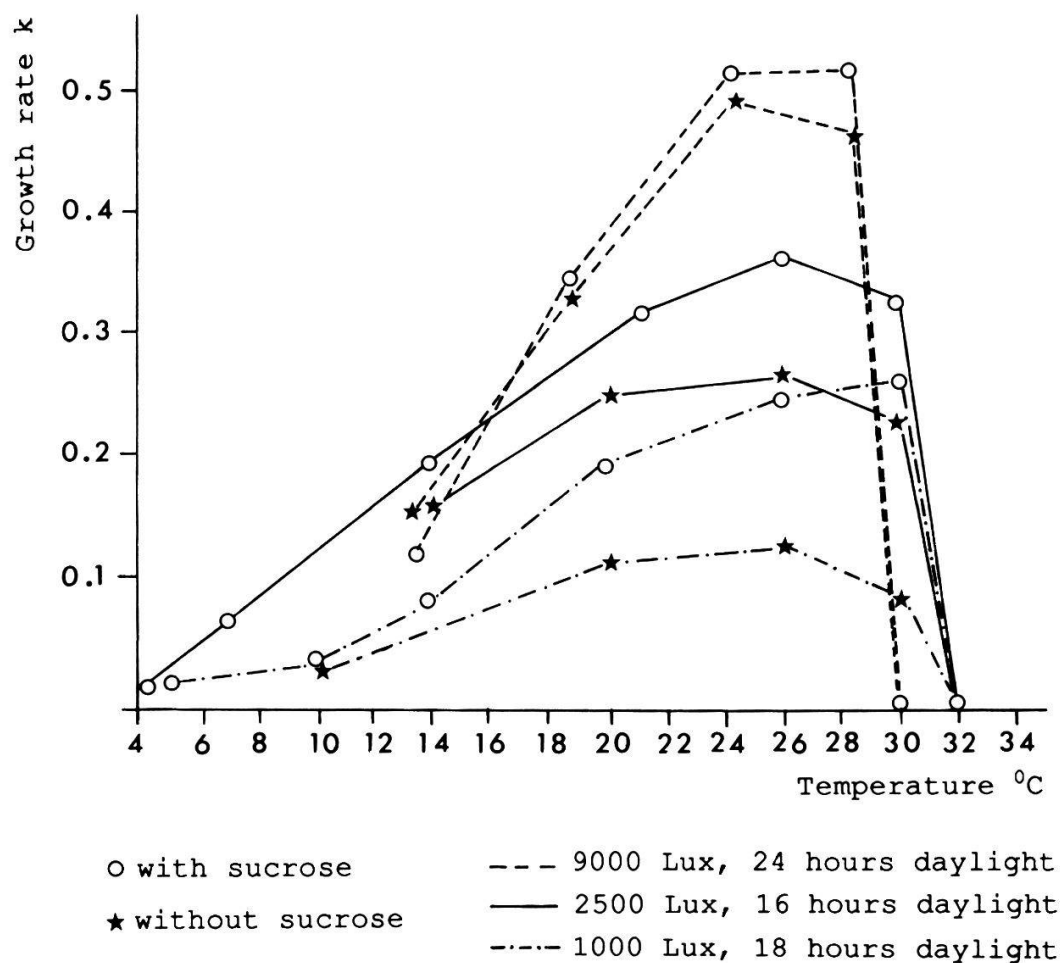


Fig. 2.25. Growth rate of *Lemna gibba* (no. 6566 and 6745) at three light intensities in solutions with and without sucrose in relation to temperature (from LANDOLT 1957, completed)

quantum yield for  $\text{CO}_2$  uptake could be obtained either by a high photon flux density of  $1.75 \text{ mmol m}^{-2} \text{ s}^{-1}$  at  $25^\circ\text{C}$  or by a moderate photon flux density of  $0.65 \text{ mmol m}^{-2} \text{ s}^{-1}$  at  $3^\circ\text{C}$ .

It is supposed that the light compensation point is similarly dependent on the temperature. This means that the lower the temperature, the lower is the compensation point.

#### 2.3.5.1.2.3. Light effect in relation to nutrient supply

ASHBY (1929a) noted light saturation of a clone of *L. minor* already at

1500 lux (at 25°C). This must be explained by the unfavourable nutrient solution. According to WHITE (1937a,b, 1938, 1939) and WHITE and TEMPLEMAN (1937), the optimal nitrogen and potassium content for L. minor is higher at higher light intensities, e.g. optimal K content at 600 lux is 0.05 mM, and at 3000 lux 5 mM. This is in accordance with the results of SWADER et al. (1975) who showed that the nitrate uptake of W. arrhiza rises with higher light intensities. LINDEMAN (1951, 1952) observed that lack of P inhibits the photosynthesis near light saturation. The inhibition was stopped within a few hours after adding  $\text{PO}_4^{3-}$  to the solution.

#### 2.3.5.1.2.4. Differences in the light effect between different species and clones

HICKS (1934) observed different light saturations (9000 lux and 15000 lux) for two clones of L. minor. WHITE (1936c) also found different responses of different clones of L. minor to light intensities. L. trisulca is able to grow at lower light intensities than L. gibba and L. minor (AMBROSE 1978). DOCAUER (1983) measured the following light saturations for 5 different species in  $\text{mmol m}^{-2} \text{s}^{-1}$ : S. polyrrhiza (0.024), L. minor

Table 2.21. Growth rate of different species of Lemnaceae at two light intensities (2000 lux and 9000 lux continuous light) in percent of the maximum growth rate at 24°C (after LANDOLT 1957)

Species (number of clones)	light intensity in lux	
	2000	9000
<u>S. polyrrhiza</u> (5)	50-55	91- 96
<u>S. punctata</u> (1)	58	95
<u>L. gibba</u> (6)	55-63	83- 96
<u>L. turionifera</u> (5)	48-63	91- 99
<u>L. minor</u> (7)	57-63	88- 98
<u>L. aequinoctialis</u> (4)	59-75	88-100
<u>L. valdiviana</u> (1)	70	101
<u>L. minuscula</u> (9)	66-86	96-105
<u>W. lingulata</u> (1)	32	53
<u>W. gladiata</u> (1)	43	68
<u>W. arrhiza</u> (1)	56	99
<u>W. columbiana</u> (1)	48	60
<u>W. globosa</u> (1)	64	95

(0.035), L. turionifera (0.047), W. columbiana (0.047), and W. borealis (0.086). Table 2.21 lists differences in the behaviour of different species and clones at two light intensities (from LANDOLT 1957). In general, the differences within a species are nearly as great as between the species. The relatively low percentage of maximum growth rates of W. lingulata, W. gladiata, and W. columbiana is remarkable. According to the results of DOCAUER (1983), W. borealis has a still lower percentage. Clones (and species) with a low percentage of the maximum growth rate at 9000 lux have a high light intensity saturation and therefore grow in open not shaded places or they grow mixotrophically. On the other hand L. minuscula or clones of L. aequinoctialis with a high percentage of the maximum growth rate at 2000 lux are able to grow in shady areas. From preliminary indications, L. trisulca also belongs to this group.

#### 2.3.5.1.2.5. Relation between light effect and the age of the fronds

When older, fronds of S. polyrrhiza grow more slowly (at  $14 \text{ J m}^{-2} \text{ s}^{-1}$ ). A short illumination of 100, 250, or  $500 \text{ J m}^{-2} \text{ s}^{-1}$  enhances the photosynthetic rate in parallel to the light intensity and the age of the fronds (GAPONENKO and STAZHETSKII 1969). The mechanism of this effect is not sufficiently known.

#### 2.3.5.1.2.6. Indirect effects of light intensity

YOSHIMURA (1952) detected a higher  $\text{NH}_4^+$  uptake in the light than in the dark for S. polyrrhiza and L. valdiviana whereas the  $\text{NO}_3^-$  uptake was slightly inhibited in the light. HILLMAN (1961a) supposes that this effect is due to the higher partial  $\text{O}_2$  pressure during photosynthesis. BLACKWOOD and LEAVER (1977) showed that in L. minor light stimulates amino acid incorporation specifically into three proteins. LIEBERT (1986a) reports a higher content of Ca, Mg, and K in L. gibba if cultivated in the light compared with in the dark.

The plasma adhesion to the cell wall of parenchyma cells containing chlorophyll of L. minor roots is lower in darkness than in the light. It is assumed that this is the result of the higher  $\text{CO}_2$  level in darkness and the subsequently lowered pH (SCHAEFER 1956).



#### 2.3.5.1.2.7. Duration of light

The growth rates of Lemnaceae rise proportionally to the length of day at least at suboptimal light intensities (figs. 2.26 and 2.27). They are highest under continuous light (ASHBY 1929a, LANDOLT 1957). Near light saturation, the increase is no longer linear. EYSTER (1966) did not observe a distinct rise of growth rate in S. polyrrhiza above 16 hours light duration at 25°C and 6000 lux. Growth rate under alternating periods of 2 hours does not differ significantly from the growth rate under a 12 hour period (ASHBY 1929a).

Some authors report damaged fronds under continuous light conditions, e.g. CLARK (1925) in S. polyrrhiza, at light intensities of 4000 and 9000 lux, and WHITE (1936c) in L. minor, at light intensities of 15000 lux. Other authors did not note any injuries to fronds under continuous light and at different intensities (e.g. LANDOLT 1957, EYSTER 1966).

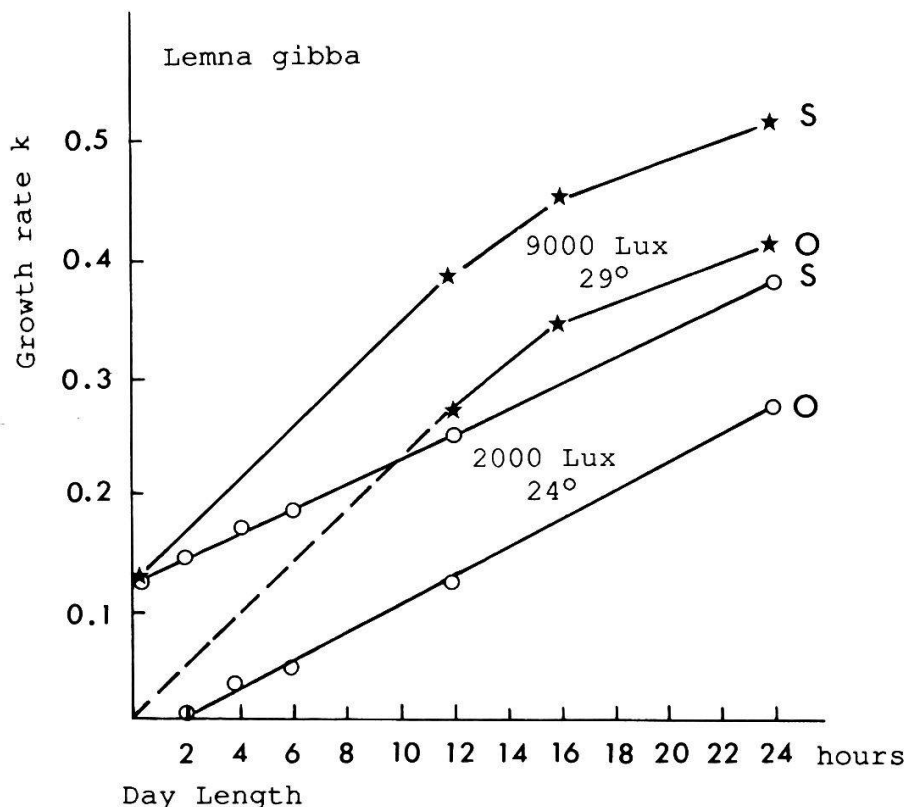


Fig. 2.26. Growth rate of Lemna gibba (no. 6566) and in relation to the length of day in solution with sucrose (S) and without sucrose (O) (from LANDOLT 1957)

Therefore, it is possible that the damaging effect must be attributed to other factors than lack of dark periods, e.g. too high temperatures, unfavourable nutrient conditions, too sudden change from conditions of low light intensity to high intensity.

Compared with continuous light, a day of 12 hours results in smaller fronds, less stomata per frond area, and smaller stomata in S. intermedia (FERNANDEZ and MUJICA 1973). The life-span of L. minor is not influenced by the length of day (WANGERMANN 1952). Differently, OSTROW and DIJKMAN (1969) observed a pronounced reduction of the life-span of S. punctata fronds with prolongation of the day.

HUBALD and AUGSTEN (1979) showed that alternating light and dark conditions enhance the activity of nitrate reductase and the content of soluble protein in W. arrhiza compared with continuous light conditions.

For other effects of length of day (especially on flowering), see chapters 2.3.5.1.2 and 2.4.3.1.5.

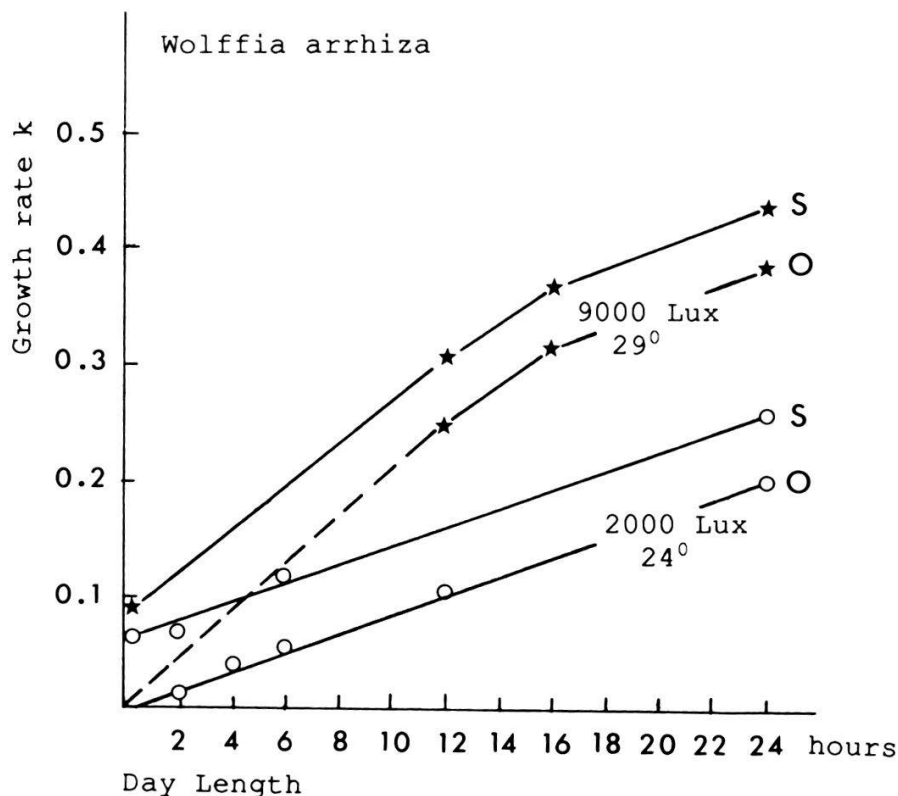


Fig. 2.27. Growth rate of Wolffia arrhiza (no. 6862) in relation to the length of day in solution with sucrose (S) and without sucrose (O) (from LANDOLT 1957)

### 2.3.5.1.3. Light effects via the phytochrome system

There is extensive literature on the effect of red and far-red light of low intensity in Lemnaceae. KANDELER (1955, 1956) revealed that flowering of the long-day plant L. gibba is stimulated by far-red illumination (720-760 nm) and inhibited by red light (600-700 nm). Inversely, far-red light prevented flowering of the short-day plant L. aequinoctialis if applied at the end of the daily short-day (SCHUSTER 1968) (see also chapter 2.4.3.1.5.). Far-red light can be replaced by blue light, red light by UV irradiation (380 nm) (OHTANI and KUGAMAI 1981) to reach appropriate effects on flowering of L. aequinoctialis.

Apart from the flowering effect, the phytochrome system regulates a series of further metabolic processes and the formation of different characteristics of Lemnaceae. ROMBACH (1976) lists the following characteristics of heterotrophically grown L. minor which are stimulated by light intensities far below the level needed for photosynthesis (light effect reversible by exposure to far-red irradiation): rate of cell division, elongation of fronds, and their multiplication. Far-red illumination given at the end of the daily main-light period on the other hand results in slower growth of roots, higher growth rate of fronds, and bigger fronds in the short-day plant L. aequinoctialis (SCHUSTER 1968). The production of resting fronds becomes inhibited by end of day far-red in L. perpusilla, another short-day plant (HUEGEL et al. 1979). The same treatment leads to a reduction of the growth rate, size of fronds and length of roots in the long-day plant L. gibba. A period of 5 minutes red light is able to neutralize the effect of far-red light (KANDELER 1963). The phytochrome control of root growth in L. gibba and L. minor is also described by CHAMURIS and NIELSEN (1983).

Furthermore, the phytochrome system regulates the nitrate reductase activity. According to LIEBERT and AUGSTEN (1986), there are low irradiance responses involved in the regulation of the enzyme in L. gibba and W. arrhiza.

Exposure to far-red light (4-5 minutes applied about one hour after normal daylight) causes a hyperpolarization of the membrane potential of L. aequinoctialis as well as of L. gibba (LOEPPERT et al. 1978, KANDELER et al. 1980). The decomposition of starch is retarded by preceding application of far-red light (KANDELER et al. 1980). The vitamin B<sub>1</sub> synthesis in L. minor is stimulated by phytochrome (P<sub>fr</sub>) (ROMBACH 1974a,b, 1976).

Phytochrome is involved in the photoregulation of anthocyanin production in S. polyrrhiza (MANCINELLI 1984, MANCINELLI and RABINO 1984). However, the authors suggest the involvement of a second photoreceptor (called cryptochrome) specific to light of the blue and UV region. Another phytochrome mediated regulation was detected by TOBIN (1981a,b). The phytochrome action rapidly affects the expression of the genes for the small subunit of RuDP carboxylase and chlorophyll a/b-protein in L. gibba. Red light induces rapid and specific increases in the amounts of RNA encoding these polypeptides. Therefore, phytochrome action is said to change either the transscription rate or the rate of degradation of these mRNAs (STIEKEMA et al. 1983a).

The phytochrome content ( $P_{fr}$ ) diminishes in darkness after exposure to red light in L. gibba, L. minor, and L. aequinoctialis. However, after a longer dark period, it rises again (ROMBACH and SPRUIT 1968, ROMBACH 1978). PORATH and BEN-SHAUL (1973) obtained similar results with S. punctata.

#### **2.3.5.1.4. Light effects not completely explainable by chlorophyll or phytochrome action**

In this chapter, all light effects will be dealt with which are not understandable by action of the chlorophyll or phytochrome systems or which have not been investigated in this respect.

ZURZYCKA and ZURZYCKI (1950) and ZURZYCKI (1957a,b, 1962) studied the influence of light on the position of chloroplasts in L. trisulca. In darkness, the chloroplasts are distributed along the cell walls. At low light intensities (10-150 lux), the plastids are along the cell walls which are perpendicular to the light incidence. At high light intensities (10000 lux and higher), they remain along the walls which are oriented parallel to the light incidence. The effect of low as well as high light intensities is caused mainly by blue light. Riboflavin is supposed to be responsible for the absorption of blue light. GABRYS et al. (1981) investigated chloroplast translocations in L. trisulca induced by a single strong pulse of blue light. With short pulses transient rearrangements of chloroplasts to a weak-light position occurred. With longer pulse duration, the initial movement to a partial strong-light position was followed by a wave of translocations to a weak-light arrangement. KAJFOSZ et al. (1983) report on chloroplast translocations in L. trisul-

ca induced by two successive blue light pulses. In contrast to L. trisulca, the O<sub>2</sub> uptake in L. aequinoctialis shows no stimulation in blue light (WITZTUM et al. 1979). Further details on light-dependent chloroplast movements are given in chapter 2.4.1.6.

Far-red light and to a lesser extent blue light elongate fronds and stalk of L. trisulca at low light intensities. This effect may partly be attributed to the phytochrome system. Red, yellow, and green light does not have any other effect than white light. Full darkness resulted in a slight elongation. High intensities of red and far-red but not of blue light also lead to elongation of the frond (ZURZYCKI 1957b). BATA and NESKOVIC (1982b) observed greatly shortened stalks and fronds of L. trisulca at high intensities of blue light compared to the effect of white and red light. This effect was not changed by the addition of kinetin, IAA, or GA.

TSUDZUKI and KONDO (1979) observed, in L. gibba, a potassium uptake rhythm which only showed up at relatively high light intensities (below 700 lux, the rhythm was rapidly damped). The amplitude of the rhythm was independent of the growth rate and of the potassium content of the medium (for details see chapter 2.5.9).

The influence of light on biosynthesis of carotinoids was studied by MONEGER (1968d) in S. polyrrhiza. The production of carotinoids (carotene, lutein, epoxylutein, violaxanthin, neoxanthin) is not fully prevented in darkness. However, light has a very stimulating effect. It seems probable that beside chlorophyll other photoreceptors are involved in the effect (compare chapter 2.5.8.2). The flavonoids vitexin and orientin are formed in S. intermedia in darkness as well as in light. However, cyanidin-3-monoglucoside is produced only in white or blue but not in red light. The vitexin content reaches the same level at light intensities of 4000 and 9000 lux. In contrast, the content of orientin and cyanidin-3-monoglucoside is lower at the lower intensity. Kaempferol behaves similarly as vitexin, quercetin similarly as orientin (McCLURE 1968). Anthocyanin in S. punctata has a high peak of synthesis at 705 nm and at 300 nm. Blue and green light are relatively ineffective. There is no evidence that the formation of anthocyanin is controlled by phytochrome (NG et al. 1964). However, MANCINELLI (1984) and MANCINELLI and RABINO (1984) assume that phytochrome as well as photosynthetic pigments are involved in biosynthesis of anthocyanin in S. polyrrhiza. In S. intermedia, a further photoreceptor (called cryptochrome) seems to be

active in addition. For further details on light-dependent anthocyanin biosynthesis see chapter 2.5.8.5.

The Hill reaction activity of isolated W. arrhiza chloroplasts is lower under red illumination compared with white light. Under blue light, it is stimulated at high growth rates and reduced at low growth rates in comparison with white light. The effect of blue light is strongly connected with an increase in the endogenous cytokinin level in fast growing cultures (EICHORN and AUGSTEN 1983a).

The synthesis of the  $5 \times 10^{-5}$  dalton plastid mRNA does not seem to be dependent on phytochrome, but is stimulated by irradiation in the blue and red regions (GRESSEL 1978).

Pigments which absorb blue light and might be considered as active in some of the mentioned processes are flavins and carotinoids (VOSKRESENSKAYA 1979).

**UV rays** stimulate the growth of S. polyrrhiza, L. gibba, and L. minor at a daily exposure of 2.5 to 10 minutes. They promote the activity of L-phenylalanine ammonia lyase and raise the anthocyanin content of S. polyrrhiza (GORDON 1977). LUKINA (1983b) also observed a stimulation of growth rates in S. polyrrhiza, L. gibba, and L. minor by UV irradiation (2-10 minutes with BUV 30 lamps). Different strains responded differently. However, BIGGS (1983) recorded a reduction of growth rate in L. minor (probably L. obscura) and a repression of flowering after UV-B irradiation (280-320 nm). Photosynthetically active radiation is required for photoreversal of these inhibitions. UV irradiation changes the colourless flavanes in S. punctata into reddish-brown (phlobaphene-like) pigments (WITZTUM 1974a). Further effects of UV irradiation in S. punctata are: lower IAA level, and abscission of daughter fronds. Exogenous supply of IAA neutralizes the effect of UV irradiation (WITZTUM 1974b, WITZTUM and KEREN 1978a, WITZTUM et al. 1978). DIJKMAN et al. (1964) observed a 20% life-span extension in L. minor and L. aequinoctialis by short exposure to UV irradiation. A longer exposure reduces the life-span. It is supposed that the UV irradiation may selectively cause the destruction of certain protoplasmic factors that shorten life-span.

HICKS (1932a) noted necrotic tissue in different Lemnaceae species after UV irradiation of at least 5 minutes at an intensity of  $0.1 \text{ J m}^{-2} \text{ s}^{-1}$ . He also reported flower induction with the same treatment in L. minor, L. turionifera (named as L. minor var. purpureus), L. trisulca, L. valdiviana, and L. minuscula, but not in S. polyrrhiza, W. gladiata, and W. bo-

realis. WANGERMANN and LACEY (1952, 1953) noted damaged fronds of L. minor after irradiation with UV rays of 250 nm. The effect is smaller in aerated cultures. The authors as well as HILLMAN (1961a) suppose that the injuries might have been caused not by the UV irradiation, but by the simultaneously developing ozone.

#### 2.3.5.1.5. Heterotrophic growth

GORHAM (1945) was the first to demonstrate that Lemnaceae are able to grow in darkness if sucrose is added to the solution. Further cultures in the dark have been carried out by GORHAM (1950), THIMANN and EDMONDSON (1949), HUTNER (1953), LANDOLT (1957), HILLMAN (1957), and many others.

The fronds of Lemnaceae grown in darkness are yellowish white, smaller than in the light, and have reduced air spaces. Spirodela and Lemna species develop only rudimentary roots (S. punctata, L. gibba, L. minor) or very small roots (S. polyrrhiza, L. aequinoctialis, L. minuscula) (LANDOLT 1957, EFRAT et al. 1975, LEUCHTMANN 1979, LIEBERT 1980a). The protein content in S. punctata is lower in fronds grown in the dark (2.5% of the dry weight) than in those grown in the light (7.8%), the amount of starch greater (50% versus 27%) (DE HEIJ et al. 1984).

The bleaching of the fronds in the dark is reversible if the plants are again transferred to the light. Young chloroplasts of S. punctata develop in the dark to amyloplasts; old chloroplasts remain unchanged for up to three months (PORATH 1979). Chlorophyll-free clones could not be induced experimentally in darkness or in light by treatments with streptomycin or 3-amino-1,2,4-triazole (SCHER and AARONSON 1958). FRICK (1972, unpubl. results) reports that cytidine desoxyriboside ( $C_{dr}$ ), a natural metabolite, quantitatively induces bleaching of the fronds of photoheterotrophically growing L. minor. The bleaching consists of a specific inhibition of plastid replication in the fronds.

Some Lemnaceae species or clones are not able to grow in the dark with the sole addition of sucrose. It has not been worked out extensively which organic substances are needed besides sucrose by different species. As HILLMAN (1957, 1961a) pointed out, the light intensity needed for sufficient production of certain vitamins or growth factors is very low. Unfortunately, most experiments dealing with growth in the dark of Lemnaceae were not made in complete darkness because the cultures had to



be controlled or the fronds counted. This resulted in short exposition to light. GORHAM (1945) found that green light had a very small effect on production of growth factors.

S. polyrrhiza (GORHAM 1945, LANDOLT 1957, HILLMAN 1961a, EFRAT et al. 1975, LEUCHTMANN 1979) and S. punctata (GORHAM 1950, HUTNER 1953, LANDOLT 1957, SCHER and AARONSON 1958, EFRAT et al. 1975, LEUCHTMANN 1979) are reported to grow with sugar alone in complete darkness.

A special effect in S. punctata was observed by McCOMBS and RALPH (1972a,b). In culture with sucrose as the only organic source (beside EDTA), growth stopped after 2-3 days following the transfer of the fronds to darkness. The fronds began to grow again 3 to 4 weeks later. Using different special treatments (cold shock, heat shock, addition of distilled water, of chloramphenicol, of actidione, or of kinetin), but also with the addition of yeast extract or with intermittent red light irradiation, the interruption of growth could be overcome. Adding kinetin to dormant S. punctata initiated increased synthesis of DNA, RNA, and protein within one hour. The authors suppose that S. punctata normally contains a growth inhibitor which is blocked by cytokinins. Since cytokinins are only formed in the light, the inhibitor still works in the dark until it is used up or inactivated after 3 to 4 weeks. Some amino acids (aspartic acid, asparagine, glutamic acid, glutamine, alanine),  $\alpha$ -ketoglutaric acid, and pyruvate also neutralize the inhibitory effect of darkness on the growth of S. punctata (BUCKLE and RALPH 1977). Differently from Spirodela, L. minor was found to grow continuously in the dark only if it is supplied additionally with casamino acids and yeast extract. With sucrose alone, the species was not able to utilize inorganic nitrate (GORHAM 1950). According to the same author, vitamin B<sub>1</sub> (thiamine) turned out to be the active substance in yeast extract. The optimum concentration amounted to 0.005 mM. The thiazole component of vitamin B<sub>1</sub> is active whereas the pyrimidine component is inefficient. BORNKAMM (1970) was able to grow L. minor with the addition of yeast extract only (casamino acids proved not to be necessary). However, the protein content was higher in solutions with supplementary casamino acids, the carbohydrate/protein ratio being 8 instead of 16. Also ROMBACH (1974a,b, 1976) and LEUCHTMANN (1979) succeeded in growing L. minor in solutions without casamino acids but with yeast extract. In contrast to these results, GORHAM (1950) had to supply casamino acids in addition to yeast extract for growth of his L. minor in darkness. It is not sure

if this difference in need of casamino acids is due to a different clone used (GORHAM possibly had a clone of L. turionifera instead of L. minor) or if other factors, e.g. culture conditions, are responsible (GORHAM used a modified Hoagland solution without ammonium). The addition of casamino acids without vitamin B<sub>1</sub> was able to delay the death of L. minor compared with a medium without casamino acids; with casamino acids, the fronds died after 25 times multiplication, without casamino acids, death was observed after 6 times multiplication of the fronds (ROMBACH 1974a). HILLMAN (1957) cultivated L. minor successfully in the dark if he added 15 or  $45 \times 10^{-4}$  mM kinetin or related compounds to the solution containing sucrose. However, his experiments lasted for only relatively few days. ROMBACH (1974a,b, 1976) showed that the cytokinins BA and kinetin ( $10^{-3}$  mM) stimulate the growth of L. minor in the dark for the first 20 days, but did not enable long-lasting growth without the addition of vitamin B<sub>1</sub>. If vitamin B<sub>1</sub> was present, the stimulating effect of BA lasted for at least 80 days. Up to  $0.1 \text{ J m}^{-2} \text{ s}^{-1}$  the stimulation of kinetin is the same, at higher light intensities it decreases (ROMBACH 1974b). Also TASSERON-DE JONG and VELDSTRA (1971b) observed, in L. minor, a stimulation of BA or kinetin only for 7 days without addition of vitamin B<sub>1</sub> under dark conditions. After 3 weeks, the cultures died. Short illumination with red light (10 minutes every 3 to 4 days) made the addition of yeast extract superfluous (GORHAM 1950, HILLMAN 1957). The effect of red light is accomplished by the phytochrome system. The vitamin B<sub>1</sub> synthesis is stimulated by phytochrome (P<sub>fr</sub>) (ROMBACH 1974a,b, 1976).

According to LEUCHTMANN (1979), L. aequinoctialis needs not only vitamin B<sub>1</sub> for continuous growth in the dark, but still another component out of the yeast extract.

LANDOLT (1957 and unpubl. results) cultivated different species under two different nutrient conditions (table 2.22). Since he did not keep absolute darkness, his results are only indicative. He grouped the investigated clones in four categories according to their different behaviour in the three solutions (table 2.23). The negative effect of amino acids and yeast extracts on S. polyrrhiza can also be observed under light conditions. Therefore, it must be concluded, that either the amino acids are not in a balanced composition, or some factors of the yeast extract are present in too high concentration for good growth in species of the group A). In group B) the species multiply with the same growth

Table 2.22. Grouping of different species and clones of Lemnaceae according to their behaviour towards yeast extracts and casamino acids in darkness (after LANDOLT 1957 and unpubl. results). Conditions and reactions are presented in table 2.23.

Clones with an asterisk (\*) were also investigated by LEUCHTMANN (1979) with essentially the same results

Group	species	clone No.
A)	<i>Spirodela polyrrhiza</i> <i>Wolffiella neotropica</i> <i>Wolffiella lingulata</i>	6581, 6593, 6613, 6627, 6731, 7003 7010, 7344* 7225 6748, 7289
B)	<i>Spirodela punctata</i> <i>Lemna gibba</i>  <i>Lemna minuscula</i>  <i>Wolffia angusta</i>	6631, 6725 6566, 6583, 6729, 6745, 6861*, 7007, 7135, 8428* 6584, 6589, 6597*, 6600*, 6726, 6747 7476
C)	<i>Lemna disperma</i> <i>Lemna obscura</i> <i>Wolffiella gladiata</i> <i>Wolffia microscopica</i> <i>Wolffia arrhiza</i> <i>Wolffia columbiana</i> <i>Wolffia globosa</i>	7223, 7782 7856 7000 8359 6862, 7014 7222 6592
D)	<i>Lemna minor</i>  <i>Lemna turionifera</i> <i>Lemna trisulca</i> <i>Lemna aequinoctialis</i>  <i>Lemna valdiviana</i> <i>Lemna minuscula</i> <i>Wolffia australiana</i>	6568, 6570, 6578*, 6579, 6580, 6591*, 6625, 6728, 7008, 7011 6573, 6601, 6619, 6727, 6736, 6853 6601, 6624, 6722, 7013 6609, 6612, 6746, 6748, 7001*, 7006, 7806* 7002, 7005 6752 7211

Table 2.23. Meaning of the categories A), B), C), and D) in table 2.22.

++ good growth, + slow growth, 0 dieing

Substances added to Hutner 1/5	A	B	C	D
1% sucrose	++	++	+	0
1% sucrose + 0.004% yeast extract + 0.08% casamino acids	+	++	++	++

rate in both solutions whereas in group C) casamino acids and yeast extract have a stimulating effect. It is possible that some or all clones of group C) might in effect need a very low amount of a growth factor in absolute darkness. The group D) with obligatory need of yeast extract and/or casamino acids can possibly be divided into two or more groups if vitamin B<sub>1</sub> is not the only factor needed for some clones. LEUCHTMANN (1979) noted a slightly inhibiting effect of casamino acids and yeast extract on his two clones of L. minuscula (6597, 6600) which are here attributed to group B). It is possible that all or some clones of L. minuscula should be changed to group A).

#### 2.3.5.2. Radiations outside the visible and UV range

##### 2.3.5.2.1. X-rays (γ-radiation)

FELDMANN (1968, 1969, 1971, 1975) succeeded in stimulating the growth rate of L. minor and in increasing the size of the frond by treatments with low intensities of X-rays. This was especially the case when light and temperature conditions were kept suboptimal. POSNER and HILLMAN (1960) did not observe any effect on L. aequinoctialis with radiation of 300 r. At higher intensities, the growth rate was inhibited, and at 5000 r no growth occurred any longer. The preference of the side on which the first daughter frond appears was often changed after irradiation. JOHNSON (1941) noted deformed or damaged fronds in L. minor as a result of short-term radiation of 1000 and 2500 r. According to LEINERTE (1969), the accumulation of Sr, Cs, and Ce is lowered with increasing irradiation doses of X-rays.

Table 2.24. Radiosensitivity of different Lemnaceae species

DL 100 = doses needed for 100% lethality

Species	DL 100 in mC kg <sup>-1</sup>	Authors
<u>W. arrhiza</u>	800	SAROSIEK and WOZAKOWSKA-NATKANIEC (1984)
<u>L. minor</u>	1806	KASINOV (1966)
<u>S. polyrrhiza</u>	2580	WOZAKOWSKA-NATKANIEC (1977a)

WOZAKOWSKA-NATKANIEC (1977a,b) investigated the tolerance of two populations of S. polyrrhiza and 14 populations of L. minor to  $\gamma$ -irradiation (with <sup>60</sup>Co). The populations showed different behaviour. S. polyrrhiza appeared to be less sensitive than L. minor. The 50% lethal dose for L. minor was achieved between 4000 and 10000 r depending on the population. The corresponding dose for S. polyrrhiza was reached between 6000 and 8000 r. Some defects could be detected already at 1000 r. L. gibba shows abnormalities at 800 r (10-60% of the fronds). This proportion was held for a long time under the same conditions. Chromosome changes within the meristem cells are assumed. The sexual descendants proved to be mostly normal (KASINOV and KASINOVA 1971a,b). The different radio sensitivities of 3 Lemnaceae species are summarized in table 2.24).

Mg and especially Ca increase the resistance of W. arrhiza to acute radiation. Therefore, high concentrations of these minerals have a protective effect on Lemnaceae which are exposed to  $\gamma$ -radiation in nature (SAROSIEK and WOZAKOWSKA-NATKANIEC 1984). LYSENKO (1981) observed a similar higher resistance of S. polyrrhiza to  $\gamma$ -radiation after pretreatments with alkylating mutagens (N-nitroso-N-methylurea, N-nitroso-N-ethylurea, ethyleneamine, diethyl sulfate). Postradiation treatment with 10 mM caffeine inhibited the protective effect of the mutagens.

#### 2.3.5.2.2. Radionucleides and other radioactive substances

Radionucleides which serve for disinfection of water are taken up by Lemnaceae to 50-60% (GUSKOVA et al. 1970) (see also chapter 3.5.3). The

uptake of radionuclides by L. gibba generally decreases with increase of the pH from 5 to 9 (EL-SHINAWY and ABDEL-MALIK 1980). S. polyrrhiza can be used as an indicator for radioactive contamination of water by measuring the content of radionuclides. However, submerged plants such as Potamogeton are more accumulative (VINTSUKEVICH and TOMILIN 1984). Lemnaceae are able to accumulate more  $^{106}\text{Ru}$  than small animals in an aquarium ecosystem (HONDA et al. 1971).

#### 2.3.5.2.3. Laser radiation

COUCH and GANGSTAD (1974) investigated the response of L. minor to  $\text{CO}_2\text{-N}_2\text{-He}$  laser radiation ( $10.6\ \mu$ ). Plants irradiated with  $100\ \text{J cm}^{-2}$  laser energy died within three weeks. Ten  $\text{J cm}^{-2}$  stopped the growth rate completely without destroying the fronds. Growth was not affected by  $1\ \text{J cm}^{-2}$  treatment.

## 2.4. DEVELOPMENT

### 2.4.1. VEGETATIVE DEVELOPMENT

#### 2.4.1.1. Morphogenesis of fronds

Environmental and endogenous factors regulate not only growth but also morphogenesis of duckweed fronds. In L. trisulca relative frond length (length/width ratio) and length of the stipe are increased, when plants were cultivated under low intensity far-red light (far red applied solely to cultures containing sugar or as an extension of the daily high-intensity white light period). Under high-intensity light conditions red as well as red plus far red have an elongating effect on frond and stipe (ZURZYCKI 1957b). Possibly, gibberellins may participate in the mediation of the cited light effects. GUERN (1965) found a stipe lengthening after application of gibberellin A<sub>3</sub> in L. trisulca and S. polyrrhiza. Another possibility to lengthen the stipe of a certain frond in L. trisulca is removal of the two daughter fronds (GUERN 1963a, 1965). In L. gibba a strong enhancing effect on stipe length is achieved, if benzyladenine is added to an ammonium-containing nutrient medium (DEKOCK et al. 1974). Salicylic acid, acetylsalicylic acid and salicylhydroxamic acid, but not benzoic acid and several other related substances have the same effect as benzyladenine (inducing in addition, however, chlorosis and growth inhibition in daughter fronds). Salicylic acid is effective only when iron and/or copper as well as ammonium are present in the nutrient medium. Addition of either iron or copper caused the salicylic acid effect to appear and the effect was more pronounced when iron or copper were increased. In S. polyrrhiza, however, benzyladenine ( $\geq 10^{-6}$  M) causes abnormal stipe growth also in the absence of ammonia (LE PABIC 1976a,b). In L. minor, the size of the frond and the length of the stipe are greatly enlarged after supply of  $10^{-3}$  M arginine (FELLER and ERISMANN 1976). The fact that arginine is one of the precursors of polyamines (see fig. 2.50) should be considered in this context.

Papillae, which can be developed on the upper frond surface above the point of root insertion and along the middle nerve in S. punctata and L. aequinoctialis, are very distinctly visible when the ethylene-releasing agent ethephon is added to the culture medium (BUECHELE and KANDELER,



SCHARFETTER and KANDELER, unpublished results). In S. punctata the effect of ethylene can be achieved also by supply of ACC (SCHARFETTER et al. 1986), EDDHA or salicylic acid (SCHARFETTER et al. 1978). Naphthaleneacetic acid reverses the effect of EDDHA.

Another characteristic, which is promoted under the influence of ethphon, ethylene, EDDHA, and salicylic acid, is the development of air spaces on the lower side of the frond (CLELAND 1974c, CLELAND et al. 1982, ELZENGA et al. 1980, PIETERSE 1976, PIETERSE et al. 1970b, 1970c). Especially in L. gibba this development gives rise to the gibbosity of the fronds. The effect of EDDHA can be reversed by benzyladenine (PIETERSE and MUELLER 1977), decomposition products of gibberellin A<sub>3</sub>, i.e. allogibberic acid, epiallogibberic acid and gibberic acid (PIETERSE 1976),  $\beta$ -naphthol (PIETERSE 1978b), and free (unchelated) copper ions (PIETERSE 1975a), but not by iron deficiency (PIETERSE 1975a). EDDHA changes the uptake of certain heavy metals into the plant. In L. gibba G3 iron uptake is increased and copper uptake decreased by EDDHA (PIETERSE 1975a). Presumably, chelation of copper is only one of the effects of EDDHA, which lead to gibbosity, because EDDHA is effective also in a medium without copper (SCHARFETTER and KANDELER, unpublished results). Manganese as an agent, which activates enzymatic ethylene release from ACC, should be investigated in this context. Preliminary investigations of ELZENGA et al. (1980) have shown that EDDHA increases the ethylene evolution in L. gibba G3. In S. punctata the formation of visible air chambers is promoted by EDDHA and SA (SCHARFETTER et al. 1978), by the anti-auxin PCIB, by kinetin, but not by ACC (SCHARFETTER et al. 1986). The auxin NAA and the blockers of ethylene synthesis AVG and AOA abolish or diminish the effect of EDDHA. Thus, EDDHA may act in an at least two-fold way: by a decrease of the endogenous auxin level and by an increase of endogenous ethylene formation. In L. minor development of intercellular air spaces needs low-intensity red light (ROMBACH 1976). In fronds grown in darkness (with or without kinetin) intercellular cavities are absent. The effect of red light is strengthened by addition of  $3 \times 10^{-6}$  M kinetin to the nutrient solution (fig. 2.28).

Cell and tissue differentiation in fronds of Lemnaceae has been studied in connection with stomata and the vascular system. FERNANDEZ and MUJICA (1973) determined the stomatal index (stomata-epidermal cell ratio) in S. intermedia grown under different regimes of light and temperature. Whereas lowering of light intensity from 7000 to 4000 lux decreases the

stomatal index, lowering of night temperature from 25 to 4°C increases this value.

In the genus Lemna stomata are inflexible after differentiation and open all the time (see chapter 2.5.1.1). The width of the stomatal fissure is fixed during development. This process can be modified by ABA supply to the nutrient medium. The mean aperture of stomata in L. minor is 73% of control after 8 days growth in the presence of  $10^{-6}$  M ABA, and 44% of control after 14 days (McLAREN and SMITH 1976).

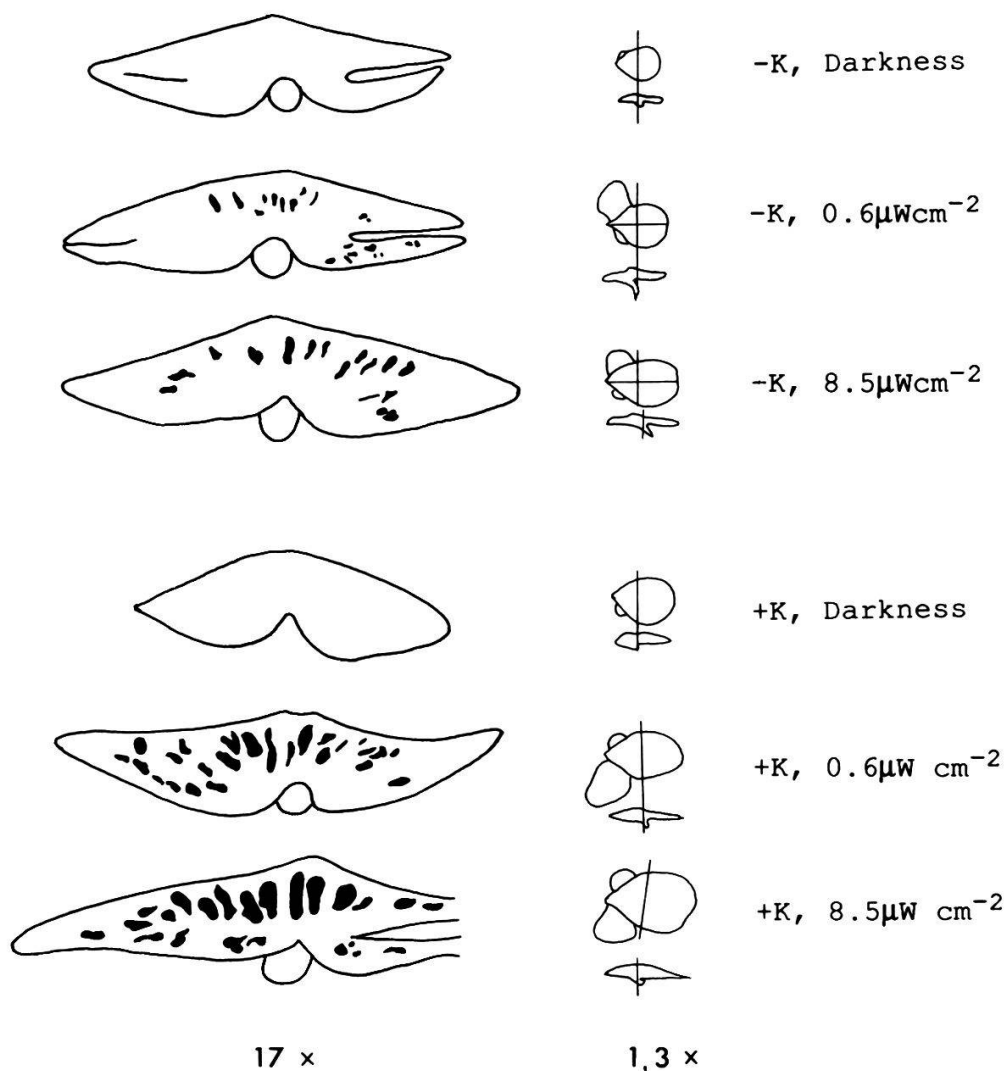


Fig. 2.28. The effect of red light and kinetin on the development of intercellular air spaces in Lemna minor (ROMBACH 1976). Fronds were grown in darkness or low-intensity red light (0.6 or 8.5 μW cm<sup>-2</sup>) without (-K) or with kinetin (+K) for 21 days. Diagrams of fronds show the intercellular air spaces in black.

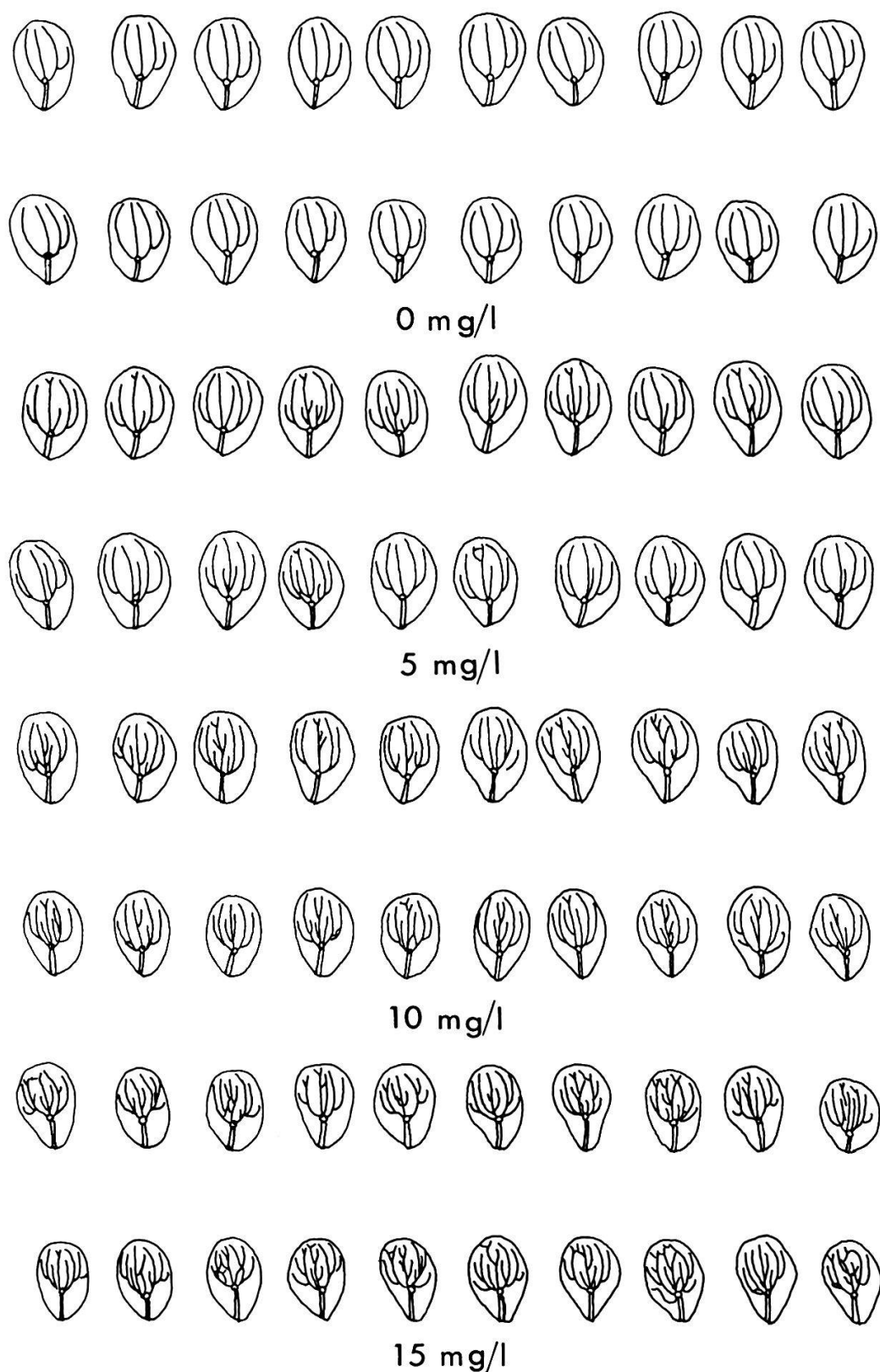


Fig. 2.29. The vascular system in fronds of *Lemna minor* as influenced by four concentrations (0-15 mg/l) of triiodobenzoic acid (SARGENT and WANGERMANN 1959).

The elaboration of the vascular system in fronds of L. minor is regulated by auxins and ethylene. Indoleacetic acid (IAA), naphthaleneacetic acid (NAA), as well as ethylene cause the production of 5 veins per frond instead of 3 or 4 in the controls. The application of triiodobenzoic acid (TIBA), an inhibitor of auxin transport, has a by far more dramatic effect (fig. 2.29). 15 mg/l of this substance increases the number of veins per frond to 10-12 (by differentiation of longer or shorter branches from the 3 veins, which arise from the "node", i.e. the insertion point of the root). 10 mg/l TIBA in combination with 0.05 mg/l NAA lead to a further increase of vein elaboration. Several other halogen-substituted benzoic acids have the same, but a less pronounced, effect as TIBA (SARGENT and WANGERMANN 1959, see also FLETCHER 1965).

The branching pattern of Spirodela and Lemna fronds is homodromous in most cases: One of the two daughter fronds, produced in one of the two pouches of the mother frond, dominates over the other during development and this dominance is preserved on the same side of the frond in all successive, vegetatively produced frond generations. In this way right-handed and left-handed clones occur, which normally are stable over decades. Nevertheless, the extent of dominance can vary and even a change of dominance can appear.

In L. trisulca the asymmetry of frond branching is expressed very weakly under high intensity light, but comes out in the following frond generations after lowering of the light intensity (GOEBEL 1921). Such colonies are spiral in shape. Supply of a sugar (sucrose, glucose or fructose), of kinetin, or an increase of medium pH (to 6-8) markedly reduces the dominance phenomenon in L. trisulca under low intensity light (BATA 1973, GUERN 1965). Gibberellin A<sub>3</sub> has a similar (GUERN 1965) or no effect (BATA 1973) in comparison to kinetin.

Experiments with excision of frond primordia have shown that there is a correlative inhibition between the different meristematic parts of a frond colony. Removal of the dominant daughter leads to development of the dominated daughter in the opposite pouch (GOEBEL 1921, GUERN 1965). In a similar way removal of grandchildren from the dominant daughter promotes development of grandchildren in the dominated daughter and vice versa. Furthermore, withdrawal of the first (= dominating) and second (= dominated) daughter or removal of grandchildren from the first and second daughter induces outgrowth of the third daughter (= second organ in the "dominating" pouch). The fourth and fifth daughter are

developed when the first three are removed (GUERN 1965). Correspondingly, kinetin or gibberellin A<sub>3</sub> (GUERN 1965) resp. the combination of sucrose with kinetin or pH increase (BATA 1973) stimulates the simultaneous production of two daughter fronds in one pouch.

Cancelling of meristem competition by optimal growth conditions may lead to a further consequence. WITZTUM (1979) has shown that in L. aequinoctialis 6746 8-10 percent of frond colonies are symmetrical under such conditions. The first two daughter fronds are both well developed and bear the dominating grandchild toward the proximal end (stipe end) of the mother frond. In this way on one side of the colony the homodromous branching pattern is interrupted by a heterodromous link and the progeny of this link shows an inversion of handedness. The same mode of branching has been found by BOECKER (1936) in a strain of L. valdiviana (collected from aquarium plants). In the southern part of Italy GIUGA (1973, and pers. comm.) collected many specimens of L. gibba colonies with symmetrical, proximally oriented grandchildren from the natural environment. In the latter case not only eutrophy of water and high intensity light may be responsible for the altered branching, but also the occurrence of certain herbicides. In addition GIUGA found another type of symmetrical frond colonies, at least in one case together with the described type. The second type has symmetrical grandchildren, which are distally oriented in relation to the mother frond. As WITZTUM (1979) pointed out, the two types of symmetrical frond colonies behave differently. The third and fourth daughter ("axillary fronds"), which are well developed in the first type of branching, are lacking in the second type. In the second type a change of handedness occurs too, but if the branching pattern is perpetuated, a regular heterodromous branching results, i.e. a change of handedness in each generation. An example of partial heterodromous branching in L. trisulca (cultured under high-intensity green light) can be taken from figure 2 in ZURZYCKI (1957b).

The change of handedness, which can be induced by X-rays (KASINOV 1973, POSNER 1961, POSNER and HILLMAN 1960), triiodobenzoic acid (POSNER 1961, WANGERMANN and LACEY 1953, WITZTUM 1979), 2,4-D (KASINOV 1973, KASINOV and KASINOVA 1974, KASINOV and PAVLOVA 1977) and - in L. aequinoctialis - by long-lasting short day (leading to double flowering fronds: DOSS 1978, WITZTUM 1979), seems to be based on a transitory appearance of the second type of symmetry. This has been shown especially in the investigations of KASINOV and co-workers and WITZTUM.

The hypothesis of WITZTUM (1979) that the "axillary frond" induces dominance in the grandchild positioned near by may be questioned in the light of Guern's results. Both developmental events, position of the "axillary frond" and of the dominant grandchild, could be under control of the same - hitherto unknown - hormonal gradients.

Another mode of determination of dominance takes place after sexual propagation. When the embryo grows out from the zygote, the handedness of the first frond (and in consequence all following frond generations) is determined at random. KASINOV (1968c), POSNER and HILLMAN (1960) and KANDELER (unpublished results) have shown that an 1:1 ratio of left-handed and right-handed progeny occur after germination of a lot of seeds.

Concluding this section, two examples of anomalous frond development may be referred to. Bell-shaped fronds, which are curved on the upper side and dip into the water with the border have been observed in S. punctata after application of IAA (1 ppm) and/or zeatin (0.025-0,25 ppm) (KERN and NAEF-ROTH 1975). In L. gibba some clones derived from X-ray (800 R) treated material show an irreversible tendency to produce monstrous individuals (between 10 and 60%) (KASINOV and KASINOVA 1971a). The following varieties occurred in all such clones: 1. Deformed individuals (proximal part of frond shortened and thickened, inflorescence often suppressed); 2. Funnel-shaped individuals (the two pouches join up to form a single pouch which is open on the upper side); 3. Scoop-shaped individuals (complete lack of stomatal epidermis; the frond consists of a single pouch with daughter fronds and inflorescence); 4. Individuals with gaps (1 or 2 gaps on one or both sides of the frond). This tendency to develop monstrous individuals persists only during vegetative reproduction and is lost after sexual propagation (self-fertilization).

#### **2.4.1.2. Morphogenesis of roots**

The number of root primordia developed at the "node" on the lower side of the frond primordium can vary in S. punctata. Addition of EDDHA or salicylic acid to the nutrient medium reduces the number of roots per frond from 2-4 to 1 (SCHARFETTER et al. 1978). A partial reversion of the EDDHA effect is achieved by blockers of ethylene synthesis AVG (aminoethoxyvinyl glycine) and AOA (aminooxyacetic acid) as well as by the ethylene-releasing agent ethephon and the ethylene precursor 1-aminocy-

clopropane-1-carboxylic acid (ACC) (SCHARFETTER et al. 1986). Naphthaleneacetic acid reduces the EDDHA effect to a very small extent only (SCHARFETTER et al. 1978).

Root growth seems to depend on the ratio of endogenous sugars to nitrogen compounds (WHITE 1937b, 1938). High intensity light and nitrate deficiency increase the length of the root in L. minor. Potassium deficiency which inhibits the net assimilation rate and enhances starch accumulation, decreases the length of the root. In accordance with these results SCHUSTER (1968) found a promotion of root growth under long day (in comparison to short day) and an inhibition of root growth by the photosynthesis blocker DCMU in L. aequinoctialis 6746. Exogenous sucrose (1-4%), however, also causes a distinct shortening of roots. Experiments with L. gibba G1 and G3 (BUECHELE and KANDELER, unpublished results) revealed that long day more than doubles the length of the root and that exogenous sucrose greatly diminishes this effect. Also under short day, with end-of-day far red, sucrose has a growth-inhibiting effect on roots of L. gibba. In S. punctata, on the other hand, not addition but removal of sucrose from the medium leads to a marked reduction of the length of the root (EFRAT et al. 1975). A complete lack of root growth occurs in S. punctata and L. gibba, when plants are cultivated heterotrophically, i.e., in darkness with a medium containing sugar (EFRAT et al. 1975, LIEBERT 1980a, 1986a). In S. polyrrhiza root growth is more or less independent from light and sucrose application (EFRAT et al. 1975). The inhibition of root length by adequate nitrogen supply has been observed in S. polyrrhiza only with increasing concentrations of  $\text{NH}_4\text{NO}_3$  but not of  $\text{KNO}_3$  (MENSCHICK 1970). Later, the different behaviour of certain Lemnaceae against varying  $\text{NH}_4\text{NO}_3$ - and  $\text{K}_2\text{HPO}_4$ -concentrations was investigated by LUEOEND (1983). In L. gibba and L. minuscula root growth is optimal, when nitrogen as well as phosphorus are present in very low concentrations. L. minor needs low phosphorus combined with moderate nitrogen and S. polyrrhiza moderate nitrogen and phosphorus concentrations for optimal root lengthening.

The effect of phytochrome on root growth has been investigated in L. gibba (G1 and G3) and L. minor (CHAMURIS and NIELSEN 1983, KANDELER 1963). A short far red irradiation given after the daily light period reduces the growth rate of roots and their attainable length. Red light given after far red reverses the far-red effect.

A treatment of plants with the auxins 2,4-dichlorophenoxyacetic acid



(ONO 1952b) or naphthaleneacetic acid (SCHARFETTER et al. 1978), with gibberellin A<sub>3</sub> (LOOS 1962), higher concentrations of benzyladenine (>0,1 ppm) (LIEBERT 1980a) or with abscisic acid (NEWTON 1972a, 1974a, 1977) reduces the length of the root in some Lemnaceae. In the case of abscisic acid not only root growth but also frond growth and frond multiplication are inhibited. The other hormones may rather work in an indirect way. In the same experiments naphthaleneacetic acid promotes the size of the frond and gibberellin A<sub>3</sub> enhances multiplication of them.

Low concentrations of benzyladenine (0.01 ppm) slightly promote root growth in L. gibba (LIEBERT 1980a). Other root-lengthening agents are EDDHA, salicylic acid, ethephon, ACC, AVG, and AOA (SCHARFETTER et al. 1978, SCHARFETTER et al. 1986). Cobaltous ions inhibit root growth in S. punctata. Some other heavy metals (Cd>Cu>Zn>Mn, Mo) inhibit light-induced root lengthening in heterotrophic cultures of L. gibba (LIEBERT 1986a).

#### 2.4.1.3. Abscission

In a series of papers Witztum and co-workers analysed the separation of daughter fronds from the mother in S. punctata (WITZTUM 1974b, WITZTUM and KEREN 1978a,b, WITZTUM et al. 1978). Two abscission zones are present in fronds of this species, one at the proximal end and one at the distal end of the stipe. In both cases the abscission layer consists of small dividing cells. Subsequent cells of mother and daughter frond, respectively, contain red-brown phlobaphene-like compounds after dying and seem to form a protective layer (for two abscission zones in S. polyrrhiza see NEWTON et al. 1978).

UV irradiation is one of the agents which activates the processes leading to abscission (preferably in the proximal abscission zone). UV lowers the IAA level drastically and in this way seems to remove the block against ethylene induction of abscission. Exogenous supply of IAA abolishes the effect of UV irradiation. The addition of 0.015-0.1 M sucrose (ca. 0.5-3.5%) to the medium has a similar effect as exogenous IAA. In this case the effect of UV on abscission is outplayed by a serious enhancement of endogenous IAA production.

A high concentration of sucrose (0.4 M, ca. 13.5%), which causes an osmotic stress situation, induces abscission in normal frond colonies and

increases the effectivity of UV. The effect of 0.4 M sucrose may come about by a change in the levels of ABA, ethylene, and IAA. The ABA content of S. punctata rises six-fold within 24 h, when plants are floated on 0.4 M sucrose (in comparison to distilled water). Application of 10 ppm ABA, on the other hand, induces abscission to a great extent. Ethylene release is greatly promoted during the first 24 h of 0.4 M sucrose treatment, but falls off to a value below the control during the second day. The ethylene outburst may be effective in frond separation, if one assumes that the osmotic stress simultaneously reduces the IAA content (compare the results of HARTUNG and WITT 1968, for Anastatica). The results of Witztum and co-workers were confirmed and extended by other authors. In S. punctata naphthaleneacetic acid, EDDHA, and salicylic acid increase the size of the colonies of fronds, i.e., inhibit frond separation (SCHARFETTER et al. 1978). In L. minor, which develops only one abscission zone, exogenous ABA accelerates abscission even at 0.2 ppm. Cellulase but not pectinase activity is increased by the ABA treatment. Benzyladenine inhibits abscission in this way enhancing the size of the colonies (OSTROW-SCHWEBEL 1973, 1979). In S. polyrrhiza separation of sub-colonies is facilitated by 1 ppm ethylene. Under 10 ppm ethylene, which simultaneously inhibits growth, only single fronds and groups with two fronds occur (control colonies consist of 2-4 fronds) (NEGBI et al. 1972). WCISLO (1963) mentioned that bacterial contaminations accelerate frond separation in L. trisulca. Possibly such infections give rise to an enhanced ethylene production. LOOS (1962) stated that gibberellin A<sub>3</sub> promotes frond separation more than frond multiplication in L. minor. Most plants consist of a single frond after cultivation with 50 mg/l gibberellin A<sub>3</sub>.

#### 2.4.1.4. Senescence and rejuvenation

Senescence is a complex of physiological processes which limits morphogenetic potentialities and life-span of a certain organ. During senescence protein degradation overcomes protein synthesis and - in interrelation with this - the hormonal state of the organ changes. The extent of senescence depends on environmental stress factors as well as on the presence of other organs which compete for nutritional and hormonal factors. On the other hand, the senescing organ may influence other parts of the organism. Especially the morphogenetic potentialities of meri-

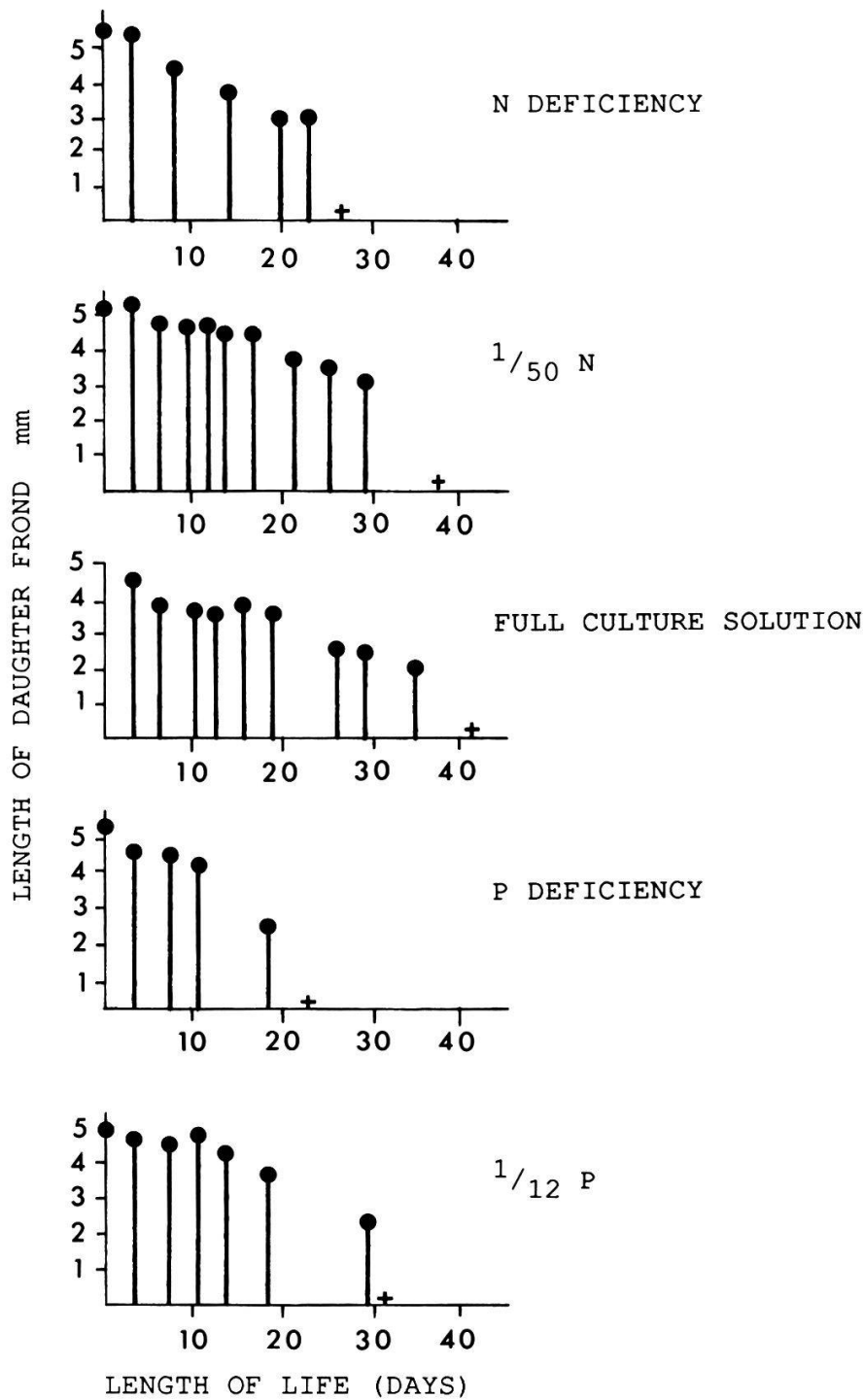


Fig. 2.30. The length of life of *Lemna minor* fronds (see +), the number of daughter fronds produced during the life-span of one mother frond (see the number of bars), and the length of daughter fronds (●) as influenced by the N- and P-content of nutrient medium (BOESZOERMENYI and BOESZOERMENYI 1957).

stems can be changed (vegetative vs. generative development) or restricted (number and size of organs produced). In principle, organ senescence itself as well as the senescence-induced meristem state are reversible, thus, a true rejuvenation occurs.

In Lemna, the life-span of fronds is reduced by nitrogen- or phosphorus-deficiency (fig. 2.30 BOESZOERMENYI and BOESZOERMENYI 1957), high temperature ( $30^{\circ}\text{C}$ ) (WANGERMANN and ASHBY 1951), UV-irradiation (2 min or more) (DIJKMAN et al. 1964), or ABA (KANG and CLELAND 1985). In the cited cases production of daughter fronds is diminished simultaneously or - at least - not enhanced. Therefore the life-shortening agents may be effective directly as stress factors. If, on the other hand, nitrogen deficiency prolongs the life-span (WANGERMANN and LACEY 1955), an indirect effect mediated by an inhibition of the development of daughter fronds may be responsible. The same can be said of short UV-irradiation (60-90 sec) which prolongs the length of life of the fronds, but diminishes their multiplication (DIJKMAN et al. 1964). Long day is another factor, which can increase or reduce life-span depending on the species or on the culture conditions (OSTROW and DIJKMAN 1969, WANGERMANN 1952). The life-shortening effect of short day in L. aequinoctialis (SCHUSTER 1968) is caused without doubt by the fact that flowers are produced under these conditions. If flowering is prevented by a short night break, life-span exceeds that under long day. The connection between flowering and frond senescence can be inferred also from results with L. perpusilla Pl46 (HUEGEL et al. 1979). Treatment of short-day cultivated plants with end-of-day far red inhibits flowering and frond senescence (see number of sister fronds produced by one mother) only to a certain degree. A night break with red light, on the other hand, inhibits flowering completely and retards frond senescence more distinctly.

The effect of stress factors on protein turnover rates was investigated in L. minor by TREWAVAS (1972b). If the plants are placed on pure water, there is a reduction in the rate of synthesis of protein and an increase (3- to 6-fold) in the rate of degradation. Similar results were obtained when only one of the following minerals were omitted from the medium: nitrate, phosphate, sulfate, magnesium, or calcium. The cited stress effects could be mediated by an increase of the ABA and decrease of cytokinin content within the plant. Supply of  $5\text{ }\mu\text{M}$  ABA to plants grown in sucrose-mineral medium has the same effects on protein turnover as the

stress factors themselves. Benzyladenine has antagonistic effects to ABA, but - surprisingly - alters in plants grown on sucrose-mineral salts only the rate of synthesis and in plants grown in pure water only the rate of degradation. An increase of endogenous ABA level and a decrease of endogenous cytokinin level 24-48 hrs after withdrawal of nitrogen from the medium has been found in L. aequinoctialis (MERTEN, HARTUNG, GRUNTZEL, LOEPPERT and KANDELER, unpublished results).

Turnover of ribosomal RNA behaves like that of proteins after transfer of plants to water or to a medium without nitrate, phosphate, calcium, or magnesium. The rate of rRNA degradation is increased and the rate of rRNA synthesis is decreased (TREWAVAS 1970). ABA ( $5 \times 10^{-6}$  M) markedly reduces the rate of rRNA synthesis but leaves the degradative rate unaltered. In plants grown on water, benzyladenine increases both the synthetic and degradative rates of nucleic acid metabolism (TREWAVAS 1970). The inhibiting effect of ABA on RNA synthesis has been found also in roots of L. minor (NEWTON 1974a).

The investigations of Trewavas were continued by Davies and co-workers in a series of papers. In spite of the increased protein degradation under nitrogen starvation the concentration of free amino acids decreases in L. minor (HUMPHREY et al. 1977). Whereas protein half life is reduced from 143 to 73 hrs under these stress conditions, the total amount of amino acids recycling seems to be increased (DAVIES and HUMPHREY 1978). Four days after set-up of nitrogen deficiency the lowering of soluble protein content of fronds ceases (COOKE et al. 1979b). The same holds true for mineral deficiency (water culture) or osmotic stress (0.5M mannitol). The rates for protein synthesis and degradation tend to return to values comparable with unstressed plants. Obviously, an adaptation to the special stress condition takes place, which includes a replacement of several proteins by others. During nitrogen starvation activities of glutamate dehydrogenase and malate dehydrogenase sustain a reduction which is stronger than under osmotic stress (mannitol). Activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and PEP carboxylase, however, are more reduced during mannitol treatment than during nitrogen deficiency (COOKE et al. 1979b). In the case of aldolase the decrease in activity after withdrawal of nitrogen is shown to be due, at least partly, to formation of a specific inhibitor, which appears to be a protein with a molecular weight of c 15.000 (DAVIES 1978).

Another factor which alters protein turnover is deuterium oxide ( $^2\text{H}_2\text{O}$ ). When plants are transferred to medium containing 50%  $^2\text{H}_2\text{O}$ , protein synthesis is inhibited for 4 h and then proceeds at a slower rate than in non-deuterated medium. Protein degradation is increased 9-10-fold initially and after 6 days in  $^2\text{H}_2\text{O}$  is still 2-3 times higher than normal. All tested enzyme activities are reduced to a similar extent by the  $^2\text{H}_2\text{O}$  treatment (COOKE et al. 1979a).

The effects of  $^2\text{H}_2\text{O}$ , nitrogen starvation, and 0.5M mannitol do not come about by an activity increase of proteinases but rather by an alteration of tonoplast permeability (COOKE et al. 1980a, b). Utilizing the different  $^3\text{H}/\text{H}$  exchange on amino acids in the cytosol and vacuole (s. transaminases in the cytosol only) the authors pointed out that the stress conditions mentioned above increase the permeability of the tonoplast for amino acids. ABA has the same effect on amino acid "leakage" from vacuole to cytosol as all the stress factors. Benzyladenine, on the other hand, reduces the effects of nitrogen deficiency and mannitol on amino acid permeation through the tonoplast. The results with amino acids have led to the hypothesis that primarily the tonoplast passage of certain substances - protons for an activation of proteinases in the cytosol or proteinases themselves - is increased, when stress factors - possibly mediated by hormonal changes - alter protein metabolism.

The effect of ABA on plasma membrane permeability seems not to be restricted to the tonoplast. ANDRES and SMITH (1976) ascertained a substantial increase of amino nitrogen in the medium within 3 h of transfer of S. polyrrhiza to  $10^{-6}$  M ABA. In L. aequinoctialis three days after ABA supply, the amino nitrogen in the medium amounts to 61% of the total in comparison to 21.9% in the control. In L. minor the values are 36.2 and 8.5%, respectively.

The refined kinetics for cytokinin action on nucleic acid and protein metabolism have been investigated on L. minor. The RNA content shows a transitory increase with a peak after 30 minutes, when plants are treated with  $10^{-5}$  M kinetin (ERISMANN and FANKHAUSER 1967, FANKHAUSER and ERISMANN 1969b). Within the same time the protein content increases too, and remains at the higher level for a long time (3 days investigated). Similar results have been found with darkened non-growing plants of S. punctata (MCCOMBS and RALPH 1972a). Within 1 hour DNA-, RNA-, and protein synthesis (measured by [ $^3\text{H}$ ] thymidine-, [ $^3\text{H}$ ] uridine-, and  $^{35}\text{SO}_4$ -incorporation, respectively) are enhanced by about  $10^{-6}$  M kinetin in this

plant material. The rate constant for RNA synthesis is doubled by kinetin and RNA degradation is diminished by 60%. Uptake of  $^{35}\text{SO}_4$ , uridine, and [ $^{14}\text{C}$ ] glucose, however, are not affected by kinetin in darkened S. punctata.

Not only cytokinins but also polyamines could be agents for inhibition of senescence and promotion of rejuvenation in Lemna. FLORES and GALSTON (1982) have shown great amounts of agmatine and spermidine to be present in vegetative and flowering L. gibba G3. GIOVANELLI et al. (1981) found spermidine in L. aequinoctialis as one of the main by-products of the methionine - 5'-methylthioadenosine cycle. Ethylene, another possible by-product of this cycle, could not be detected in these experiments.

The effect of sugars on senescence may be rather complex. Lemna minor cultivated without sucrose for 4 weeks shows a reduced rate of rRNA synthesis and an increased rate of rRNA degradation in comparison to plants grown on sucrose-containing medium (TREWAVAS 1970). This result may be considered in connection with the fact that sucrose application causes a drastic increase of the endogenous IAA level in S. punctata (WITZTUM et al. 1978). But the growth promotion rendered possible by supply of sugars in consequence can lead to an exhaustion of endogenous and exogenous nitrogen reserves and in this way to an acceleration of senescence. Yellowing - as an indication of senescence - in fronds of S. punctata is retarded by 1% glucose only when the nutrient medium is renewed frequently. When, however, the glucose containing medium is not changed, a great acceleration of thylakoid and chlorophyll degradation proceeds (GROB and EICHENBERGER 1973). Transfer of yellowing fronds to fresh (glucose-free or glucose-containing) medium induces a regeneration of thylakoids and regreening.

Sugars which enhance senescence directly are galactose (DEKOCK et al. 1979) and sorbose (STROTHER 1981). Mature and young Lemna fronds become progressively chlorotic and growth is arrested in the presence of one of these sugars. In the case of galactose the changes in mineral content of fronds has been followed up. The potassium content is lowered and levels of sodium, calcium, magnesium, and iron are raised. Largely the same changes in mineral composition of L. gibba have been found after application of 1 ppm ABA (DEKOCK et al. 1978). Benzyladenine (1 ppm) can partially counteract the ABA effect. LIEBERT (1977), investigating the ABA dependence of mineral content in the same species (L. gibba) but using another nutrient medium, has stated a drastic decrease of  $\text{K}^+$  level and



only a slight decrease of  $\text{Ca}^{++}$  level after 13 days of plant cultivation with 1 ppm ABA. A decrease of  $\text{K}^{+}$  and increase of  $\text{Ca}^{++}$  content has been ascertained in plants grown for 26 days in an unchanged, pure inorganic medium (LIEBERT 1980b).

Stress factors such as (high) temperature, intense photosynthetically active radiation, UV-B radiation, and high osmoticum, induce the production of several "stress proteins" in L. minor (BIGGS and KOSSUTH 1985). The cell wall protein extensin is one of the stress-induced proteins. High concentrations of  $\text{CuSO}_4$  (1 mM) enhance breakdown, among others, of the large and small subunits of ribulose diphosphate carboxylase, 26 kDa-light harvesting chlorophyll a/b apoprotein, and the  $\text{Q}_B$  thylakoid protein (MATTOO and CONLON 1985). These effects appear to be independent of ethylene production that is markedly stimulated in the presence of 1 mM  $\text{CuSO}_4$  (see chapter 2.5.7.2).

The effect of frond senescence on daughter frond development was first investigated by ASHBY and WANGERMANN (1949). Daughter fronds are produced within the whole life-span of a given mother, but fronds of such a series successively produced from one mother frond behave differently. The later during the lifetime of the mother frond the daughter frond is developed, the smaller the adult frond remains (compare fig. 2.30) and the shorter is its own life span. This gradual reduction in the size of successively produced daughter fronds holds true in L. minor (BOESZOERMENYI and BOESZOERMENYI 1957, BOSS et al. 1963a, WANGERMANN 1952, WANGERMANN and ASHBY 1951, WANGERMANN and LACEY 1953), in long-day cultivated L. aequinoctialis 6746 (SCHUSTER 1968), and in L. aequinoctialis 6746, held at 30°C and 12 h light per day (CLAUS 1972). Another mode of daughter frond development is seen in L. aequinoctialis 6746, when grown under short day, or short day with night break (SCHUSTER 1968), and in L. aequinoctialis "clone 1", held at 25°C and 12 h light per day (BOSS et al. 1963a). In this case, some or several daughters - first produced by the mother - reach about the same size of frond. After that, further daughters with smaller fronds are produced by the mother. This "one-step reduction" of daughter frond development may be an interesting hint to a special type of frond senescence (fig. 2.31).

The restriction of morphogenetic potential induced by the senescent frond in its daughter fronds is reversed during the development of granddaughter and great-granddaughter fronds. A rejuvenation takes place especially in the firstborn fronds. These fronds become larger than

those of their mother. In this way small lateborn fronds produce a progeny of maximum size within a line of about four first-daughter generations (WANGERMAN and ASHBY 1951, compare vol. 1, fig. 2.11).

A special case of rejuvenation combined with a change in the morphogenetic program is the proliferation of disordered cell material. When intact mature Lemna fronds are grown on agar medium with a certain combination of growth substances, outgrowth of calli takes place. Whereas in L. gibba G3 10 mg/l 2,4-D combined with 1 mg/l N<sup>6</sup>-s( $\Delta^2$ -isopentenyl)-adenine (2iP) induces callus formation (CHANG and CHIU 1976, 1978), only 0.1 mg/l 2,4 D together with 10 mg/l 2iP is effective in L. aequinoctialis (CHANG and HSING 1978). In L. gibba large friable undifferentiated

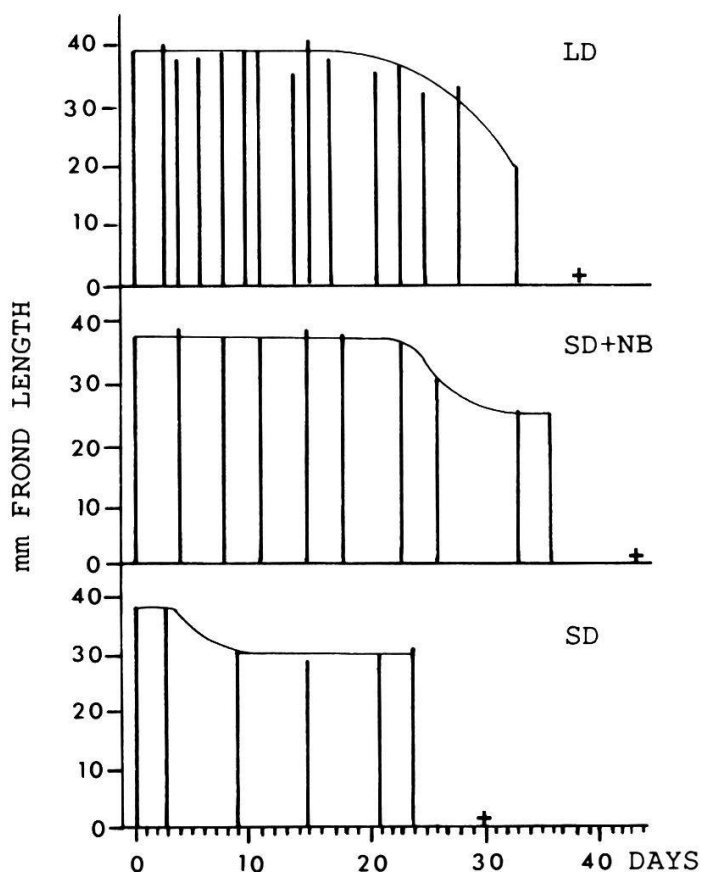


Fig. 2.31. Senescence and frond development in Lemna aequinoctialis 6746 (after SCHUSTER 1968). Frond lengths of a mother frond (first bar) and its daughter fronds (following bars) are plotted at the time of frond appearance. The cultures of single mother fronds were held under long day (LD), short day with night break (SD+NB), or short day (SD). The end of the life-span of the mother frond is marked by +.

calli were obtained by the end of 8 weeks. The callus could be subcultured and proliferated in fresh medium under illumination or in the dark. In L. aequinoctialis 3 months were needed for callus induction. Up to now a complete regeneration of fronds from callus tissue has been achieved only in L. gibba. A basal medium supplemented with indoleacetic acid (4 mg/l) and kinetin (1 mg/l) was able to induce frond regeneration. A regenerated strain, named L. gibba strain Sinica No. 1, exhibited a slower growth rate but no distinct morphological features.

#### 2.4.1.5. Chloroplast development

Ultrastructure of chloroplasts at various developmental stages has been investigated using electron microscope techniques. Meristematic cells of S. polyrrhiza and L. minor contain proplastids with only a few thylakoids (RAO 1968, FRICK and JONES 1976, LE PABIC 1972, WROBLEWSKI 1973). By contrast, the primordial cells of W. arrhiza show no proplastids, but well-developed chloroplasts containing grana (ANDERSON et al. 1973). In mature frond tissues of S. polyrrhiza grana stacking is more marked in the palisade parenchyma than in the spongy parenchyma (LE PABIC 1972). Chloroplasts of root cap, epidermis, cortex, and endodermis in L. minor exhibit only few grana thylakoids (WROBLEWSKI 1973). When frond colonies of S. punctata are darkened for a longer time, the chloroplast ultrastructure in mature fronds remains unchanged, but in young green fronds a retrogressive development occurs. Chloroplasts are transformed to etioplasts with a prolamellar body. Both crystalline and vesicular forms of prolamellar body can be observed (PORATH 1979). The reverse process has been investigated in L. minor. As in other plants, a radiation of thylakoids from the prolamellar body can be seen at an early stage of de-etiolation. Plastid division occurs only in primordial cells of green and etiolated plants (FRICK and JONES 1976).

In the mutant 1073 of L. aequinoctialis No. 6746, which is incapable of photosynthesis and ammonium ion uptake, the grana of chloroplasts contain many more thylakoid membranes than the wild-type chloroplasts. In addition, tubular clusters are present in mutant chloroplasts, which differ from prolamellar bodies found in etioplasts (MONSELISE et al. 1984). Supply of L-cystine normalizes the ultrastructural features of the mutant, but not its physiological behaviour (MONSELISE et al. 1986). Greening of etiolated plants under continuous white or red light pro-

ceeds after a lag phase of 20-24 h (S. polyrrhiza and S. punctata) (PO-RATH and BEN-SHAUL 1973) or 10-12 h (L. minor) (FRICK 1975). In all cases the lag phase can be eliminated by a pre-illumination with a short red light given about the length of lag period before the onset of continuous light. Phytochrome seems to mediate the effect of short red light, because far red given simultaneously with or after red reduces the red light effect. When L. minor is grown under light of different spectral qualities, grana stacking is highest under blue light. The appearance of appressed thylakoid membranes also increases after blue irradiation (MAEENPAEAE and ARO 1986).

Mature Lemna chloroplasts (as well as whole plants) possess an outstanding resistance against extremely high centrifugal forces ( $350.000 \times g$  for 15-60 min). Thylakoids are not so greatly stretched as in spinach leaf chloroplasts. This may be due to the presence of a denser stroma in the duckweed chloroplast (BEAMS et al. 1979).

Several changes of chloroplast ultrastructure take place in L. minor when plants are cultivated in a medium containing atrazine (0.25 ppm), a specific inhibitor of Hill reaction (BEAUMONT et al. 1980). The number of grana per chloroplast and the length of grana lamellae increases, whereas the number of lamellae per granum decreases. Intralamellar vesicles disappear and outgrowths are formed at the ends of the chloroplast. The stroma is reduced and accumulates osmiophilic globules.

Many agents - metabolic and growth inhibitors, metabolic intermediates, as well as high temperature - can cause a severe inhibition of chloroplast development, visible as production of bleached daughter fronds. A first group of bleaching substances are inhibitors of protein synthesis (chloramphenicol, cycloheximide, tetracycline, streptomycin, and kanomycin) and inhibitors of nucleic acid synthesis (fluorodeoxyuridine, nalidixic acid, and aminotriazole). Azaguanine and azauracil are effective only when given together with certain ribonucleosides. The effects of most of the cited substances are reversible after removal. This means that mature cells of bleached fronds regreen in the absence of the inhibitor. Only effects of fluorodeoxyuridine and aminotriazole are irreversible. Bleaching of growing fronds by aminotriazole (59  $\mu\text{M}$ ) and kanamycin (5  $\mu\text{M}$ ) is specific in a sense that frond growth is not inhibited by these agents (FRICK 1972, FRICK and JONES 1975).

Some intermediates of nucleic acid metabolism are another group of bleaching substances. Cytidine deoxyriboside (Cdr), uridine deoxyribo-

side, and guanosine deoxyriboside, all induce quantitative bleaching in L. minor, when grown under continuous light in a sucrose-containing medium (FRICK 1978). Cdr-induced bleaching is not accompanied by a reduction in frond multiplication rate and is fully prevented by an additional supply of thymidine, cytidine, or uridine. These facts are interpretable by the assumption that Cdr interferes with biosynthesis of pyrimidine nucleotides and acts in a compartment which provides DNA precursors for plastids only. Further analysis of Cdr effect has shown that plastid replication is affected in the first place (PIZZOLATO and FRICK 1979). The number of plastids in cells of comparable size is reduced during bleaching by more than 50%, their volume by 10-30%.

A third group of substances capable of inhibiting chloroplast development are certain nitrophenols (PRICE and WAIN 1976). White fronds are produced by L. minor, when 4-hydroxy-3-nitrobenzoic acid, 4-hydroxy-3-nitrobenzaldehyde, or 4-hydroxy-3-nitrobenzyl alcohol is added to the medium.  $10^{-5}$  M of these substances are effective to 69, 72, and 66%, respectively.

Growth-inhibiting concentrations of benzyladenine ( $10^{-6}$ - $10^{-4}$  M) are a further possibility to inhibit elaboration of chloroplast ultrastructure primarily in young frond primordia. Bleached fronds of S. polyrrhiza grown in the presence of  $10^{-6}$  M benzyladenine contain plastids with only a few thylakoids (LE PABIC 1976b). Mature fronds, however, can also show a partial loss of pigments and thylakoids after treatment with high benzyladenine concentrations. Accumulation of starch is greatly enhanced under these conditions. The content of phospholipids increases and the surfaces of the thylakoid membranes are more appressed (LE PABIC 1976b, 1980).

S. punctata, grown in light at 37°C for a period of 3-6 days, loses the ability to synthesize chlorophyll, to propagate, and to evolve  $O_2$ . Grana thylakoids are destroyed and the number of droplets in the chloroplast increased. This "heat bleaching" appears first in younger fronds. Plants, kept in the dark at 37°C for at least 5 days, are not affected by the thermal treatment (PORATH and BEN-SHAUL 1971). The bleaching by exposure to raised temperatures is reversible in Spirodela (SCHER and AARONSON 1958).

Not only proplastids and developing chloroplasts are sensitive to inhibiting factors. Ultrastructure, protein, and pigment content of mature chloroplasts are the primary sites, which are attacked by senescence-

promoting agents (see chapters 2.4.1.4, 2.5.7.3 and 2.5.8.3). The degradation of chloroplast ultrastructure under the influence of nitrogen or mineral deficiency was demonstrated by GROB and RUFENER (1969) and HUBALD and AUGSTEN (1977b). Destruction of the chloroplast becomes irreversible latest when the envelope bursts on account of excessive starch deposition. Such a process has been found in L. minor after applying  $10^{-4}$  M kinetin (ERISMANN and WEGNER 1967) and in L. gibba senesced artificially by 0.1% galactose (DEKOCK et al. 1979). Another monosaccharide which causes bleaching in mature and young fronds of L. minor is sorbose (STROTHER 1981). Toxicity of galactose is counteracted by glucose added simultaneously and the effect of sorbose can be diminished by fructose.

#### 2.4.1.6. Movement of chloroplasts

After reviewing the developmental processes of chloroplasts the movements of these organelles within the cell may also be reported. Under dark conditions, chloroplasts in the mesophyll layer of L. trisulca are distributed more or less at random (apostrophe position). In unilateral, low-intensity light, the chloroplasts migrate to the periclinal cell walls lying approximately perpendicular to the direction of illumination (epistrophe position). Under high-intensity light, the chloroplasts move to the anticlinal cell walls (parastrophe position). Other environmental factors as well as endogenous rhythms have no definite influence on the movements of chloroplasts in L. trisulca (ZURZYCKA and ZURZYCKI 1953). In experiments with white light the transition to epistrophe was accomplished with a light intensity as low as 9 lux. In the range from about 800 to 12000 lux epistrophe is replaced gradually by parastrophe. The velocity of movement increases with the "jump" of light intensity, i.e. the difference between the initial and the final light intensity. The migration from parastrophe (at 13300 lux) to epistrophe (at 40 lux), for example, is completed within about 15 min. The movement from parastrophe (5500-13300 lux) to apostrophe proceeds through an epistrophe transition stage occurring 30 min after the beginning of darkness. Apostrophe then is reached after a further 30-60 min (ZURZYCKA and ZURZYCKI 1953). The path of single chloroplasts from epistrophe to parastrophe has been followed by a kinematographic method. The path is very complicated; direction as well as rapidity of the movement change constantly (ZURZYCKI and ZURZYCKA 1953). During the parastrophe - epistrophe reaction, on the



other hand, the paths are rather straight, and the velocity of movement is fairly constant, ranging from 0.6 to 1.0  $\mu/\text{min}$  (ZURZYCKA and ZURZYCKI 1957). Under dark conditions, transient rearrangements of chloroplasts can be induced by short light pulses. An 0.8 s pulse of strong blue light ( $30 \text{ W m}^{-2}$ ) causes a transitory migration to the weak-light position (epistrophe). With longer pulse duration (8 s), a biphasic response takes place: The initial movement to a partial strong-light position is followed by a wave of translocation to a weak-light arrangement (GABRYS et al. 1981).

The blue component of light is responsible for induction of the oriented chloroplast movements in L. trisulca. The action spectra for weak-light and strong-light responses are identical and have a major peak at 450 nm and two lower ones at 485 and 380 nm (ZURZYCKI 1962). Also in S. polyrrhiza and many other plants only blue light is effective (INOUE and SHIBATA 1973). Consequently, the photosynthetic mutant, strain 1073, of L. aequinoctialis which has a block in the electron transport chain between plastoquinone and cytochrome f is capable of light-induced chloroplast movements (WITZTUM et al. 1979). A flavin has been proposed to be the main photoreceptor for chloroplast orientation (HAUPT 1982). When the fluence rate dependence of chloroplast translocations is tested with blue light, some rearrangement occurs even under an intensity near  $0.15 \text{ mW m}^{-2}$ . At temperatures of 20 to  $25^{\circ}\text{C}$  maximum weak-light arrangement is achieved approximately  $150 \text{ mW m}^{-2}$  and complete strong-light position is obtained in the range  $3\text{--}10 \text{ W m}^{-2}$  (ZURZYCKI et al. 1983). At a temperature of  $5^{\circ}\text{C}$ , however, the optimum intensities of blue light for the weak-light and strong-light positions are shifted to lower values (HAUPT and WEISENSEEL 1966, WEISENSEEL 1968a).

In healthy, growing material of L. trisulca the light-controlled displacement of chloroplasts depends not only on the intensity and wavelength of radiation but also on the direction of polarization. Under high-intensity light, chloroplasts avoid the side walls situated parallel or nearly parallel to the E-vector of light (fig. 2.32). From this reaction pattern it can be concluded that the responsible photoreceptor molecules are oriented, their vector of absorption being parallel to the cell surface (ZURZYCKI 1969, ZURZYCKI and LELATKO 1969). In some L. trisulca material (especially from old cultures in the autumn) the action dichroism cannot always be observed. The appearance of undulations in the cytoplasmic membrane has been considered to be the cause for this



behaviour. EDTA, ATP, and phosphate buffer of pH 4.7 are agents which can restore the action dichroism in otherwise not reacting material (HAUPT and WEISENSEEL 1967, ZURZYCKI 1969).

The speed of chloroplast displacements can be influenced by strong red light. The action spectrum of this photokinetic activity reveals a peak at 680 nm. Thus, photosynthetic pigments seem to mediate this light effect (ZURZYCKI 1962, 1964). The source of energy for all types of chloroplast rearrangements is ATP as revealed by studies with metabolic inhibitors and by ATP-feeding experiments (ZURZYCKI 1965, ZURZYCKI and ZURZYCKA 1955). Respiration as well as photosynthesis can contribute to the ATP delivery. Thus, the effect of strong red light on the photokinetic activity of chloroplasts obviously comes about by photosynthetic ATP formation. The effects of temperature on the velocity of chloroplast movements (WEISENSEEL 1968b, ZURZYCKA and ZURZYCKI 1950) may also be explained by the dependence on temperature of energy generation.

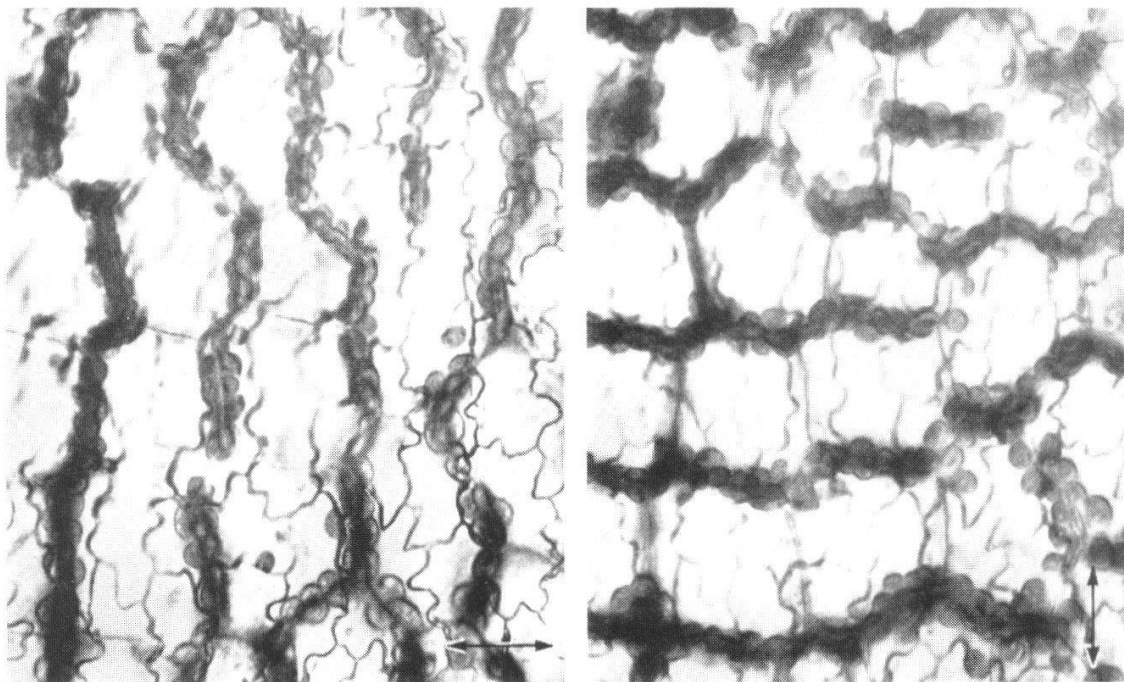


Fig. 2.32. Arrangement of chloroplasts in the same cells induced by polarized light. Arrows indicate the plane of the E-vector of light. Cells with chloroplasts in low light intensity<sub>2</sub> position were irradiated 1 hour with an intensity of  $38 \text{ Wm}^{-2}$  polarized blue light (ZURZYCKI 1969).

The principal mechanism of chloroplast displacement in Lemna seems to be the passive transport with the creeping or streaming cytoplasm (ZURZYCKA and ZURZYCKI 1957, ZURZYCKI and ZURZYCKA 1953). The light regime decides, in which particular regions of the ectoplasm chloroplasts are anchored. An indicator for the ability of ectoplasm to attach chloroplasts should be the low transferability of chloroplasts by centrifugation. In fact, weak light and to some degree also strong light diminish the passive displacement of chloroplasts by centrifugation (ZURZYCKI 1960, 1962). On the other hand, the transition from epistrophe to parastrophe or apostrophe needs a local weakening of cytoplasm stickiness. Ions which diminish the viscosity of cytoplasm,  $K^+$  and  $Li^+$ , accelerate especially the transitions from epistrophe to parastrophe and apostrophe, whereas  $Mg^{2+}$  and  $Ca^{2+}$  retard the same reactions (ZURZYCKA and ZURZYCKI 1951). As an early link in the transduction chain from light signal to response ZURZYCKI (1972) envisaged glyoxylate to be an "attractant". This compound is assumed to be formed from glycine by the membrane-bound deaminating enzyme glycine oxidase, which is activated by blue light via FMN. In correspondence with the hypothesis, an enhancement of  $O_2$  uptake by very low blue light intensities has been demonstrated in DCMU-treated plants (ZURZYCKI 1970, 1972; see chapter 2.5.1.2). Chloroplasts should accumulate at those regions in the cytoplasm which are enriched in glyoxylate. In Funaria leaves, a glyoxylate solution applied unilaterally causes a preference of chloroplasts to the cell wall facing the test solution (GODZIEMBA-CZYŻ 1973).

The influence of chloroplast displacements on the optical properties of leaves has been elaborated in detail (ZURZYCKI 1961). The work of ZURZYCKI (1953, 1955a, 1955c) with regard to the influence of chloroplast rearrangements on photosynthesis has been reported in chapter 2.5.1.1.1.

#### 2.4.2. Dormancy

True dormancy can be characterized as an developmental intermission, in which growth and other developmental processes are arrested by certain internal conditions. In Lemnaceae dormancy lasts for some time in turions, turion-like fronds, and seeds. Turions are small rootless fronds, which sink to the bottom of the water because of reduced or absent air spaces and a high starch content. They have been found in S. polyrrhiza (HEGELMAIER 1868, VAN HOREN 1869), L. turionifera (LANDOLT 1957, 1975), N-2 type strains of L. aequinoctialis (BEPPU and TAKIMOTO 1981c), W. brasiliensis (LANDOLT 1957), W. arrhiza (HEGELMAIER 1868), and W. columbiana (LANDOLT 1957). Also W. borealis, W. australiana, W. angusta, and W. globosa are able to form turions (LANDOLT 1986). Small, thick, fleshy, non-growing fronds, which contain much starch but float, are functionally comparable with turions and therefore can be called turion-like or resting fronds. They occur in S. punctata (HARRISON 1964, SCHARFETTER and KANDELER, unpublished results), L. perpusilla (KANDELER and HUEGEL 1974a), L. gibba (VAN HOREN 1869), L. minor (SCHULZ 1962), and W. gladiata (PIETERSE et al. 1970d).

Recently, SMART and TREWAVAS (1983b) compared ultrastructural features of turions and vegetative fronds in S. polyrrhiza. Turions show smaller air spaces, smaller cell vacuoles, thicker cell walls, and accumulation of numerous starch grains. No distinct quantitative differences were found for ground cytoplasm, endoplasmic reticulum, mitochondria, chloroplasts (envelope, stroma, and thylakoids) and lipid droplets.

##### 2.4.2.1. Induction of turions and turion-like fronds

Most factors inducing development of turions or turion-like fronds in Lemnaceae are agents which simultaneously promote senescence in the mature parts of the plant. This holds true for several forms of mineral deficiency or imbalance, as well as for the effects of short day, red light, and abscisic acid.

Turion induction in S. polyrrhiza has been found after exhaustion of minerals (CZOPEK 1963a, HENSSEN 1954), withdrawal or lowering of nitrogen (JACOBS 1947, MALEK and COSSINS 1983a, MALEK and ODA 1979, NEWTON and DUFFEY 1975, NEWTON et al. 1978, PERRY 1968), sulfate deficiency (MALEK and COSSINS 1983a), and phosphate deficiency (AUGSTEN and JUNGNICHEL

1983, JUNGnickel 1978, 1986a). Different clones, however, behave differently and, in addition, an endogenous seasonal factor can influence the extent of turion production. Nitrogen deficiency was effective in only two of four clones investigated by PERRY (1968). In clone P115 (ETH No. 7505), used by MERTEN and KANDELER (unpublished results), turion induction by nitrogen deficiency proceeded from January to March, but not during May. HENSSEN (1954) obtained turions by mineral deficiency during the whole year, but turion formation in full nutrient medium occurred in the "Gr" clone during the winter only. In a similar way turion formation in L. turionifera can be observed after exhaustion of minerals, especially phosphate deficiency. Under short day conditions, turions of some clones are formed under otherwise optimal conditions (LANDOLT, not publ.). BEYER (pers. comm.) observed turion formation during winter in a clone from Southern California even under long day and otherwise optimal conditions. It is not investigated whether turion formation is induced by an endogenous rhythmic factor or by the air pollution which is greater during the winter.

Short day hastens senescence in mature fronds and induces development of turion-like fronds in L. perpusilla P146 (ETH No. 7507) (HUEGEL et al. 1979). The short-day effect is truly photoperiodic, because a short night break with red light cancels both responses. Another light program, which abolishes the short-day effects on senescence and production of turion-like fronds in L. perpusilla, is short irradiation with far red at the end of the daily light period. The connection between senescence and formation of dormant storage organs seems to be very close in L. perpusilla, as can be seen by the fact that first-daughter fronds are the last which reach the dormant state (HUEGEL et al. 1979).

Far red at the end of short day inhibits turion development also in S. polyrrhiza (MERTEN and KANDELER, unpublished results). Furthermore, when the infrared content (beyond 750 nm) of irradiation is relatively high during long day, production of turions in S. polyrrhiza is reduced (DEUTCH and RASMUSSEN 1974). Far red as well as infrared may be effective by lowering the level of active phytochrome. Continuous blue light diminishes turion formation in S. polyrrhiza in comparison to red light (MALEK and ODA 1979). The light effect may act through an influence on the degree of senescence, because blue light in comparison to red shifts the ratio of protein to carbohydrate synthesis to the former (VOSKRESENSKAYA 1979). In accordance with this assumption are the facts that W.

arrhiza cultivated under continuous blue light contains distinctly more cytokinins than under red light (EICHORN and AUGSTEN 1980), and that turion formation in S. polyrrhiza (induced by nitrate or sulfate deficiency) is prevented by addition of isopentenyl adenine or benzyladenine (MALEK and COSSINS 1983a).

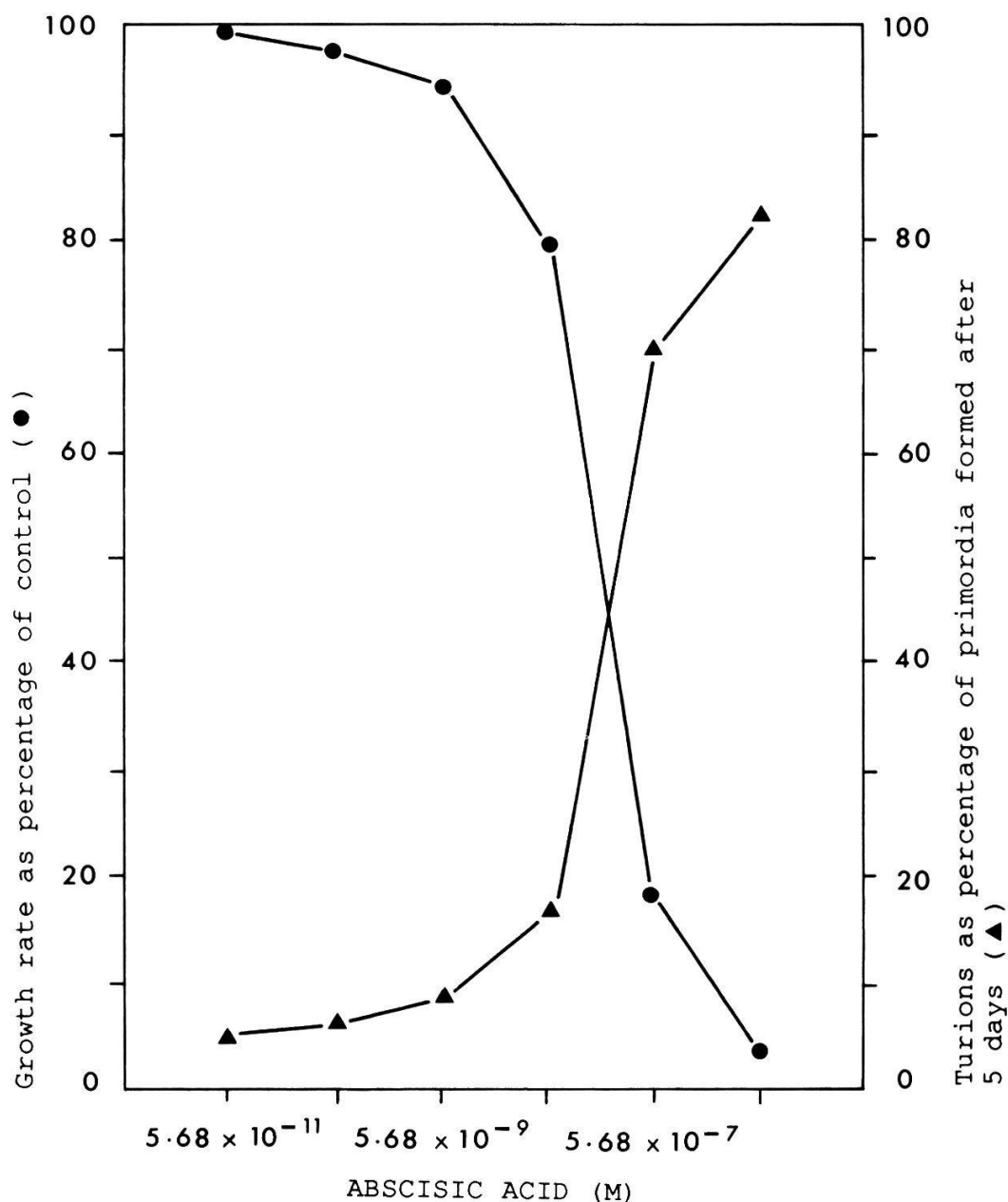


Fig. 2.33. Effect of abscisic acid on growth and turion formation (STEWART 1969). (●) growth rate; (▲) turion ratio. (All points are the mean of three determinations.)

Absciscic acid (ABA) as a senescence-promoting hormone induces turions in S. polyrrhiza (fig. 2.33) (PERRY and BYRNE 1969, SAKS et al. 1980, SMART and TREWAVAS 1983a, STEWART 1969) and in L. turionifera (VAN OVERBEEK et al. 1968), and turion-like fronds in S. punctata and L. gibba (SCHARFETER and KANDELER, unpublished results). No effect of ABA has been found in one of four clones of S. polyrrhiza (PERRY and BYRNE 1969) and in W. gladiata (PIETERSE et al. 1970d). Presumably a high cytokinin content of plants hinders the realization of the ABA effect. Kinetin applied simultaneously with ABA prevents turion formation in S. polyrrhiza (STEWART 1969). In old cultures of S. polyrrhiza ABA is released from the plants to the medium, in this way acting not only within the ABA-synthesizing individuals but also on their neighbours. The time of appearance of released ABA in the solution was correlated with the beginning of turion formation in the old culture (SAKS et al. 1980). SMART and TREWAVAS (1983a) stated that only frond primordia <0.7 mm long, i.e., before the stage of rapid cell expansion, could be induced by ABA to change morphogenesis to turion formation. During about 3 days the effect of ABA is reversible. Plants transferred to ABA-free medium after this time did not produce turions. Cytokinins and ABA are not the only hormones which influence senescence and turion formation in Lemnaceae. Gibberellin A<sub>3</sub> retards senescence as well as induction of turion-like fronds in L. perpusilla (HUEGEL et al. 1979).

As in senescence sugars can have an inhibiting or promoting effect on turion production. In L. perpusilla short-day induced development of turion-like fronds is prevented by addition of sucrose to the medium. Simultaneously, senescence of mature fronds is retarded (HUEGEL et al. 1979). A promotion of turion production by exogenous sucrose or glucose, on the other hand, has been shown in S. polyrrhiza (CZOPEK 1963a, HENSSSEN 1954, NEWTON and DUFFEY 1975, NEWTON et al. 1978), W. gladiata (PIETERSE et al. 1970d), and W. arrhiza (GODZIEMBA-CZYŻ 1969). Furthermore, factors which increase the endogenous production of photosynthates, as high intensity light, long daily light period and CO<sub>2</sub> enrichment, promote turion formation in S. polyrrhiza (JACOBS 1947). The effects of exogenous and endogenous carbohydrates on turion formation may come about in part indirectly by increasing growth and, therefore, exhaustion of minerals in the medium. Sugars and photosynthesis-promoting agents, however, are turion inducing also under growth-inhibiting conditions. They increase the turion-inducing effect of low night temperature

(10-15°C) (JACOBS 1947), and mineral deficiency (NEWTON and DUFFEY 1975, NEWTON et al. 1978).

#### 2.4.2.2. Dormancy and germination of turions

Turions in comparison to vegetative fronds of S. polyrrhiza are characterized by a lack of many proteins as revealed by polyacrylamide gel electrophoresis (PERRY 1963, SMART and TREWAVAS 1984a, see chapter 2.5.7.3.4). The capacity for photosynthesis is lowered drastically (CZOPPEK 1967. See, however, BEER 1985). Also in turions of W. arrhiza rates of gas metabolism are reduced. The water content of these turions amounts to 78.9% in comparison to 97% of vegetative fronds (GODZIEMBA-CZYŻ 1970). In turion-like fronds of W. gladiata the content of gibberellins (similar to GA<sub>1</sub>, GA<sub>2</sub>, GA<sub>3</sub> and GA<sub>8</sub> in the chromatograms) is lowered distinctly and another fraction with gibberellin-like activity is raised (PIETERSE 1972, PIETERSE et al. 1971a).

Turions of S. polyrrhiza are very tolerant to anaerobic conditions, but not resistant to air drying. They are tolerant to temperature extremes of -8°C and 50°C for at least 24 hrs. Two days at -12°C or one day at 60°C, however, are lethal (JACOBS 1947). Interestingly, there are some observations that turions are able to survive in India in ponds that dry out provided that they are completely covered by mud (B. GOPAL, pers. comm.). In W. arrhiza floating fronds are more resistant to desiccation than turions and resistance to low temperatures is similar in turions and vegetative (floating and immersed) fronds. Only darkness is endured better by turions (GODZIEMBA-CZYŻ 1970).

The dormant state of turions lasts to a different degree depending on the kind of turion-inducing factors, culture conditions during turion development, and genetic properties of the clone. Turions of S. polyrrhiza strain 0-381 induced by low temperature possess a dormancy, which is broken either by storage in N-deficient medium and darkness (>12 days) or by storage under continuous light (>20 days). Turions of the same strain, however, which are induced by long-lasting nitrogen deficiency, need no further treatment, before germination can proceed in N-containing medium (SIBASAKI and ODA 1979). Supply of sucrose or another sugar (NEWTON and DUFFEY 1975) or gibberellin A<sub>3</sub> (LACOR 1969) during induction of turions removes the requirement of chilling to break dormancy. Long day can replace cold treatment to break dormancy in strain 16-



62 of S. polyrrhiza, but not in strain 8-64 (PERRY 1968).

In principle, the following factors break dormancy in turions of S. polyrrhiza: certain metabolic inhibitors, osmotic stress, nitrogen deficiency in combination with sucrose, chilling, long day, gibberellins and cytokinins. HENSSEN (1954) has shown that a treatment of turions with 0.1% potassium cyanide or 0.2% 2,4-dinitrophenol (40-48 hours) breaks dormancy at any time. Similarly, dormancy can be broken by treatment (1-2 hours) with 3% sodium sulfocyanate, 95% ethanol or 0.1% IAA (YOSHIMURA 1950). Germination of turions amounts to 90-100% within 2 days, if treated with the osmoticum polyethylene glycol 6000 (5-20%) for 4 days prior to the germination test. Mild drying of turions in air has a similar, but less pronounced effect (MALEK 1981). Turions of strain 0-381, which were stored in the turion-inducing medium (1/20 nitrate Hoagland's solution with 1% sucrose) or in 1% sucrose solution, reached full germination capacity about 20 days after onset of turion formation. In 1/20 nitrate medium lacking sucrose, however, turions need 51 days to reach partial germination ability (MALEK and ODA 1980). For chilling the following temperature programmes have been found to be effective in different strains of S. polyrrhiza: 14 days at 10°C or below (JACOBS 1947), 20-30 days at 4°C (NEWTON et al. 1978, PERRY 1968), 28 days at 5,5°C (HENSSEN 1954), and 1 month at 0-3°C, turions in water (CZOPEK 1962, 1964a). When turions are stored at 25°C, dormancy can last 6 months or longer (JACOBS 1947). Turions collected by HENSSEN (1954) during autumn from the natural location remained in the dormant state for 10 months, when stored at 20°C in the dark. Some germination capacity, however, began to appear already after 4 months, when turions were given light during the germination test. Application of long day or continuous light during turion storage is a further agent for shortening of the dormant state. Unchilled turions of strain 16-62 germinate within 21 days only when held under long day, but not under short day. Supply of 10 ppm gibberellin A<sub>3</sub> has about the same effect as long day. Gibberellin and long day, given together, cause an immediate germination as after chilling (PERRY 1968). Dormant turions, strain 0-381, stored under continuous light germinate after a lag period of about 24 days. When, however, the turions are darkened during the first 6, 12, or 18 days of storage period, then the onset of germination is delayed to a corresponding extent (SIBASAKI and ODA 1979). A positive effect of gibberellin A<sub>3</sub> as well as of kinetin on breaking dormancy has been demonstrated by LACOR

(1969). The highest germination rate in light is obtained when both gibberellin and kinetin are added to the medium. When the dormancy of young turions has been varied by a different phosphorus supply during turion development, benzyladenine strongly increases germination independent of the dormant state (JUNGNICKEL 1986a).

For light and some other factors it is important to distinguish between effects on breaking dormancy as a precondition of germination and on germination itself. Light promotes germination also in turions which have been chilled for an adequate time (HENSSEN 1954, NEWTON et al. 1978). Ten minutes of white or red light per day are sufficient to stimulate turion germination. Phytochrome seems to mediate the light effect, because far red irradiation after red abolishes the effect of red light, at least partially (CZOPEK 1962). Kinetin ( $10^{-6}$  M) is another agent which promotes germination of chilled turions. The substance is effective in the dark as well as in combination with a short red light. Gibberellin A<sub>3</sub> ( $10^{-5}$ - $10^{-7}$  M) inhibited dark or red light-stimulated germination in these experiments (CZOPEK 1964a). Benzyladenine and isopen-tenyladenine render germination of osmotically pretreated turions possible even in pure water (MALEK and COSSINS 1983a). Without added cytokinins these turions need at least supply of the anions which were omitted from the medium during turion induction for germination. Turions formed in nitrate-deficient medium germinate in 1 mM Ca(NO<sub>3</sub>)<sub>2</sub>, but not in 1 mM MgSO<sub>4</sub>. The reverse holds true for turions which were produced in a sulfate-deficient medium. DUDLEY (1983) observed germination of turions of L. turionifera in old cultures if phosphorus was added to the solution.

The floating up of turions to the surface of the water is made possible by gas bubble formation in the light (JACOBS 1947). The bubbles seem to consist of photosynthetically evolved O<sub>2</sub>, as photosynthetic rates of submerged turions correlate positively with their surfacing rate. Enriched CO<sub>2</sub> concentrations increase photosynthesis as well as surfacing (BEER 1985).

#### 2.4.2.3. Dormancy and germination of seeds

In contrast to turions, seeds are more or less resistant to desiccation. In Israel, WITZTUM (1977) observed fruiting L. gibba in a shallow pool which were desiccated completely by August. Seeds taken from this loca-

tion germinated within a week in the laboratory under continuous light at 25°C. In the natural environment the seeds survived until the next rainy season and germinated during December and January. Seeds of L. gibba, however, collected in August from a Czechoslovakian fishpond and kept under dry conditions at room temperature for more than one year showed only a very small percentage of germination (REJMANKOVA 1976). Control seeds stored in diluted medium in the dark exhibited 70% germination under long day at the same time, and lost their viability only during the course of the following ten months.

Seeds of L. gibba are able to germinate soon after ripening. Germination does not occur in the dark, but a short dark pre-treatment seems to be necessary for rapid germination. Increasing water temperature (17° to 33°C) promotes the rate of germination (REJMANKOVA 1976).

Similar results have been found with seeds of L. aequinoctialis 6746 (GLICKMAN and POSNER 1966, POSNER and HILLMAN 1962). No germination proceeds without light, also when the medium has been complemented with yeast extract plus casein hydrolysate, kinetin, or gibberellin A<sub>3</sub>. On the other hand, germination takes place only after a lag period of 6-7 days, when short day, long day, or continuous light is applied without preceding 1-4 days darkness. Gibberellin A<sub>3</sub> enhances the germination rate under such conditions. The most rapid germination is achieved when 4 days darkness (at >10°C) are followed by continuous high-intensity light (800 ft-c). Kinetin or - especially - casein hydrolysate in combination with lower light intensity (200 ft-c) can replace the requirement of high-intensity light. In S. intermedia germination has been observed in darkness in a medium complemented with casein hydrolysate, yeast extract, kinetin, and gibberellin A<sub>3</sub> (ROSSI 1969).

In L. perpusilla P146 germination proceeds only after chilling of seeds, for example, by keeping the culture in a refrigerator (4-6°C) for one month (KANDELER 1975). Releasing the embryo from its seed coat, however, makes germ-bud development possible without chilling on a sucrose- and kinetin-containing medium (KANDELER and HUEGEL 1974a). A germination-inhibiting substance, presumably ABA, seems to be located chiefly in the seed coat (and possibly the pericarp).

### 2.4.3. Flowering

#### 2.4.3.1. Flower induction

Using the advantages of Lemnaceae as axenically cultivable small water plants, several workers examined the effects of diverse organic and inorganic substances on flower induction. From the summary of these investigations (table 2.25) one can make a first survey on the factors which have an influence on flower initiation. As in other plants flowering depends on all the known phytohormones, their metabolism and interactions. Nitrogen and micro nutrient supply, light intensity and colour, as well as day length and temperature are the main environmental conditions, which are tested by the plant to come to the "decision" to flower or not. In table 2.25 the rough direction (positive or negative) of substance effectiveness is entered. It should be taken into consideration, however, that many agents work at a certain concentration range, under certain experimental conditions, or in certain species or strain only. In dependence of concentration and experimental conditions one and the same substance may even have a flower-promoting or flower-inhibiting effect. In some cases the classification of agents is rather hypothetical (EDDHA and salicylic acid, for instance). These and other details are discussed in the following chapters. A more circumstantial review on physiology of duckweed flowering has been given recently (KANDELER 1985).

##### 2.4.3.1.1. The hormonal state

Phytohormones seem to play a central role in flower induction. Presumably they are important for mediation, integration, and processing of environmental stimuli as well as for internal coordination of flowering with other developmental processes.

All the known phytohormones modify the extent of flowering if added to the nutrient medium of duckweed cultures (table 2.25). In L. gibba G3 (long-day plant = LDP) flowering is promoted by gibberellin A<sub>3</sub> ( $10^{-6}$ - $10^{-5}$  M) under long-day conditions (CLELAND and BRIGGS 1969, CLELAND et al. 1982, OOTA 1965, OOTA and TSUDZUKI 1971). Higher concentrations of gibberellin A<sub>3</sub> ( $10^{-4}$  M), however, as well as the inhibitor of gibberellin synthesis, CCC, are flower inhibiting (CLELAND and BRIGGS 1969,

Tab. 2.25. Substances influencing flower initiation in Lemnaceae

+ promoting  
 - inhibiting  
 0 not or only slightly promoting

LDP long-day plants  
 SDP short-day plants

Experiments with amino acids of low concentration ( $10^{-6}$  M) are not included in the table (see text).

	LDP	SDP	References	
			LDP	SDP
A. Hormones. Inhibitors and promoters of hormone synthesis				
Gibberellin A <sub>3</sub>	+/-	-	9,48,58,89,90	15,25,26
CCC	-	+	9	37
Allogibberic acid	-	-	59	69
Abscissic acid	+/-	+/-	70,89,90,99	15,37
Zeatin (and other cytokinins)	+/-	+/-	48,58,61,89,90,106	1,15,16,18,45,48,86,95
Indoleacetic acid	+/-	-	48,58,90	15,18
Ethephon	-		59,70	
ACC	+/-	+/-	70,103	103
AVG, AOA	-		70	
Co <sup>2+</sup>	-	-	70	17
Polyamines		-	104	
MGBG		+	104	
B. Some heavy metals, chelating agents, and phenolic substances				
Mn <sup>2+</sup>	+		42	
Fe		+		17,22,43,74,101
o-Phenanthroline, $\alpha,\alpha'$ -dipyridyl, azide (iron reagents)	-		49	
EDDHA (compare also EDTA, group C)	+	+	3,60,71	17,43,74
Salicylic acid, benzoic acid, acetyl-salicylic acid	+	+	2,6,7,8,10,27,54,55,61,71,77,79,90,106	2,10,15,27,41,78,94
8-Hydroxyquinoline		+		96,98
Tannic acid		+		97
Dicoumarol		+		93
4-Hydroxycoumarin		+		93
2-Hydroxy-1-phenyl-1,4-pentadione		+		105
Phenylglyoxal		+		105
Chlorogenic acid	-		84	
$\beta$ -Naphthol	-		102	
Benzo (a) anthracene	-		4	

Table 2.25 (continued)

	LDP	SDP	References	
			LDP	SDP
C. Substances involved in nitrogen metabolism				
NO <sub>3</sub> <sup>-</sup>	+/-	+/-	14	14
MoO <sub>4</sub> <sup>2-</sup>	+	-	5	76,83
WO <sub>4</sub> <sup>2-</sup>	-	+	87	76,83
Cu <sup>2+</sup> (and other sulfhydryl blockers:	+/-	+	23,42,87	23,72,75
Ag <sup>+</sup> , Hg <sup>2+</sup> , iodoacetamide)				
Salicylaldoxime, diethyldithiocarbamate, hydroxyquinoline (copper chelators)	+		49	
EDTA	+	+/-	21,22,28,51,53	20,43,44,75
NH <sub>3</sub>	-	-	33	24,80
Urea		-		80
Casamino acids		-		80
Cysteine, cystine, arginine, phenylalanine, tryptophane	-	0/-	46,47	65,80,81
Aspartate, glutamate	0	+/-	46,47	64,65,80,81,83
Alanine, glycine, serine	-	+/-	46,47	64,65,80,81,83
Threonine, proline, iso-leucine	-	0/-	46,47	65,80,81
Lysine	+/-	0/-	46	65,80,81
Leucine, tyrosine, valine, methionine hydroxyproline, histidine	-	0	46,47	65,81
Asparagine, glutamine		+/-		65,80,81,83
D. CO <sub>2</sub> , carbohydrates, and energy budget				
CO <sub>2</sub>	+/-	-	29	65
Sucrose	+/-	+/-	21,30,31,38,52	13,26,38,62,63,64,65,66,67,72,73
Glucose, fructose	0/-	0/-	38,50	38,62,63,64
NADH, NADPH	+	-	35	35
Ascorbic acid	-	+	35	35
DCMU	+	+/-	32	68,72,73
ATP,ADP	+	+/-	32,33,38	34,40,65,67

Table 2.25 (continued)

	LDP	SDP	References	
			LDP	SDP
Arsenate (and other respiratory inhibitors)	-		30,49	
2,4-Dinitrophenol	-	-	49	40
Glucose-6-P (and other respiratory intermediates)		+		65
E. Effectors of membrane transport				
Acetylcholine, eserine	-	+	36,55	36
Valinomycin, gramicidin	+		56	
Li <sup>+</sup>	-	+	34	34
Ca <sup>2+</sup>	+	+/-	100	19,23,63
PO <sub>4</sub> <sup>3-</sup>	+	+/-	100	17,19,23,63,67,101
F. Some further substances				
Ferricyanide, ferrocyanide, CN	+	+	77,87	75,76,82,83
Nicotinic acid	+	+	91	91
cyclic AMP	+	+	39,50,55,57	67
Isoproterenol (and other catecholamines)	+		52,55	
Vitamin K <sub>5</sub>		+		92
5-Fluorodeoxyuridine	-		85	
5-Fluorouracil	-	-	85	12
2-Thiouracil	-	+/-	85	11,12
Cycloheximide, ethionine, chloramphenicol		-		12
Agar	-		88	

**References:**

- |  |                            |
|--|----------------------------|
| 1 BENNINK AND DE VRIES 1975)                 | 6 CLELAND 1974c            |
| 2 BEPPU and TAKIMOTO 1981b)                  | 7 CLELAND 1979             |
| 3 BHALLA and SABHARWAL 1972a)                | 8 CLELAND and AJAMI 1974   |
| 4 BHALLA and SABHARWAL 1974)                 | 9 CLELAND and BRIGGS 1969  |
| 5 BUECHELE and KANDELER, unpublished results | 10 CLELAND and TANAKA 1979 |
|  | 11 DOSS 1975c              |



Table 2.25 (continued)

12	DOSS 1975d	59	PIETERSE 1976
13	ESASHI and ODA 1964	60	PIETERSE et al. 1970b
14	ESASHI et al. 1972	61	PIETERSE and MUELLER 1977
15	FUJIOKA et al. 1983b	62	POSNER 1967b
16	GUPTA and MAHESHWARI 1969	63	POSNER 1969a
17	GUPTA and MAHESHWARI 1970a	64	POSNER 1970
18	GUPTA and MAHESHWARI 1970b	65	POSNER 1971
19	HALABAN and HILLMAN 1970a	66	POSNER 1973b
20	HILLMAN 1959b	67	POSNER 1973a
21	HILLMAN 1961c	68	POSNER et al. 1977
22	HILLMAN 1961b	69	PRYCE 1973b
23	HILLMAN 1962	70	SCHARFETTER et al. 1986
24	HILLMAN and POSNER 1971	71	SCHARFETTER et al. 1978
25	HODSON and HAMNER 1971	72	SCHUSTER 1968
26	HUEGEL et al. 1979	73	SCHUSTER and KANDELER 1970
27	KAIHARA et al. 1981	74	SETH et al. 1970
28	KANDELER 1962	75	TAKIMOTO and TANAKA 1973
29	KANDELER 1964a	76	TAKIMOTO and TANAKA 1976
30	KANDELER 1967	77	TANAKA and CLELAND 1980
31	KANDELER 1968	78	TANAKA and CLELAND 1981
32	KANDELER 1969b	79	TANAKA et al. 1979a
33	KANDELER 1969a	80	TANAKA and TAKIMOTO 1975
34	KANDELER 1970	81	TANAKA and TAKIMOTO 1977
35	KANDELER 1971a	82	TANAKA and TAKIMOTO 1978
36	KANDELER 1972	83	TANAKA et al. 1979b
37	KANDELER and HUEGEL 1973	84	UMEMOTO 1971
38	KANDELER et al. 1975	85	UMEMURA and OOTA 1965a
39	KESSLER and STEINBERG 1973	86	VENKATARAMAN et al. 1970
40	KHUDAIRI and MAENG 1973	87	WATANABE and TAKIMOTO 1977
41	KHURANA and MAHESHWARI 1978	88	CLELAND 1985
42	KRAJNCIC 1976a	89	CLELAND et al. 1982
43	MAHESHWARI and GUPTA 1967	90	FUJIOKA et al. 1985
44	MAHESHWARI and SETH 1966b	91	FUJIOKA et al. 1986b
45	MAHESHWARI and VENKATARAMAN 1966	92	KAIHARA and TAKIMOTO 1985a
46	NAKASHIMA 1964	93	KAIHARA and TAKIMOTO 1985b
47	NAKASHIMA 1965	94	KHURANA and MAHESHWARI 1983a
48	OTA 1965	95	KHURANA and MAHESHWARI 1983b
49	OTA 1969	96	KHURANA and MAHESHWARI 1984
50	OTA 1972a	97	KHURANA and MAHESHWARI 1986a
51	OTA 1972b	98	KHURANA et al. 1986
52	OTA 1974	99	KRAJNCIC 1985
53	OTA 1975b	100	MAENG and BAE 1984
54	OTA 1975c	101	MORI 1984b
55	OTA 1977a	102	PIETERSE 1978b
56	OTA 1977b	103	SCHARFETTER et al. 1987
57	OTA and KONDO 1974	104	TSAO and JIN 1985
58	OTA and TSUDZUKI 1971	105	SUZUKI et al. 1985
		106	WATANABE et al. 1983

CLELAND et al. 1982, OOTA and TSUDZUKI 1971). Likewise the decomposition products of gibberellin A<sub>3</sub> after autoclaving, allogibberic acid, gibberic acid and epiallogibberic acid, suppress flowering in L. gibba G3 (PIETERSE 1976). In the short-day plant (SDP) L. perpusilla Pl46 short-day induced flowering shows a distinct reduction with  $5 \times 10^{-7}$  M gibberellin A<sub>3</sub> (HUEGEL et al. 1979). The same results were found in the SDP L. aequinoctialis 381 with 1 ppm gibberellin A<sub>3</sub> (FUJIOKA et al. 1983b). CCC on the other hand, can force flowering in the SDP L. aequinoctialis 6746 under long day (KANDELER and HUEGEL 1973, TSAO et al. 1985). High concentrations of gibberellin A<sub>3</sub> ( $10^{-4}$  M) and gibberellin decomposition products are both flower inhibiting in L. aequinoctialis 6746 (HODSON and HAMNER 1971, PRYCE 1973b). The opposite behaviour of the two photoperiodic reaction types against low concentrations of gibberellin A<sub>3</sub> and against CCC may be explained by the hypothesis that the endogenous accumulation of gibberellins is different in the two groups of plants and must be corrected by day length in a different way to reach a flower-inducing level. In fact, uptake of [<sup>3</sup>H]-gibberellin A<sub>1</sub> reaches a higher saturation level in L. aequinoctialis 6746 than in L. gibba G1 (AL-SHALAN and KANDELER 1979, HARTUNG and KANDELER 1976). Moreover, preliminary determinations of the endogenous gibberellin content in both species cultivated under the same conditions showed higher values in strain 6746 than in strain G1 (LADENBURGER, BAUER and KANDELER, unpublished results). Recently, TSAO et al. (1985) revealed that the level of gibberellins in L. aequinoctialis 6746 decreases after transfer of plants to short day.

Abscisic acid (ABA) is another hormone which has an opposite effect in the LDP L. gibba G3 and the SDP L. aequinoctialis 6746.  $10^{-8}$ - $10^{-7}$  M ABA inhibit long-day induction of flowering in L. gibba G3 (CLELAND et al. 1982, SCHARFETTER and KANDELER, unpublished results). In L. aequinoctialis 6746  $2 \times 10^{-9}$ - $2 \times 10^{-8}$  M ABA strengthen the flower-inducing effect of CCC under long-day conditions (KANDELER and HUEGEL 1973). Correspondingly, the level of ABA in strain 6746 increases transiently about threefold, when plants are transferred to short day (TSAO et al. 1985). In both species frond senescence has the same effect as ABA. Daughter fronds, which are produced under long day during the second part of the life-span of a mother frond, failed to flower in L. gibba G1, but came into flower in L. aequinoctialis 6746 in spite of the non-inductive day length (fig. 2.34) (KANDELER et al. 1974). In the day-neutral plant S. polyrrhiza

frond senescence has the same effect on flowering as in L. aequinoctialis (LACOR 1970). A low concentration of ABA (0.1 mg/l) has a flower-promoting effect in this species, when given under long day and in combination with EDDHA (KRAJNCIC 1985). In contrast to the above cited results FUJIOKA et al. (1983b) found a flower-inhibiting effect of low ABA concentrations in the SDPs L. aequinoctialis 151 and 381, when flowering under continuous light was forced by addition of benzoic acid to the nutrient medium. KRAJNCIC (1985) demonstrated some promotion of flowering by 0.1-1 µg/l ABA in the LDP L. minor. 10 µg/l ABA, however, were inhibitory in all investigated photoperiodic types: the LDPs L. minor and L. gibba, the long-short-day plant W. arrhiza and the day-neutral plant S. polyrrhiza.

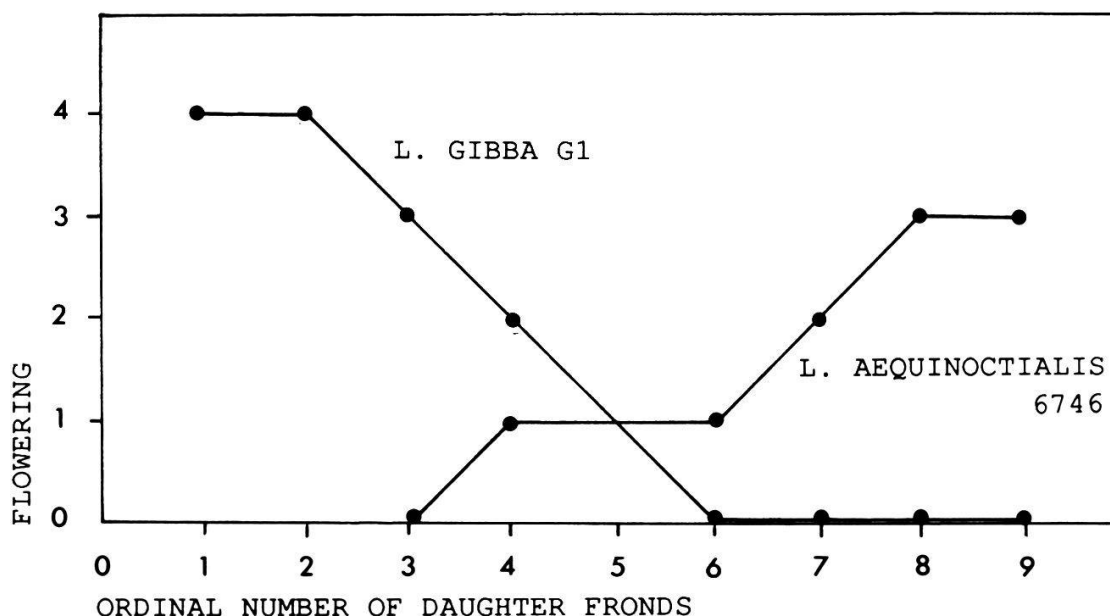


Fig. 2.34. The contrary influence of mother frond senescence on flower induction in daughter fronds of Lemna gibba G1 and L. aequinoctialis 6746 (after data from KANDELER et al. 1974). Single mother fronds (four in both species) were cultivated under long-day conditions, and the resulting daughter fronds (with ordinal numbers 1 to 9) were examined for flowering. Senescence of the mother frond is responsible for the lack of flowers in late-produced daughter fronds of L. gibba, as the series of first-daughter frond generations (derived from the same mothers and cultivated in parallel under the same conditions) forms flowers all the time. In L. aequinoctialis, first-daughter fronds do not flower under long day, but daughter fronds with a higher ordinal number (i.e., produced by a senescing mother frond) can produce a flower in spite of the non-inductive day length.

Cytokinins promote flowering in the LDPs L. gibba (CLELAND et al. 1982, OOTA 1965, OOTA and TSUDZUKI 1971, PIETERSE and MUELLER 1977) and L. minor (WATANABE et al. 1983) as well as in the SDPs L. aequinoctialis (FUJIOKA et al. 1983b, GUPTA and MAHESHWARI 1969, 1970b, KHURANA and MAHESHWARI 1983b), L. perpusilla (BENNINK and DE VRIES 1975, and W. microscopica (MAHESHWARI and VENKATARAMAN 1966, VENKATARAMAN et al. 1970) and in the long-short-day plant W. arrhiza (KRAJNCIC 1983). Benzyladenine has the highest flower-promoting effectiveness of 5 different cytokinins in W. microscopica (VENKATARAMAN et al. 1970). In L. gibba  $10^{-6}$  M kinetin enhances flowering only under short-day conditions (in combination with gibberellin, OOTA 1965), but inhibits flower initiation under long day (OOTA and TSUDZUKI 1971).

Indoleacetic acid (IAA) causes inhibition of flowering in L. gibba and L. aequinoctialis (FUJIOKA et al. 1983b, GUPTA and MAHESHWARI 1970b, OOTA 1965, OOTA and TSUDZUKI 1971), but a weak flower-promoting effect of  $10^{-9}$  M IAA has been found in L. gibba (OOTA and TSUDZUKI 1971).

Ethephon (Ethrel) as an ethylene releasing agent is flower inhibiting in L. gibba G3 (PIETERSE 1976). The ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), however, promotes flowering in L. gibba G1 as well as in L. aequinoctialis 6746, when plants are induced to low or moderate flowering by other agents (SCHARFETTER et al. 1987). The optimum concentration is lower in L. gibba ( $10^{-8}$  M) than in L. aequinoctialis ( $10^{-7}$ - $10^{-6}$  M). Higher ACC concentrations are inhibitory in both species. In S. punctata O5, ethylene-releasing substances (Ethrel, ACC) and inhibitors of ethylene formation (AVG, AOA,  $\text{Co}^{2+}$ ) are effective only in the case that flowering has been induced by EDDHA (SCHARFETTER et al. 1986). Then both groups of substances greatly inhibit flower induction. It is suggested that flower initiation depends on an intermediate ethylene level, but only after accomplishment of certain other flower-inducing processes. EDDHA may reduce the level of free auxin (see next chapter) and, simultaneously, may adjust ethylene formation to a flower-promoting level. A flower-inhibiting action of  $\text{Co}^{2+}$  were found also in L. aequinoctialis (GUPTA and MAHESHWARI 1970a).

Summarizing the results hitherto, it can be stated that levels of gibberellins and ABA have to be changed in an opposite way for flower initiation in LDPs and SDPs and seem to be under photoperiodic control. Cytokinins and auxins, on the other hand, exert their effects on flowering more or less independently from the photoperiodic type of plant. Possi-

bly, certain polyamines belong to the first of the two groups of growth regulators. In L. aequinoctialis, the endogenous spermidine content decreases during a 4 day dark induction (putrescine increases concomitantly). Correspondingly, exogenous spermidine and spermine inhibit the short-day induction of flowering more strongly than putrescine. The inhibitor of spermidine and spermine synthesis, MGBG, induces flowering under continuous light (TSAO and YIN 1985). Generally, polyamines are known to delay senescence and, therefore, counteract the effects of ABA and ethylene. Polyamines and ethylene can inhibit their formation mutually (see PALAVAN et al. 1984). Thus, the higher demand of ethylene formation for flowering in the SDP L. aequinoctialis (see above) may be related to the required reduction of polyamine synthesis in this plant.

#### **2.4.3.1.2. Some heavy metals, chelating agents, and phenolic substances**

Manganese and iron seem to be of special importance for flowering in Lemnaceae. Omission of manganese or iron from the nutrient medium lowers or prevents flowering in L. aequinoctialis (GUPTA and MAHESHWARI 1970a). In strain 6746 of L. aequinoctialis a temporary lowering of the iron level during short-day induction inhibits flowering without an effect on vegetative frond multiplication (HILLMAN 1961b). Especially under continuous darkness, the flower-retarding effect of medium dilution is abolished by supply of iron or phosphate ions. No other component of Huter's medium is effective in such a way in strain 6746 (MORI 1984a, 1984b). Furthermore, excessive doses of iron can force flowering in the Sohna strain of L. aequinoctialis and in W. microscopica under otherwise non-flowering conditions (GUPTA and MAHESHWARI 1970a, MAHESHWARI and GUPTA 1967, SETH et al. 1970). In L. gibba G3 PIETERSE et al. (1970b) found no inhibition of flower initiation by deletion of iron from the medium, but some iron reagents (o-phenanthroline,  $\alpha$ ,  $\alpha'$ -dipyridyl, azide) inhibit flowering in this plant (OOTA 1969).

Subsequently the strong effect of some chelating agents on flowering may be reported. In LDPs L. gibba (PIETERSE et al. 1970b), L. minor (BHALLA and SABHARWAL 1972), and S. punctata (SCHARFETTER et al. 1978), as well as in SDPs L. aequinoctialis (GUPTA and MAHESHWARI 1970a, MAHESHWARI and GUPTA 1967) and W. microscopica (SETH et al. 1970) flower initiation is rendered possible (under certain experimental conditions) only when EDTA (ethylenediaminetetraacetic acid) or EDDHA (ethylenediamine-di-o-hydro-

xyphenylacetic acid) is added to the nutrient medium. This effect can be made understandable by the well-known fact that such chelating agents increase the availability of iron and manganese for plants (for copper decontamination compare chapter 2.4.3.1.3). In L. gibba G3 addition of EDDHA to the medium doubles the iron content of the plants (PIETERSE 1975a), and this is also the case in L. aequinoctialis 6746, when the plants are cultivated under short day conditions (KHURANA and MAHESHWARI 1984). Under long day, EDTA is more effective than EDDHA to increase the endogenous iron level of L. aequinoctialis. In flower induction, EDDHA is distinctly more effective than EDTA in most cases, but in strain 6746 of L. aequinoctialis long-day flowering is achieved by EDTA but not by EDDHA (YUKAWA and TAKIMOTO 1976). Interestingly, ELZENGA et al. (1980) found an enhancement of ethylene production in L. gibba G3 after addition of EDDHA to the medium (preliminary result).

A group of substances, whose effectivity is very similar to that of EDDHA, are salicylic acid (SA), benzoic acid (BEA), and acetylsalicylic acid (ASA). The flower-promoting activity of SA was found by CLELAND (1974c; CLELAND and AJAMI 1974), when he tested with axenic L. gibba cultures the phloem sap of Xanthium plants for flower-inducing agents. In continuation of this work several authors confirmed the flower-inducing or flower-promoting effect of SA, BEA, and ASA in LDPs L. gibba (CLELAND and BEN-TAL 1982, CLELAND and TANAKA 1979), S. punctata (SCHARFETTER et al. 1978), L. minor (KAIHARA et al. 1981, WATANABE et al. 1983), and S type strains of L. aequinoctialis (BEPPU and TAKIMOTO 1981b, KAIHARA et al. 1981); moreover in many short-day-dependent strains of L. aequinoctialis (BEPPU and TAKIMOTO 1981b, CLELAND and TANAKA 1979, FUJIOKA et al. 1983b, KAIHARA et al. 1981, KHURANA and MAHESHWARI 1978), in the SDP W. microscopica (MAHESHWARI and KHURANA 1978, KHURANA and MAHESHWARI 1983a), and in the day-neutral plants S. polyrrhiza (KHURANA and MAHESHWARI 1980), L. obscura (CLELAND et al. 1982), and L. aequinoctialis K type strain (BEPPU and TAKIMOTO 1981b). With only one exception, SA generally is more effective than BEA in LDPs (CLELAND 1974c, KAIHARA et al. 1981), whereas BEA is more effective than SA in SDPs (KAIHARA et al. 1981, WATANABE et al. 1981, WATANABE and TAKIMOTO 1979).

Analysing the effect of SA in L. gibba G3 CLELAND and BEN-TAL (1982) found an immediate decrease of flowering after removal of SA from the medium. Thus, for maximal effectiveness SA must be present in the medium



for the entire experiment. An explanation for this behaviour is given by the observation that [ $^{14}\text{C}$ ] SA after uptake is partially converted to one or more bound forms and then the free and bound SA are stored within the cells (presumably the vacuoles) without further transport or metabolism (BEN-TAL and CLELAND 1982). Correspondingly, FUJIOKA et al. (1983a, 1985) found no positive correlation between the endogenously produced level of BEA and flowering in L. aequinoctialis strains 151, 381, and 321, and in L. gibba G3.

Besides SA and BEA, certain other phenols (respectively derivatives of phenols) have been found to induce or promote flowering in Lemnaceae. 8-Hydroxyquinoline increases the critical day-length in L. aequinoctialis (KHURANA and MAHESHWARI 1984) and makes long-day flowering possible in W. microscopica (KHURANA et al. 1986). With  $10^{-5}\text{M}$  tannic acid L. aequinoctialis 6746 flowers to a high degree even under continuous light (KHURANA and MAHESHWARI 1986a). Dicoumarol has a strong flower-promoting effect especially under near critical photoperiods in strains 6746 and 441 of L. aequinoctialis, but is highly effective also under continuous light in strain 151 of the same species (KAIHARA and TAKIMOTO 1985b). 4-Hydroxycoumarin, a component of dicoumarol, is active like dicoumarol in strain 151. A flower-inducing substance, which has been isolated from Pharbitis purpurea, 2-hydroxy-1-phenyl-1,4-pentadione, as well as phenylglyoxal, a component of this substance, show flower-inducing activity as high as benzoic acid in strain 151 (SUZUKI et al. 1985). In contrast, chlorogenic acid and  $\beta$ -naphthol are phenols, which abolish flowering in the LDP L. gibba (UMEMOTO 1971, PIETERSE 1978b). The flower-inhibiting effect of benzo(a)anthracene in L. gibba may be mentioned as an appendage in this context (BHALLA and SABHARVAL 1974).

At least in the case of 8-hydroxyquinoline and EDDHA there are arguments that the flower-inducing activity may be based on chelation of heavy metals. 8-Hydroxyquinoline increases the iron content of L. aequinoctialis 6746 more strongly than EDTA and EDDHA, increases the copper content instead of a decrease caused by EDTA and EDDHA (see chapter 2.4.3.1.3), and has a higher flower-inducing activity than EDTA and EDDHA in the same experiments (KHURANA and MAHESHWARI 1984). Long ago, MAHESHWARI and GUPTA (1967) demonstrated that in another strain of L. aequinoctialis the flower-inducing effect of EDTA and EDDHA can be mimicked by supply of high doses of ferric citrate. In W. microscopica, iron uptake is higher with Fe-EDDHA than with EDTA + Fe-citrate, and, correspondingly,



long-day flowering is achieved with Fe-EDDHA, but not with EDTA + Fe-citrate (SETH et al. 1970).

Presumably, chelation of heavy metals is not the only way, in which phenols act on flower induction. Activation or inhibition of IAA-oxidases, formation of conjugates with IAA or with polyamines, and free radical scavenging (leading to a lower ethylene formation) are further processes, which - singly or in combination - may participate in the action mechanism. A further working point for SA and related substances may be the signalling action of oligosaccharides. DOHERTY et al. (1987) have shown that ASA abolishes the effect of oligosaccharides on the activity of serine proteinases in tomato leaves. Agar (CLELAND 1985) as well as oligosaccharides (ALBERSHEIM et al. 1987) inhibit flowering in L. gibba G3, and the effect of agar is nullified by SA (see chapter 2.4.3.1.8). Interestingly, TAKIMOTO (1987) has reported that BEA is bound to apiofuranosyl-1,6-glucopyranoside, aspartate, isocitrate, and malate only, when supplied to L. aequinoctialis 151.

In any case, lowering of the endogenous free auxin level seems to be one of the major consequences of application of flower-promoting phenols. When in L. gibba G3 flowering is induced by supply of SA, a distinct part (40%) of endogenous IAA is converted to a bound form (EPSTEIN and BENTAL 1985). SA itself may react with IAA, as SA is converted in part to one or more bound forms after uptake (BENTAL and CLELAND 1982). From p-hydroxybenzoic acid, which has virtually no effect on flowering in L. gibba, a higher percentage remains as the free acid after uptake (CLELAND and KANG 1985). In S. punctata, the effects of EDDHA on flowering and several other developmental processes are counteracted by the auxin NAA (SCHARFETTER et al. 1978). The anti-auxin PCIB while not acting on flowering mimics the EDDHA effects on seven vegetative attributes in S. punctata (SCHARFETTER et al. 1986). The lack of flower-inducing activity of PCIB may be caused by the fact that EDDHA changes not only the auxin but also the ethylene metabolism. The similarity of SA and EDDHA effects has been explained by the assumption that EDDHA is degraded by the plant to SA-like fragments (CLELAND et al. 1982).

#### 2.4.3.1.3. Significance of nitrogen metabolism

As in many other plants, nitrogen supply has a distinct influence on flower initiation in Lemnaceae. ESASHI et al. (1972) investigated the

effect of different nitrate concentrations and found strong effects especially under conditions of high-intensity light (7000 lux). In the SDP L. aequinoctialis 6746 flowering under short day is optimum at medium or high nitrate concentrations, whereas optimal vegetative growth is achieved at lower  $\text{NO}_3$  levels under the same conditions. In continuous light the same strain needs relatively low or medium concentrations of nitrate for good flowering (medium or high concentrations for vegetative growth). In the LDP L. gibba G3 the nitrate effect on flowering depends on the pH of nutrient medium. At pH 5.5 optimal long-day flowering occurs with medium nitrate supply (optimal vegetative growth with high nitrate supply). At pH 4.2 a high  $\text{NO}_3$  level is needed for optimal flowering and a medium  $\text{NO}_3$  level for vegetative growth. In summary, the claims of vegetative and generative development for nitrate supply are different in both photoperiodic reaction types and under diverse experimental conditions.

Several observations have led to the hypothesis that activity of nitrate reductase is of great importance for flowering in Lemnaceae (TAKIMOTO and co-workers). Deletion of molybdate, an activator of nitrate reductase, from the medium makes long-day flowering possible in the SDP L. aequinoctialis 6746 (TAKIMOTO and TANAKA 1976, TANAKA et al. 1979b). In the LDP L. gibba G1, on the other hand, addition of molybdate to the nutrient medium enhances long-day flowering (BUECHELE and KANDELER, unpublished results). Correspondingly, tungstate, a specific inhibitor of nitrate reductase, promotes long-day flowering in L. aequinoctialis, but suppresses flowering in L. gibba (TAKIMOTO and TANAKA 1976, TANAKA et al. 1979b, WATANABE and TAKIMOTO 1977).

More or less identical with the effect of tungstate is that of copper and other sulfhydryl blockers ( $\text{Ag}^+$ ,  $\text{Hg}^{++}$ , iodoacetamide), which may also work through inhibition of nitrate reductase. All these agents induce long-day flowering in L. aequinoctialis 6746 (HILLMAN 1962, SCHUSTER 1968, TAKIMOTO and TANAKA 1973, NASU et al. 1984). The effect can be cancelled by supplemental addition of cysteine (TAKIMOTO and TANAKA 1974). In L. gibba G3 the same SH inhibitors inhibit flowering under continuous illumination (HILLMAN 1962, WATANABE and TAKIMOTO 1977). Copper chelators as salicylaldehyde, diethyldithiocarbamate and hydroxyquinoline, on the other hand, enhance flowering in L. gibba G3 (OTA 1969). Certain effects of EDTA on flowering - inhibition of long-day flowering in L. aequinoctialis 6746 and promotion of flowering in L.

gibba G3 - are also caused by sequestration of free copper from the medium (HILLMAN 1961d, 1962). In both species,  $\text{Cu}^{2+}$  uptake is prevented almost completely by EDTA (TANAKA et al. 1982a, NASU et al. 1983. Compare also KHURANA and MAHESHWARI 1984).

In their later publications TAKIMOTO and co-workers rejected the original hypothesis that SH inhibitors act on flowering by blocking nitrate reductase (TAKIMOTO and TANAKA 1976, TANAKA and TAKIMOTO 1978, WATANABE and TAKIMOTO 1977), because under their experimental conditions lowering of nitrate concentration in the medium does not act like inhibitors of nitrate reductase, but rather like molybdate (that is flower inhibiting in L. aequinoctialis 6746). This discrepancy, however, can be explained by the assumption that nitrate has a twofold function for flower-inducing processes. On the one hand, nitrate may be effective by being a substrate of nitrate reductase and in consequence a material for protein synthesis, in this way leading to a retardation of senescence and the connected hormonal changes (lowering of gibberellin/ABA balance). On the other hand, nitrate may have a more direct influence on the hormonal state of the plant. In L. aequinoctialis 6746 the presence of nitrate is needed for an adequate cytokinin synthesis. Twenty-four - 48 hours after omission of  $\text{NO}_3^-$  from the medium the level of cytokinins is lowered in this plant (LOEPPERT, GRUNTZEL and KANDELER, unpublished results). Recently, TANAKA et al. (1986a) confirmed that the dependence on concentration of copper-, silver-, tungstate-, and molybdate-deficiency-effects on nitrate reductase activity (inhibition) is about the same as in the corresponding effects on long-day flowering (promotion) in L. aequinoctialis 6746. They now conclude again that one of the flower-inducing actions of copper, silver, tungstate, or the deletion of molybdate is to suppress the nitrate assimilation. There is also a relation between effects of ferricyanide on flowering and nitrate reductase activity as with the other above-named agents (TANAKA et al. 1986a). In hybrids of strain 6746 with strain 371 of L. aequinoctialis, however, only the ferricyanide effectivity, but not the copper and silver effectivity is maintained (BEPPU and TAKIMOTO 1983). Furthermore, ferricyanide acts on flowering in both photoperiodic reaction types in the same way, i.e., not in an opposite way as the other nitrate reductase inhibitors. Therefore, the principal action mechanism of ferricyanide in connection with flowering seems to differ from that of nitrate reductase inhibitors (for the details of ferricyanide effects see chapter 2.4.3.1.8).

Other nitrogen sources, which have been tested for their effect on flower initiation, are nitrite, ammonia, urea, casamino acids, glutamine and asparagine. All these substances abolished long-day flowering in L. aequinoctialis 6746 induced by  $\text{Cu}^{2+}$ ,  $\text{Ag}^+$  or ferricyanide (TANAKA and TAKIMOTO 1975). In connection with the effect of ammonia TANAKA et al. (1982a) have stated that this substance inhibits the uptake of copper from the medium (see also NASU et al., 1983). Ammonia is flower inhibiting in strain 6746 also under short-day conditions when the nutrient medium is diluted (1/10 conc.) and complemented with 1% sucrose (HILLMAN and POSNER 1971). In the LDP L. gibba, strains G1 and G3, ammonia suppresses flowering without a simultaneous effect on the vegetative multiplication of fronds (KANDELER 1969a).

The effectivity of single amino acids was investigated by several authors. As can be seen from tab. 2.25 most of the amino acids inhibit flower production in the LDP L. gibba, if given in a concentration range of  $10^{-5}$ - $10^{-3}$  M (NAKASHIMA 1964, 1965). Aspartate and glutamate have a small effect only and lysine, which is flower inhibiting in itself, can partially or wholly reverse the floral inhibition caused by some other amino acids. In L. aequinoctialis 6746 a group of amino acids, aspartate, glutamate, alanine, glycine, and serine, has a clear promotive effect on flowering in reverting the sucrose-induced inhibition (POSNER 1971) or supporting the inductive effect of a single 96 h dark period (TANAKA and TAKIMOTO 1977). All the other amino acids are not, or only slightly, promotive under these conditions. When the above named amino acids are tested with the same strain under long-day conditions, they cause an enhancement or an inhibition of flower initiation, depending on culture conditions (suboptimal concentration of ferricyanide or Mo-deficient medium on the one hand or slightly higher concentration of ferricyanide or tungstate addition on the other hand) (TANAKA et al. 1979b, TANAKA and TAKIMOTO 1975). MAENG and KHUDAIRI (1973), working with a lower concentration of amino acids ( $10^{-6}$  M), found, for strain 6746, a flower promoting action of serine, threonine, and tryptophane and a flower-inhibiting effect of cysteine, if amino acids were added to the nutrient medium during the last dark period of three inductive short-day cycles. KRAJNCIC and DEVIDE (1982b) studied the effect of amino acids in the LDP L. minor, strain Barje, and the day-neutral plant S. polyrrhiza, strain Petanjci. Using the same low concentration as MAENG and KHUDAIRI, they observed flower-promoting effects with glutamate, alanine, glycine,

serine, threonine, and some other amino acids under long day in both species. Under short day, however, preferably other amino acids are flower enhancing in S. polyrrhiza: valine, proline, iso-leucine, and histidine.

Aspartate, glutamate, alanine, glycine, threonine, and some other amino acids are repressors of nitrate reductase in tobacco (FILNER 1966). Assuming the validity of this statement for Lemnaceae, one can speculate that flower inhibition in the LDP and flower promotion in the SDP by such amino acids comes about in the same way as the effect of tungstate, Mo-deficiency, and sulfhydryl blockers, namely by lowering of nitrate reductase activity. The observation that one and the same amino acid can exert a positive or negative effect depending on experimental conditions, may be explained by the fact that the molecule is substrate for protein synthesis and effector for enzyme repression at the same time.

#### **2.4.3.1.4. Significance of carbohydrates and energy-generating processes**

Under long-day conditions, addition of 1% sucrose to the nutrient medium causes an inhibition of flowering in LDPs L. gibba G1 and G3 (HILLMAN 1961c, KANDELER 1968, OOTA 1974), but - especially in combination with a copper supply - a flower promotion in the SDP L. aequinoctialis 6746 (ESASHI and ODA 1964, SCHUSTER 1968, SCHUSTER and KANDELER 1970). Under short day, however, supplemented with a short far-red irradiation at the end of the daily light phase, sucrose enhances flowering considerably in L. gibba G1 (KANDELER 1968). L. aequinoctialis 6746, precultured under long day with sucrose and grown in diluted ammonium-containing Hutner medium, shows a suppression of flowering with sucrose under short day (POSNER 1967b, 1969a, 1970).

Glucose and fructose seem to be effective like sucrose only when the plants were precultured with sucrose: in L. gibba G3 under long day (compare OOTA 1972a and KANDELER et al. 1975), as well as in L. aequinoctialis 6746 under short day or long day respectively (compare POSNER 1970 and KANDELER et al. 1975).

CO<sub>2</sub> enrichment of the air or high-intensity light results in the same effect as sucrose in most of the above-named situations. CO<sub>2</sub> (3.5%) inhibits long-day flowering, but enhances flowering under short day with end-of-day far red in L. gibba G1 (KANDELER 1964a). In L. aequinoctialis 6746 1% CO<sub>2</sub> inhibits flowering like sucrose under short day (POSNER

1971). High-intensity light inhibits flowering under long day in L. gibba G1 (KANDELER et al. 1975). In L. aequinoctialis 6746 high-intensity light in combination with copper makes flowering possible under long day, if a Hoagland-type medium is used (ESASHI and ODA 1964, SCHUSTER 1968). On the contrary, with a 1/2 strength Hutner-sucrose-medium (ammonium during preculture only) high-intensity light inhibits flower initiation in strain 6746 under long day (POSNER et al. 1977).

The correspondence between certain effects of sucrose, CO<sub>2</sub> enrichment, and light intensity suggests that photosynthesis is the physiological process which mediates CO<sub>2</sub>- and light-intensity effects and works by delivering of assimilates. In fact, the effects of high-intensity light, cited above for L. aequinoctialis 6746, can be cancelled by application of the photosynthesis inhibitor DCMU (SCHUSTER 1968, SCHUSTER and KANDELER 1970, POSNER et al. 1977). There are, however, some experimental results which cannot be explained by the assumption that photosynthetically active light acts on flowering by production of assimilates. Short-day as well as long-day flowering can be achieved by DCMU in L. aequinoctialis 6746 although sucrose is present in the nutrient medium (POSNER 1973b, POSNER et al. 1977). (Compare experiments under long day with another nutrient medium where DCMU is not effective in the presence of sucrose; SCHUSTER and KANDELER 1970). The DCMU-mediated lowering of photosynthetically produced reductants may be responsible for this effect (POSNER et al. 1977). Addition of NADPH or NADH to the medium suppresses long-day flowering in strain 6746 whereas ascorbic acid promotes it (KANDELER 1971a). In connection with these statements it should be remembered that in L. aequinoctialis a low nitrate reduction is a prerequisite for long-day flowering (chapter 2.4.3.1.3).

During the light phase DCMU lowers the size of the ATP pool in L. aequinoctialis 6746 (GOWER and POSNER 1979). The DCMU effect on flowering in this strain under long day - reversion of high-intensity-light inhibition of flowering (see above) - is, however, not mediated by this reduction of the ATP pool, because the DCMU action proceeds also after addition of ATP to the medium (POSNER et al. 1977). Nevertheless, ATP or ADP supply has a distinct effect on flowering in strain 6746 under certain other experimental conditions. The percentage of flowering increases, when ATP is applied during the last dark period of three inductive short-day cycles (KHUDAIRI and MAENG 1973). Correspondingly, the uncoupler of oxidative phosphorylation 2,4-dinitrophenol diminishes flow-



ering, when applied during the same time. Long-day flowering of strain 6746, on the other hand, when induced in Pirson-Seidel medium with copper, is drastically reduced by addition of ADP to the medium (KANDELER 1970). In reverting the sucrose inhibition of flowering under short day not only ATP, but also other adenylates, inorganic phosphate, glucose-6-phosphate, and other respiratory intermediates are effective in strain 6746 (POSNER 1971, 1973a).

In L. gibba G1 the flower-promoting effect of DCMU (which blocks noncyclic electron transport, but permits cyclic photophosphorylation) in 1/10 Pirson-Seidel medium can be imitated by application of ADP (KANDELER 1969b). Several uncouplers of oxidative and/or photophosphorylation as arsenate, atebirin, and 2,4-dinitrophenol inhibit flower initiation with or without promoting effect on frond multiplication in this species (KANDELER 1967, OOTA 1969). The effects of arsenate and atebirin as well as the flower-inhibiting effects of sucrose and ammonia can be cancelled by addition of ATP or ADP (KANDELER 1969a, KANDELER et al. 1975). ADP is more effective than ATP in most cases, possibly because of the action of adenylate kinases. ATP and ADP were effective in compensating atebirin inhibition, but not so AMP, adenosine, or adenine. Taking all the results together it seems likely that not only assimilates and photosynthetic reductants but also ATP from photophosphorylation or oxidative phosphorylation can contribute to the physiological processes which induce or inhibit flower initiation in Lemnaceae.

#### 2.4.3.1.5. Photoperiodism and effects of coloured light

Depending on the species, clone, or environmental conditions, the effect of daylength on flowering can be strong, moderate, or lacking in Lemnaceae. A qualitative response is seen in the LDPs L. gibba (KANDELER 1955), and L. turionifera (LANDOLT 1957; BENNINK et al. 1970, named as L. minor), in the SDPs L. aequinoctialis (most strains, YUKAWA and TAKIMOTO 1976) and L. perpusilla (HUEGEL et al. 1979), and in the long-short-day plant W. arrhiza (KRAJNCIC and DEVIDE 1980). A quantitative daylength effect was found in the LDPs S. polyrrhiza (one strain only, KRAJNCIC and DEVIDE 1982a), S. punctata (SCHARFETTER et al. 1978) and S type strains of L. aequinoctialis (BEPPU and TAKIMOTO 1981b), and in the SDPs L. aequinoctialis (strain 151, WATANABE and TAKIMOTO 1979) and Wolffia brasiliensis (MAHESHWARI and SETH 1966b). Day-neutral plants are



S. polyrrhiza (most strains, KRAJNCIC and DEVIDE 1980) and L. aequinoctialis (K type strain 351, YUKAWA and TAKIMOTO 1976). Strain 6746 of L. aequinoctialis behaves as a qualitative SDP under some experimental conditions, but as a day-neutral plant under others (HILLMAN 1959b), and therefore can be denoted as conditional SDP. Strain Sohna of W. microscopica shows a qualitative short-day response, when cultivated in Hoagland medium, but only a quantitative response in Bonner-Devirian medium (VENKATARAMAN et al. 1970). Critical daylengths and the minimal number of inductive light-dark cycles vary from strain to strain and are listed in table 1 in KANDELER (1985).

Most of the effects of coloured light, which have been pointed out in Lemnaceae, are summarized in table 2.26. Blue, red, and far red are the spectral regions, which are preponderantly active in light regulation of flowering, and their effect depends on the timing within the daily light-dark cycle. At least in part the effects of red and far red are phytochrome-mediated: The effect of short far-red irradiation given at the end of the daily light phase (short day), which is flower-inducing in L. gibba G1 and flower-inhibiting in L. aequinoctialis, can be reverted by a following short red light (KANDELER 1962, PURVES 1961, SAJI et al. 1982). Also the effect of a short night break with red light during long night, which induces flowering in L. gibba and suppresses flowering in L. aequinoctialis, is cancelled by subsequent short far red under certain experimental conditions (KATO 1979a, HILLMAN 1966, ISHIGURI and ODA 1974). The distinctness of these phytochrome effects varies in dependence of strain properties and environmental conditions. The end-of-day far-red effect is lacking in strain G3 of L. gibba and in blue-light cultivated L. aequinoctialis 6746 (KANDELER 1968, ISHIGURI and ODA 1976). Further modifying factors of the end-of-day far-red effect in strain 6746 are light intensity and duration of the main light period as well as the copper content of the nutrient medium (SCHUSTER 1968). Night break effectivity is demonstrable in strain G3 only under threshold conditions for flowering (CLELAND and BRIGGS 1968, KANDELER 1968, KATO 1979a, NAKASHIMA 1968).

If far red is applied not at the beginning but during the course of a long dark period, then the effectivity of far-red irradiation decreases gradually with time in L. gibba G1 (KANDELER 1956). Therefore, an hour-glass mechanism may be responsible for the change in sensitivity to this phytochrome effect. Night break effectivity of red light, however, de-

Table 2.26. Effects of blue, red, and far-red irradiation on flowering in Lemna gibba (LDP) and Lemna aequinoctialis (SDP)

+ promotion  
 - inhibition  
 0 no effect in comparison to control  
 in the same paper (compare reference number)

LD long-day  
 SD short-day  
 Low Int. low intensity

Main light period					End-of-day treatment		Night break treatment	
white	white with far red	blue	red	far red	far red	red after far red	red	far red after red
<u>L. gibba G1</u>								
control (LD) 9, 11	+							
control (SD) 11	9,11 control (SD) 9,10,11				9,10,11	- 10	+	
<u>L. gibba G3</u>								
control (LD) 11	+	control (LD) 2,8	- 2,8	- 2,8				
control (SD) 11,12	11 control (SD) 1,11				0 11		0/+ 11/1,12	0/- 1/12
<u>L. aequinoctialis 6746</u>								
control (SD) 15,17			control (SD) 3,6,7		- 7,15	+	- 3,6,7, 15,17	0 3,7,15
control (LD) 13,17		control (SD) 5,6,7	control (LD) 2,4,8,13,17	+	0 7		- 5,6,7	+
Low Int.: + 17		+	Low Int.: + 17	2,8,13				5,7
<u>L. aequinoctialis 335, 421, 391</u>								
control (LD) 18		0 18		0 18				
Low Int.: 0 18								
<u>L. aequinoctialis T-101</u>								
control (SD) 14		- 14	0 14	- 14				
control (LD) 14		0 14	0 14	0 14				
<u>L. aequinoctialis 441</u>								
control (SD) 16					- 16	+	- 16	0 16

#### References:

- |                           |                             |
|---------------------------|-----------------------------|
| 1 CLELAND and BRIGGS 1968 | 10 KANDELER 1962            |
| 2 ESASHI and ODA 1966     | 11 KANDELER 1968            |
| 3 HILLMAN 1959a           | 12 KATO 1979a               |
| 4 HILLMAN 1965            | 13 ODA 1962                 |
| 5 HILLMAN 1966            | 14 OHTANI and ISHIGURI 1979 |
| 6 HILLMAN 1967            | 15 PURVES 1961              |
| 7 ISHIGURI and ODA 1976   | 16 SAJI et al. 1982         |
| 8 ISHIGURI et al. 1975    | 17 TAKIMOTO 1973            |
| 9 KANDELER 1956           | 18 YUKAWA and TAKIMOTO 1976 |

depends on a circadian clock. A brief interruption of a long dark period with red irradiation has a maximal flower-inhibiting effect in L. aequinoctialis 6746, when applied 7 or 9 hours after the beginning of darkness (HILLMAN 1959a, PURVES 1961). In L. gibba G3 a 30 min light interruption, given at various times during a 51 h dark period after a 9 h short day, shows two maxima of effectivity: 12 and 36 hours after the beginning of a dark period (NAKASHIMA 1968). The significance of endogenous rhythms for light regulation of flowering, which is demonstrated by such results, is discussed further in chapter 2.5.9.

Spectral composition of radiation during the main light period also has a strong influence on flower initiation. Supplementation of white light with far red enhances long-day induction of flowering in L. gibba (KANDELER 1956, 1968). Far red or red as the sole source of light during long day, however, cannot induce flowering in strain G3 of L. gibba. Only blue light (ESASHI and ODA 1966) or violet and green (ISHIGURI et al. 1975) have similar effects as white under long day in this plant. When far red and red are applied together during continuous light, a ratio of one to one is optimal for flowering in strain G3. With mixed blue and red light the optimal ratio for flowering amounts to 4.9 (ESASHI and ODA 1966). All these results can be interpreted by the assumption that an intermediate level of active phytochrome is needed during the main light period for optimal flowering in L. gibba.

In the SDP L. aequinoctialis 6746 long-day flowering can be achieved when blue or far red is used instead of white or red (HILLMAN 1965, ISHIGURI et al. 1975, ODA 1962). Under continuous light with two combined spectral regions optimal flowering occurs at values far red/red = 14.8 and blue/red = 13.5 respectively (ESASHI and ODA 1966). Therefore, a relatively low level of active phytochrome seems to allow long-day flowering in strain 6746. Another possibility to achieve long-day flowering in strain 6746 is reduction of white or red light intensity (TAKIMOTO 1973). Both cited effects, long-day flowering by use of blue or far-red irradiation or by lowering of light intensity, are restricted to strain 6746. Other strains of L. aequinoctialis, 335, 391, 421, and T-101, cannot be brought to flower under long day with these special light programmes (OHTANI and ISHIGURI 1979, YUKAWA and TAKIMOTO 1976). In strain T-101 short-day flowering is even suppressed when blue or far-red irradiation is used instead of white or red (OHTANI and ISHIGURI 1979).

#### 2.4.3.1.6. Effects of temperature

Flower induction proceeds in L. gibba G3 with optimal rate, if culture temperatures between 26 and 31°C are used (OOTA 1973). Especially night temperature seems to be critical. Lowering of the temperature to 21°C has a flower-inhibiting effect only when applied during the second half of the daily light-dark cycle. Under continuous light, the same diurnal change in sensitivity to temperature proceeds, if acetylcholine, eserine, sodium lauryl sulfate, ouabain, or indoleacetic acid is added to the culture medium (HOSHINO 1979, OOTA 1973, OOTA and HOSHINO 1974).

In comparison to L. gibba L. aequinoctialis needs lower night temperatures for flowering. A night temperature of 23°C instead of 25°C can compensate the flower-inhibiting effect of sub-critical dark periods in many strains of this species (BEPPU and TAKIMOTO 1981b). In strain 6746 of L. aequinoctialis flower initiation by inductive skeleton photoperiods is optimal at 23°C. Temperatures above and below 23°C markedly diminish flowering (DOSS 1975). Under conditions of full short day a 4 hour period of 33°C (instead of 28°C) is flower-inhibiting when given during the dark period but not during the light period (HILLMAN 1959b). Long-day flowering in a copper-containing medium can be achieved in strain 6746 with a culture temperature of 27°C but not 29.5°C (HILLMAN 1959b). In W. microscopica, a quantitative SDP, a temperature of 22°C has to be used for optimal flower induction (RIMON and GALUN 1968b).

#### 2.4.3.1.7. Effectors of membrane transport

Feeding experiments with axenically cultivated Lemnaceae have revealed many further substances, which influence flowering in a specific way (this means without, or only relatively small, simultaneous effects on vegetative multiplication). Most interesting are some effectors of membrane transport (tab. 2.25E). Acetylcholine, a transmitter substance which depolarizes the postsynaptic membrane in many animal nerves, suppresses flowering in L. gibba G1, but promotes long-day flowering in L. aequinoctialis 6746 (KANDELER 1972). The acetylcholine action seems to be not simply a drug effect, because eserine, an inhibitor of acetylcholine esterase, acts like acetylcholine, presumably by increasing the endogenously produced level of acetylcholine. The occurrence of acetylcholine in L. gibba G3 has been demonstrated by HOSHINO and OOTA (1978).

With a special light program OOTA (1977a) tested the effect of acetylcholine in strain G3. He found that this substance eliminates the effect of photoperiodic "L1-phase", which works as the dawn signal for entrainment of the circadian clock (see chapter 2.5.9.). Other membrane effectors, the  $K^+$ -ionophores valinomycin and gramicidine, can substitute for the light requirement of "L1-phase" (OOTA 1977b). A further argument that effectors of membrane transport exert their effects on flowering through an influence on the circadian rhythm, comes from experiments with lithium chloride. On the one hand, lithium acts like acetylcholine on flowering in L. gibba and L. aequinoctialis (KANDELER 1970). On the other hand, this ion lengthens the period of the free-running  $K^+$ -uptake rhythm in L. gibba G3 (KONDO and TSUDZUKI 1980c).

Lowering of calcium or phosphate concentration in the nutrient medium allows or enhances long-day flowering in L. aequinoctialis 6746 (HILLMAN 1962), but is flower-inhibiting under short day in combination with sucrose and ammonium (HILLMAN and POSNER 1971, POSNER 1969a). In the same plant material calcium and phosphate ions inhibit the leakage of one or more flower-promoting substances, when plants are transferred to distilled water for 4 hours during long night (HALABAN and HILLMAN 1970a, 1971). Thus, calcium and phosphate may influence flowering by changing the conditions for membrane transport. In L. gibba G3, lowering of calcium or phosphate concentration diminishes the flower-promoting effect of suboptimal salicylic acid concentrations (MAENG and BAE 1984). DMSO, rendering the cell membrane more permeable to small molecules, has a similar effect as low  $Ca^{2+}$  or phosphate in  $NH_4^+$ -free 1/2 Hutner medium.

#### 2.4.3.1.8. Some further factors

Ferricyanide has a flower-promoting effect in both L. gibba G3 and L. aequinoctialis 6746. In L. gibba some short-day flowering (TANAKA and CLELAND 1980, WATANABE and TAKIMOTO 1977) and in L. aequinoctialis flowering under long day (TAKIMOTO and TANAKA 1973, 1976, TANAKA and TAKIMOTO 1978, TAKIMOTO et al. 1979b) is made possible. Ferrocyanide and cyanide have the same effect as ferricyanide in strains G3 and 6746 (TAKIMOTO and TANAKA 1976, TANAKA and CLELAND 1980, TANAKA and TAKIMOTO 1978, TANAKA et al. 1983), indicating that released cyanide may be the active component within the plant. In L. gibba ferricyanide and salicylic acid show a synergistic interaction. Applied together, they induce some flow-

ering even under light-dark cycles with only 3 hours light (TANAKA and CLELAND 1980).

Nicotinic acid induces flowering in L. gibba G3 and L. aequinoctialis 151 and 381 when they are grown in one tenth-strength M medium under continuous light (FUJIOKA et al. 1986b). The effect is modified by supply of hormones. GA<sub>3</sub> and ABA are inhibiting in both species, GA<sub>3</sub> being more active in L. aequinoctialis and ABA being more active in L. gibba. Zeatin increases the effect of nicotinic acid in L. aequinoctialis, but decreases this effect in L. gibba. IAA has a relatively small inhibiting effect in both species. Preliminary studies indicate that nicotinic acid is present in Lemna in significantly higher levels than is benzoic acid (FUJIOKA et al. 1986a). Little or no difference in the endogenous levels of nicotinic acid between vegetative and flowering Lemna has been found. Another flower-promoting agent in L. gibba and L. aequinoctialis is cyclic adenosine-3'-5'-monophosphate (cyclic AMP) a "second messenger" in animal cells. Addition of cyclic AMP to the nutrient medium enhances flowering in L. gibba G3 under a variety of experimental conditions (KESSLER and STEINBERG 1973, OOTA 1972a, 1977a, OOTA and KONDO 1974). In L. aequinoctialis 6746 cyclic AMP acts like other adenine derivatives in reverting the sucrose inhibition of flowering under short day (POSNER 1973a). Endogenous cyclic AMP may be involved in flower initiation, because adenylyl cyclase-activating catecholamines such as isoproterenol and epinephrine have a similar effect as exogenous cyclic AMP in strains G3 and 6746 (IVES and POSNER 1982, OOTA 1974, 1977a).

In L. aequinoctialis, strains 151 and 6746, vitamin K<sub>5</sub> is a further flower-inducing agent, when plants are held in one tenth-strength M medium under continuous light (KAIHARA and TAKIMOTO 1985a). The substance acts synergistically with benzyladenine (strain 151), benzoic acid, ferricyanide (both strains), copper, and high-intensity light (strain 6746).

Inhibition of flowering has been found in L. gibba G3 by some antimetabolites of nucleic acid synthesis. At certain concentrations 5-fluorodeoxyuridine (blocker of DNA replication), 5-fluorouracil (blocker of DNA replication and transcription), and 2-thiouracil (blocker of DNA transcription) reduce flowering percentage without effect on frond multiplication (UMEMURA and OOTA 1965a).

Short-day flowering in L. aequinoctialis 6746 is inhibited to some extent by 5-fluorouracil and 2-thiouracil as well as by some antimetabo-



lites of protein synthesis (cycloheximide, ethionine, and chloramphenicol), when applied for only 2 hours during the first four inductive dark periods (DOSS 1975c). The strong flower-inhibiting effect of a short night break, however, is diminished by a two hour treatment with 2-thiouracil or actinomycin D (DOSS 1975b).

Concluding this chapter, a flower-inhibiting effect of agar may be mentioned. In L. gibba G3 various agars and even the most highly purified are very inhibitory to flowering (CLELAND 1985). The effect of 0.5% agar can be abolished by supply of 3.2  $\mu$ M salicylic acid.

#### 2.4.3.2. Flower and fruit development

In many plants photoperiodic requirements for flower induction and further development of flower primordia are different, but Lemnaceae belong to a group of plants, which need the same daylength for initiation as well as further growth of the flower (compare type 3 in: VINCE-PRUE, Photoperiodism in Plants, McGraw-Hill, 1975). In the LDP L. gibba G3 at least 6 long days are required for reaching mature flowers, whereas a minimum of two long days is sufficient to induce the formation of flower primordia (CLELAND and BRIGGS 1967). When plants of strain G1 were transferred from continuous light to short day, then in the generation, which is the last but one at the beginning of short-day treatment, shrunken primordia of stage II (stamens and pistil fully meristematic) dominate. In the following generation only dried stage I primordia (without organ differentiation) or no flower residues can be seen (KANDELER, unpublished results). Obviously, flower primordia dye and shrink immediately after transfer of plants to non-inductive photoperiodic conditions. This holds true also in SDPs L. aequinoctialis and L. perpusilla. A regression or abortion of flower primordia after transfer to long day or night-break treatment has been observed in these species by several authors (BENNINK and DE VRIES 1975, DOSS 1975a, HILLMAN 1959a, 1969c, KIRKLAND and POSNER 1974, SATO and ODA 1977, SHIBATA and TAKIMOTO 1975). The inhibition of flower differentiation in SDPs by long day can be overcome by some treatments: low temperature SATO and ODA 1978), low light intensity (HILLMAN 1969c, KIRKLAND and POSNER 1974), blue light, DCMU (KIRKLAND and POSNER 1974), or benzyladenine (BENNINK and DE VRIES 1975). In L. aequinoctialis 6746 at each temperature there is a certain threshold level of light intensity, which is without any inhib-



iting or promoting activity for flower-bud development (SHIBATA and TAKIMOTO 1975).

The statement that flower initiation and further flower development are influenced in the same manner seems to be valid not only for the effect of day-length. This can be drawn from the general rule that conditions, which are flower-inducing to only a small extent (s. low flowering percentage), simultaneously lead to an increase in dried up flower primordia. In L. gibba G1 and G3 this has been found time and again in experiments, where flowering was modified by very different agents (for example far red irradiation, medium composition, CO<sub>2</sub>, sucrose, ATP and ADP, DCMU, arsenate, and atebrin) (BUECHELE and KANDELER, unpublished results). In L. aequinoctialis 6746 long-day flowering has been induced only to a certain degree by supply of CCC plus ABA (KANDELER and HUEGEL 1973) and also in this case appearance of aborted flower primordia was enhanced.

The equilibrated development of male and female flower organs depends on a certain balance of gibberellins and ethylene. Hormones are produced by the flower primordium only in part and must be completed by hormonal imports from the leaf. When flower primordia of L. gibba G1 are explanted to an agar medium which contains minerals, sucrose, and kinetin, then the development of the pistil preponderates. The two stamens remain relatively small. A normalization of in vitro flower development, due to a promotion of stamen growth, is achieved by addition of gibberellin A<sub>3</sub> to the agar medium, whereas CCC (blocker of gibberellin synthesis), ethephon (ethylene-releasing agent), or IAA enhance feminization by a further promotion of pistil growth (HUEGEL 1976a, 1976b). Explants of L. aequinoctialis 6746 cultured on the above cited minimal medium preferably develop the stamens. This masculinization can be corrected by supply of CCC or ethephon which allow better pistil growth also in strain 6746 (HUEGEL 1976a,b). Low concentrations of kinetin as well as ABA enhance the one-sidedness of in vitro sex expression in both species, presumably by a more general promoting resp. inhibiting effect on organ development (HUEGEL 1976c). The opposite behaviour of L. gibba and L. aequinoctialis may be caused by a different endogenous production or accumulation capacity of gibberellins (compare the statements in connection with photoperiodic flower induction, chapter 2.4.3.1.1), but ethylene metabolism may also be different in the two species. The tendency to feminization of L. gibba has been observed in several strains also in

sterile cultures, i.e. in vivo (LANDOLT, unpublished results).

DNA duplication in connection with microsporogenesis has been investigated in W. microscopica using autoradiographic methods (RIMON and GALUN 1968b). Unexpectedly, optimal incorporation of tritiated thymidine into the nuclei of future pollen cells was obtained when labelling was done at a stage at which the whole floral bud was not yet anatomically recognizable. The authors conclude that thymidine -<sup>3</sup>H incorporation into the nuclei of microspores occurs in all probability during one of the premeiotic mitotic interphases.

The physiology of pollination and fruit development has not yet been investigated in Lemnaceae. HUEGEL (1974) mentioned a special development of pistil and parthenocarpic development of seeds in L. gibba, when advanced flower primordia were explanted on to an agar medium with 16% sucrose. POSNER (1962b) induced some aberrant strains of L. aequinoctialis 6746 by X-irradiation which produced very few seeds and these always lacked embryos. These strains may be a good material for further investigations.

## 2.5. METABOLISM

### 2.5.1. Generation of energy

#### 2.5.1.1. Photosynthesis

##### 2.5.1.1.1. Factors limiting or restricting photosynthesis

In older work photosynthesis has been investigated by measuring dry-weight increase or growth rate (see chapter 2.3.5.1.). ZURZYCKI (1953, 1955a,c) used a microtechnique based on a capillary tube respirometer for photosynthesis measurements in L. trisulca. He showed that during the first 15-30 minutes of low-intensity illumination photosynthesis depends on the rearrangement of chloroplasts. O<sub>2</sub> production increases with the displacement of chloroplasts from a random position (apostrophe in darkness) to a flat position (epistrophe in weak light). Under high light intensities gas exchange proceeds with a constant rate independent of simultaneous changes in chloroplast arrangement (transition to profile position, parastrophe in strong light). Epistrophe lasts between 10 and 800 lux, full parastrophe is reached approximately in those light intensities (12000 lux) in which a further increase of intensity has no influence on photosynthesis (for analysis of chloroplast movements see chapter 2.4.1.6).

A farther reaching adaptation to different light intensities seems to exist on the basis of developmental processes. Plants of S. polyrrhiza precultivated under 1400 lux show a saturation of photosynthesis at about 4400 lux. Exposure to strong light (10000-50000 lux for 12 h) leads to a substantial increase (to 60-85%) in the rate of photosynthesis, which is not eliminated by a subsequent exposure to 1400 lux (GAPO-NENKO and STAZHETSKII 1969).

A further limiting factor for photosynthesis, effective in floating plants only, is the behavior of stomata on the upper side of the fronds. In S. punctata stomata close during water deficit and - to a various extent - after application of ABA, metabolic inhibitors (CMU, FCCP), or ionophores (valinomycin, nigericin), but not after darkening (SEVERI and BARONI FORNASIERO 1982, 1983b). In Lemna, however, stomata are not movable and remain open under all conditions (HABERLANDT 1887, WAGNER 1973,

cit. in HUBER and SANKHLA 1979). For example,  $10^{-5}$  M ABA added to the medium for 24 h has no effect on the degree of opening in L. gibba G1 (HARTUNG, personal comm.). In L. minor CMU, FCCP, valinomycin, and nigericin are not effective as in S. punctata (fig. 2.35). Nevertheless, young stomata of L. minor, which are still viable and occur especially in the basal part of fronds, exhibit a weak movableness (HABERLANDT 1887, REUTER 1948).

As in other plants light intensities considerably above those experienced during growth inhibit photosynthesis reversibly. This so-called photoinhibition has been found in L. trisulca (ZURZYCKI 1957a), L. minor (LINDEMAN 1979), and L. gibba (NILSEN and DANIELSEN 1984, OEGREN et al.

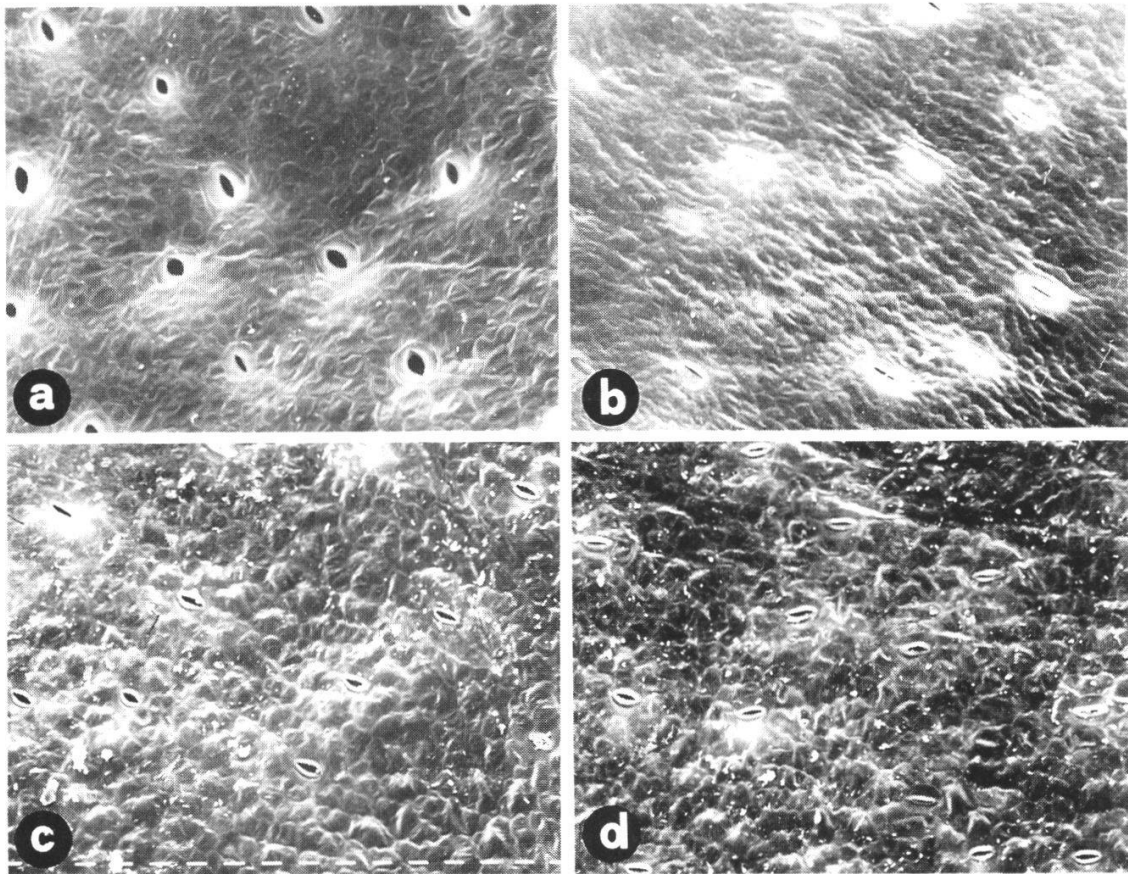


Fig. 2.35. Movability of stomata in Lemnaceae (SEVERI and BARONI FORNASIERO 1983b). In Spirodela punctata a 6 hour treatment with CMU, FCCP, valinomycin, or nigericin results in a strong closing of stomata (b) in comparison to untreated controls (a). In Lemna minor, on the other hand, treated fronds (d) and control fronds (c) show about the same degree of aperture (SEM pictures; bar = 10  $\mu$ m).  
(With permission of Dr. A. Severi)

1984, OEGREN and OEQUIST 1984a, b). Factors which restrict photosynthesis such as chilling (2-3°C. LINDEMAN 1979, OEGREN et al. 1984), or N<sub>2</sub> atmosphere (NILSEN and DANIELSEN 1984) lower the light intensity necessary for the inhibitory effect of bright light. The papers dealing with L. gibba come to the conclusion that inactivation of photosystem II-mediated electron transport occurs during photoinhibition. Polarization of light (both linear and circular) has no effect on photosynthesis in L. trisulca (ZURZYCKI 1955b).

In S. punctata and L. minor maximum rates of O<sub>2</sub> production and <sup>14</sup>CO<sub>2</sub> uptake are reached at 35°C (WEDGE and BURRIS 1979). The range of favourable temperatures is broader in L. minor than in S. punctata, but at 10°C and 45°C there is no O<sub>2</sub> evolution in either species. At 35°C S. punctata evolves 1.6 µl O<sub>2</sub>/h/mg fresh wt. (L. minor: 1.1 µl O<sub>2</sub>/h/mg fresh wt.). 12-hour experiments with L. minor grown in continuous light showed that a diurnal fluctuation in photosynthesis does exist, with a peak in photosynthesis appearing at midday.

Availability of CO<sub>2</sub> is another factor which determines the rate of photosynthesis in many cases. The great differences in accumulation of photosynthetic starch appearing in L. gibba after 3 h cultivation in open dishes, open or cotton-plug closed vessels, may be caused mainly by differences in CO<sub>2</sub> supply (KOENIGSHOFER, personal comm.). When the CO<sub>2</sub> concentration of the atmosphere is raised from 300 to 1000 ppm, under high-intensity light conditions (17000 lux, equivalent to 240 µE m<sup>-2</sup>s<sup>-1</sup> PAR; temperature 27±1°C) the photosynthetic rate increases from about 107 to 165 mg CO<sub>2</sub>/h/g dry wt. in S. punctata, from 160 to 250 mg CO<sub>2</sub>/h/g dry wt. in L. minor, and from 135 to 300 mg CO<sub>2</sub>/h/g dry wt. in L. valdiviana (LOATS et al. 1981). Correspondingly FUHRER and ERISMANN (1983) found a highly significant correlation between CO<sub>2</sub> input (100-700 ppm) and dry weight increase in L. minor. In L. gibba the net assimilation rate increases with CO<sub>2</sub> enrichment of the air till 1500 ppm CO<sub>2</sub> (ANDERSEN et al. 1985, BJOERNDAL and NILSEN 1985). A dramatic deterioration of CO<sub>2</sub> supply for photosynthesis results from vacuum infiltration of fronds. O<sub>2</sub> evolution in L. minor (211 µmoles/h/g/ fresh wt. under 18000 lux of incandescent light, at 25°C) is eliminated completely by vacuum infiltration, whereas O<sub>2</sub> absorption in the dark is not lowered by such treatment (McDONALD 1975). FILBIN and HOUGH (1985) concluded from in situ investigations that in field populations of L. minor an average of 86% of fixed carbon was from aqueous inorganic carbon. ESHEL and BEER (1986),

however, demonstrated in laboratory experiments with S. polyrrhiza that carbon assimilation from the aqueous phase amounts only up to 5% of that from the air.

Restriction of photosynthesis can be achieved by many other factors: limitation of nitrogen supply (INGEMARSSON et al. 1984), inactivation of glutamine synthetase (JOHANSSON and LARSSON 1986a, b), phosphate deficiency (LINDEMAN 1951), feeding with sodium acetate (BRUNOLD and ERISMANN 1968), salt stress with sodium chloride (HUBER and SANKHLA 1979), supply of ABA (BAUER et al. 1976, McLAREN and SMITH 1976, TILLBERG et al. 1981), fumigation with SO<sub>2</sub> (LOATS et al. 1981), "synthetic smog", that is a mixture of ozone and hexene (ERICKSON and WEDDING 1956), application of certain herbicides (BIELECKI and SKRABKA 1976), and preculture with the morphactin "chloroflurenol" (TREICHEL 1974b).

The effect of reduced nitrogen supply on photosynthesis in L. gibba is apparent only when calculated on a dry weight basis, but not on a chlorophyll basis. Thus reduced nitrogen works in a rather indirect way (presumably restricting the differentiation of chloroplasts). The light compensation point was unaffected by the nitrogen regime (INGEMARSSON et al. 1984). Nevertheless, blocking of glutamine synthetase by MSO (L-methionine-D,L-sulphoximine) decreases CO<sub>2</sub> fixation in L. gibba to 50% within 100-150 min (JOHANSSON and LARSSON 1986a, b). The inhibiting effect of P-deficiency on photosynthesis in L. minor can be abolished within a few hours by supply of phosphate. At rate limiting light intensities reactivation is greater at 650 nm than at 700, 711, and 717 nm (LINDEMAN 1951, 1972). Sodium acetate ( $\geq 10^{-3}$  M) taken up from the medium may consume photosynthetic ATP and NADPH for assimilation and therefore compete with CO<sub>2</sub> fixation. In addition, fatty acids are produced from assimilated acetate and may inhibit some photosynthetic reactions as ATP synthesis, carboxylation of RuDP, and dephosphorylation of FDP (BRUNOLD and ERISMANN 1968).

High concentrations of NaCl (85 mM) slow down the photosynthetic electron transport (HILL reaction) in L. minor, but have no effect on the pattern of <sup>14</sup>CO<sub>2</sub> fixation (HUBER and SANKHLA 1979). Exogenous ABA acts in a similar way (BAUER et al. 1976, McLAREN and SMITH 1977). As NaCl increases endogenous ABA level up to 1.5 times and exogenous ABA lead to an endogenous ABA concentration about twice as high as in the control plants, salt stress may act on photosynthesis through the "stress hormone" ABA (HUBER and SANKHLA 1979, SANKHLA and HUBER 1979). Preliminary



experiments of McLAREN (cited in McLAREN and SMITH 1977) indicate that ABA decreases the chloroplast membrane permeability to 3-phosphoglyceric acid. This could be the explanation for the fact that starch is accumulated in chloroplasts of ABA-treated plants (McLAREN and SMITH 1976). Fumigation with 0.75 ppm  $\text{SO}_2$  during preculture for 8 h each day leads to an inhibition of net photosynthesis in S. punctata and L. valdiviana, but not in L. minor (LOATS et al. 1981). L. minor seems to oxidize  $\text{SO}_2$  to  $\text{SO}_4^{2-}$  (BRUNOLD and ERISMANN 1976) more efficiently than the other species. The inhibition of photosynthesis after fumigation with 1 ppm ozone can be explained by chlorophyll degradation. In the case of an ozone-hexene mixture, however, reduction of photosynthesis exceeds that of chlorophyll loss (ERICKSON and WEDDING 1956). Herbicides which inhibit photosynthesis are Prometryne, Ametryne, Diuron (DCMU), Fluometuron (CEDENO-MALDONADO and LIU 1976), and Afalon (50% Linuron), Gramoxone (20% paraquat), Simazine and Krezamon (50% DNOC) (BIELECKI and SKRABKA 1976). The morphactin, 2-chloro-9-hydroxyfluorene-carboxylate-(9) (chloroflurenol) influences photosynthesis and related processes only if the plants are treated with the substance for at least 9 days. Morphogenetic changes seem to be involved during this time. Photosynthetic  $\text{O}_2$  production,  $\text{CO}_2$  consumption, and  $^{14}\text{CO}_2$  incorporation all are reduced after prolonged morphactin treatment, whereas starch, ATP, and chlorophyll content increase (TREICHEL 1974b).

#### 2.5.1.1.2. Steps of the photosynthetic process

In several cases Lemnaceae have been used to investigate certain steps of the photosynthetic process: action of the two photosystems, the chain of electron transport, generation of reduction energy, production of ATP, assimilation of  $\text{CO}_2$ , and light respiration.

The presence of the Emerson enhancement effect in L. minor and L. gibba shows that photosystems I and II work as in other plants (LINDEMAN 1973, CHATURVEDI et al. 1982). Capacity for photosynthetic  $\text{O}_2$  evolution depends on the mode of preillumination. Lemna illuminated for 5 to 7 days every 15 min by a 1 ms strong polychromatic flash has no capacity for  $\text{O}_2$  evolution, and stroma thylakoids of these plants are unfused. Three to 6 minutes of illumination with photosynthetic actinic light are enough to induce photosystem II-activity and a fused state of stroma thylakoids (STRASSER 1974). The author hypothesizes that a manganese reducing sys-



tem may be created during the induction period.

The DCMU-sensitive step in photosynthetic electron transport has been investigated with S. punctata (MATTOO et al. 1981, 1984a). DCMU seems to bind reversibly to a 32 kDa polypeptide, which is the main thylakoid protein. Plants depleted of this protein by incubation with low levels of chloramphenicol (WEINBAUM et al. 1979a) show a selective inhibition of  $e^-$  transport at the reducing side of photosystem II, especially between the primary quencher and plastoquinone pool. The 32 kDa protein may be a "proteinaceous shield" covering the primary electron acceptor of photosystem II and acting as a regulator of electron flow between photosystem II and photosystem I. The protein is not rate limiting for  $CO_2$  fixation. Photoassimilation of  $CO_2$  remains at 86% of controls in chloramphenicol-treated plants containing less than 20% of 32 kDa protein (WEINBAUM et al. 1979a). Biosynthesis, turnover, and light induction of the 32 kDa protein have been investigated extensively (chapter 2.5.7.3.2). The binding site of triazine herbicides in photosystem II seems not to be identical with the 32 kDa protein but located in close connection (GRESSEL 1982). In L. minor 1.0 ppm atrazine reduces photosynthetic  $O_2$  evolution to 50%. Lower concentrations (0.02-0.10 ppm) however, distinctly stimulate photosynthesis (BEAUMONT et al. 1976).

A block between plastoquinone and cytochrome f in the photosynthetic electron transport system has been found in a mutant strain 1073, which was isolated from an x-irradiated culture of wild type strain 6746 of L. aequinoctialis (SHAHAK et al. 1976). Isolated chloroplasts of the mutant are able to reduce diaminodurene (DAD) or phenylenediamine (lipophilic electron acceptors at the level of plastoquinone) but not its own cytochrome f. Electron paramagnetic resonance analysis of mutant chloroplasts reveals a lack of the "Rieske" iron-sulfur center signal (MALKIN and POSNER 1978). This Rieske center may be either absent or defective in the mutant.

Moderately high Hill activity of crude chloroplasts was demonstrated in L. minor and W. borealis as early as 1950 (CLENDENNING and GORHAM 1950). DYAR (1953) then used blue tetrazolium as an in vivo indicator of the Hill reaction and showed photochemical reduction of the tetrazolium salt to be located especially in grana-like regions of chloroplasts in fronds and roots of L. minor. Manganese requirement during culture of L. minor for optimum Hill reaction of chloroplasts has been found to be  $5 \times 10^{-2}$  ppm (EYSTER et al. 1958). Hill activity of isolated chloroplasts

from W. arrhiza was determined by EICHHORN and AUGSTEN (1983a). Plants were cultivated in chemostat steady state populations under continuous white, blue, or red light. Deviations in Hill activity caused by blue or red light preculture do not exceed 10-15% of white light control. Supply of boron (0.5-1 ppm) to the nutrient medium, however, reduces Hill activity to a greater extent (to about 50% with material grown under red light and high growth rate conditions). Isolated chloroplasts from L. gibba have been used for measurement of Hill activity in connection with photoinhibition of photosynthesis (see chapter 2.5.1.1.1). Pretreatment of plant material with high photosynthetic photon flux density reduces the capacity of whole chain electron transport, as well as efficiency of electron transport to the primary electron acceptor Q of photosystem II, measured as variable chlorophyll fluorescence at 20°C (OEGREN and OEQUIST 1984a). ABA supply during preculture of L. minor has an inhibiting effect on the Hill reaction of isolated chloroplasts (BAUER et al. 1976). A method to prepare isolated chloroplasts from S. polyrrhiza is given by BAHL (1971).

A method for determination of minute amounts of reduced and oxidized NADP and NAD from S. polyrrhiza was worked out by MONEGER et al. (1977) and VERMEERSCH et al. (1977b). Oxidized forms of pyridine nucleotides are largely predominant and NADPH represents a high proportion of reduced forms (plants cultivated with or without addition of sucrose). Sucrose feeding provokes a decrease in content of all pyridine nucleotides (D'HARLINGUE et al. 1976, LECHEVALLIER et al. 1977). Culture of S. polyrrhiza under weak red light leads to a decrease of the NADPH/NADP ratio (measurements from the whole tissue as well as from isolated chloroplasts (LECHEVALLIER et al. 1977, VERMEERSCH et al. 1977a). Plants cultivated in a medium enriched with calcium (20-40 mI/l calcium instead of 4) have an increased total pyridine nucleotide content and a decreased NADPH/NADP ratio (LECHEVALLIER 1977a).

A small part of photosynthetic reduction energy is used for driving the xanthophyll cycle. Within the chloroplast violaxanthin is reduced to antheraxanthin and zeaxanthin, and these substances are re-transformed to violaxanthin by oxygen (fig. 2.36). In L. gibba G3 zeaxanthin is accumulated only during the first 10-20 minutes of illumination (transition phase) and - under steady state conditions - in the case of high light intensities (> 10000 lux) (SIEFERMANN 1971, 1972). Under the conditions used in these experiments CO<sub>2</sub> consumption is saturated at 35000 lux.

Energy charge of the adenylate system,  $ATP + 1/2 ADP/ATP+ADP+AMP$ , is not affected by light intensity (500-4400 lux) and remains nearly constant throughout a 1 day period when plants of *L.gibba* G3 in mixotrophic culture are exposed to short day, continuous light, or continuous darkness. Depletion of sucrose from the medium, at least under continuous light and continuous darkness, causes no change in energy charge (KONDO and NAKASHIMA 1979). Correspondingly, EICHHORN and AUGSTEN (1977a) found only slight changes of energy charge after supply of diverse carbohydrates

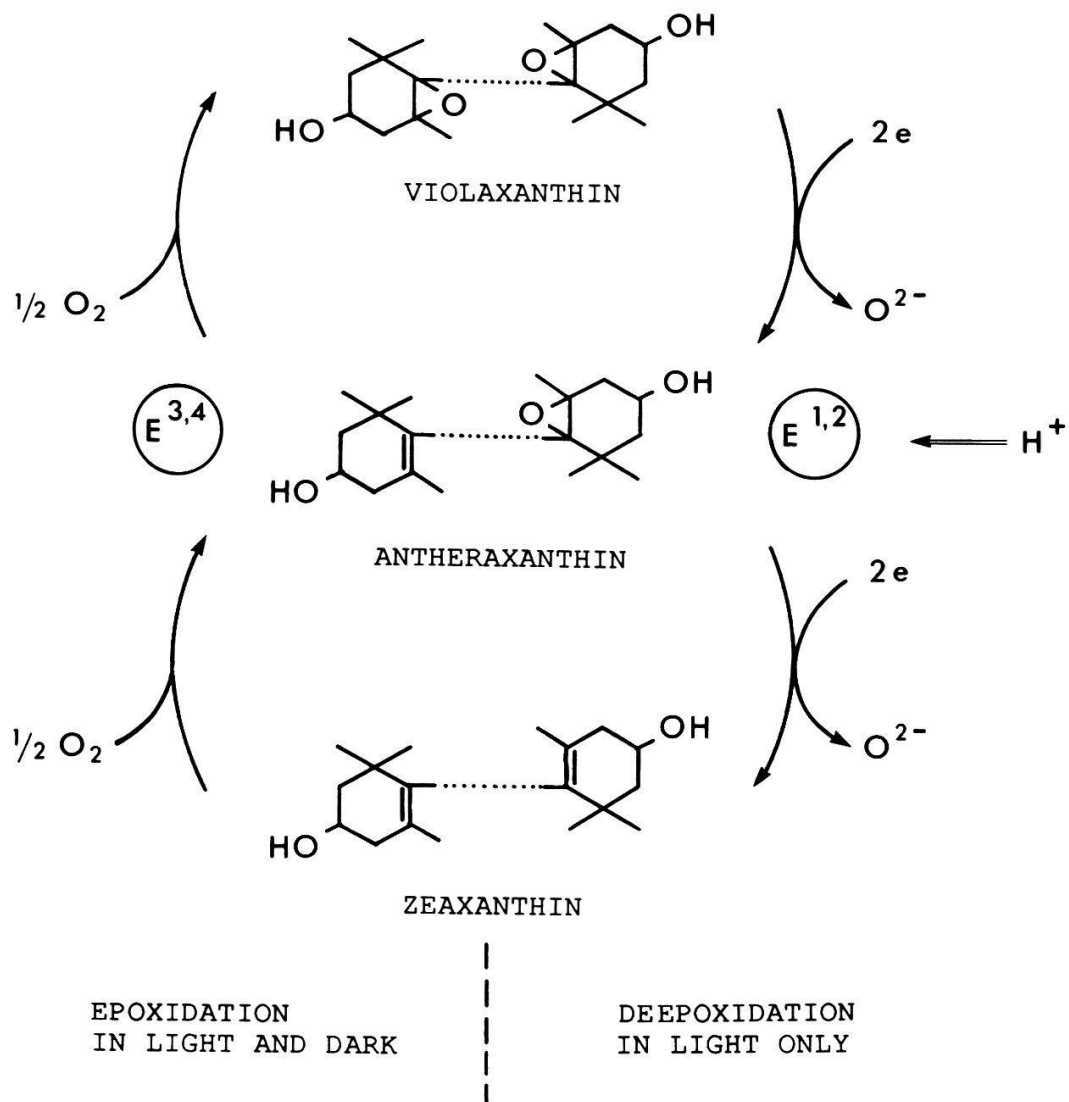


Fig. 2.36. Scheme of the xanthophyll cycle (after SIEFERMANN 1972).  $E^{1,2}$  are pH-dependent enzymes of deepoxidation;  $E^{3,4}$  are enzymes of epoxidation.

to W. arrhiza cultures under light and dark conditions. In L. aequinoctialis pool sizes of ATP were determined by GOWER and POSNER (1979). Wild type fronds (strain 6746) show a higher ATP level than the photosynthetic mutant (strain 1073) under light and dark conditions. DCMU (1  $\mu$ M; not effective in the mutant) lowers rather than raises the ATP level in wild type plants during prolonged exposure to light. Supply of  $\text{NH}_4\text{NO}_3$  instead of  $\text{KNO}_3$  to tenth-strength Hutner's medium (containing 1% sucrose) increases the ATP pool in both strains. In steady-state populations of W. arrhiza adenosinephosphate pools and energy charge are higher in blue-light cultivated plants, as compared with plants cultivated in red light (EICHHORN and AUGSTEN 1977b). Protein and chlorophyll content, however, are also elevated under blue light.

Photosynthetic  $\text{CO}_2$  assimilation proceeds through the reactions typical for  $\text{C}_3$  plants as indicated by the lack of Kranz leaf anatomy (RAGHAVENDRA and DAS 1976), the pattern of  $^{14}\text{CO}_2$  fixation (BAUER et al. 1976) and a distinct photorespiration (ERISMANN 1972). In L. minor BAUER et al. (1976) found labelling to be highest in 3-phosphoglyceric acid and sugarphosphate-esters after a  $^{14}\text{CO}_2$  pulse of 10 sec. Relatively fast labelling occurs also in some amino acids: alanine, aspartate, serine, and glycine (BAUER et al. 1976, ERISMANN and KIRK 1967). Supply of ammonium instead of nitrate changes the steady-state levels of photosynthetically produced sugars and amino acids. The formation of sucrose is favoured at the cost of glucose and fructose. Ammonium increases aspartic acid and glutamic acid (ERISMANN and KIRK 1969). Carbonic anhydrase, catalyzing the reversible hydration of  $\text{CO}_2$ , shows a very low activity in L. trisulca, but an intermediate activity in L. minor and W. columbiana. The results correspond with those of other submersed or floating macrophytes (WEAVER and WETZEL 1980).

Much work has been done by Ziegler and co-workers to investigate the light-induced increase in the activity of NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (NADP-GPD) in L. gibba. Activation of the enzyme by light proceeds within some minutes and needs high light intensities (11-37  $\text{mW.cm}^{-2}$ ) for an optimal effect. Simultaneously, activity of the NAD-dependent GPD decreases (ZIEGLER et al. 1968). The light induction seems to be mediated by chlorophyll (SCHMIDT-CLAUSEN and ZIEGLER 1969) and depends on an operating non-cyclic electron transport. DCMU and some other metabolic inhibitors block the light effect completely (ZIEGLER and ZIEGLER 1966). Actinomycin C has no influence, but chloram-

phenicol (2.5 mg/ml) suppresses the light activation of NADP-GPD (ZIEGLER and ZIEGLER 1965). On the basis of further kinetic studies for light activation and dark inactivation and of their earlier results the authors proposed a model in which activation of NADP-GPD is accomplished by association of four subunits of the enzyme to the activated tetrameric form. NADPH, created during the non-cyclic electron flow, could be the allosteric effector (SCHMIDT-CLAUSEN et al. 1969). Blue light (450-500 nm) not only has an activating effect on NADP-GPD through chlorophyll, but also an inhibiting effect as shown under optimum light-inducing conditions by elimination of particular bands of wavelengths from the complete spectrum (SCHMIDT-CLAUSEN and ZIEGLER 1969). Blue-light enhanced respiration with a concomitant decrease of photosynthetic  $O_2$  evolution could be the cause for this phenomenon.

Photorespiration was substantiated in L. minor by  $CO_2$  outburst phenomena occurring after darkening in cultures held under pure oxygen (fig. 2.37) or in light after short  $CO_2$  fixation and a following change from  $N_2$  to  $O_2$  fumigation (ERISMANN 1972). Also several other criteria suggest the presence of photorespiration in L. minor (FILBIN and HOUGH 1985). The ratio  $\alpha$  between oxygenation and carboxylation of ribulose-1,5-diphosphate was calculated by FUHRER and ERISMANN (1984) on the basis of  $CO_2$  compensation concentration and the intercellular  $CO_2$  concentration. This ratio  $\alpha$  is lowered when plants are transferred from 25 to 15°C. Supply of ammonium instead of nitrate, however, has no influence on  $\alpha$ . Under conditions allowing a low photorespiratory  $^{14}CO_2$  release only (2%  $O_2$  fumigation), DCMU increases photorespiration in L. gibba significantly (CHATURVEDI et al. 1982). The authors come to the conclusion that electrons from the oxidation of glycolate are donated to photosystem I when the electron supply from water is low. Glycolate oxidase in L. minor has a high specific activity (FREDERICK et al. 1973). The enzyme activity is doubled in plants grown with an ammonium nitrogen source as compared with plants grown on nitrate. In addition, the quaternary structure of glycolate oxidase is altered. Ammonium-consuming plants contain a high-molecular-weight form of enzyme (about 500000). After transition to nitrate nutrition molecular weight of glycolate oxidase amounts to about 250000 (EMES and ERISMANN 1982). As expected,  $NH_4^+$  plants evolve more  $^{14}CO_2$  from labeled glycolate than  $NO_3^-$  plants (MARQUES and ERISMANN 1984, MARQUES et al. 1985). Formate is labeled only from (2- $^{14}C$ ) glycolate and in  $NH_4^+$  plants it is markedly less labeled in the light than

in the dark. TILLBERG (1980) found a stimulation of growth in L. gibba G3 by supply of 0.5 mM glyoxylate or glycolate and interpreted the effect as mediated by an inhibition of photorespiration.

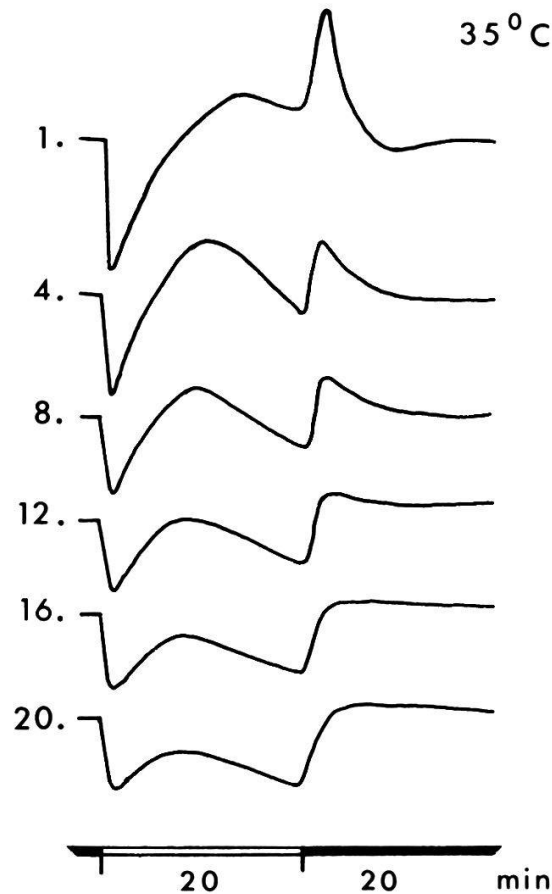


Fig. 2.37. Photorespiration in Lemna minor as shown by the course of CO<sub>2</sub> evolution during repeated light-dark changes (20:20 min) under pure oxygen (ERISMANN 1972). Curve sections from every fourth light-dark period are shown. The magnitude of CO<sub>2</sub> outburst at the beginning of the dark phase corresponds to the value of transient CO<sub>2</sub> evolution during the preceding light phase. Weakening of both phenomena during the course of the experiment may be caused by an exhaustion of substrate for photorespiration. (A short supply of glycolate to the plants can restore the CO<sub>2</sub> outburst phenomena.)

#### 2.5.1.2. Respiration

In L. aequinoctialis respiration of intact plants has been compared with gas exchange of isolated mitochondria (LOEPPERT 1983). In both cases  $O_2$  consumption shows the same dependence on dissolved oxygen concentration. Half-maximum respiration rate ( $K_m$ ) for intact plants is reached at  $1.15 \pm 0.08 \mu M O_2$  and for isolated mitochondria at  $1.07 \pm 0.06 \mu M O_2$ . From this fact it can be concluded that diffusion of oxygen within the tissue is obviously not rate-limiting for respiration in Lemna. At  $2.5 \mu M$  dissolved  $O_2$  (in equilibrium with 1%  $O_2$  in air) oxygen consumption amounts to about 70% of maximum rate. Plants of Lemna spec., grown heterotrophically and fumigated with argon containing about 1%  $O_2$ , show an adaptation to such conditions. After a lag-period of 10 days, growth rate is recovered up to approximately one-half of the aerobic rate (SIEGEL 1961).

The ATP level of intact L. aequinoctialis plants decreases roughly in

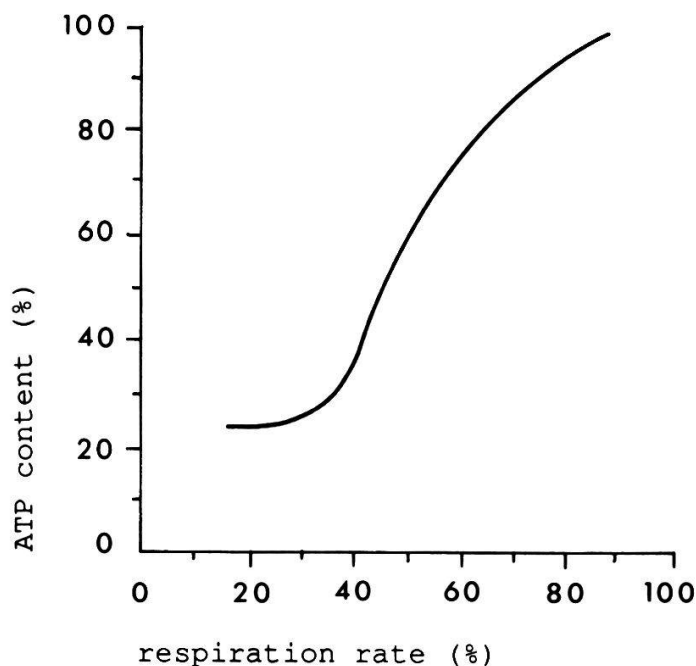


Fig. 2.38. ATP content of intact Lemna aequinoctialis plants as a function of respiration rate (LOEPPERT 1983). Values of respiration rate and ATP content were determined at various oxygen concentrations and then used for a plot expressing both parameters as percentage of the maximum value.



parallel with the respiration rate (fig. 2.38) when the oxygen concentration is reduced below  $10 \mu\text{M O}_2$  (LOEPPERT 1983). Both, the  $\text{CN}^-$  sensitive and the  $\text{CN}^-$  insensitive pathway participate in respiration of Lemna, as an inhibition of respiratory gas exchange can be achieved by simultaneous supply of  $\text{CN}^-$  and SHAM (salicylhydroxamic acid) only (LOEPPERT, personal comm.; ULLRICH-EBERIUS et al. 1983). Cyanide, given alone, has no, or even an enhancing, effect on respiration in L. aequinoctialis (LOEPPERT, personal comm.). In L. gibba  $\text{CN}^-$  inhibits or stimulates respiration depending on its magnitude. In sucrose-fed plants with high respiration ( $7-9 \mu\text{M O}_2 \text{ g}^{-1} \text{FW h}^{-1}$ ),  $\text{O}_2$  uptake rate is inhibited more than 60% by  $\text{CN}^-$ ; in plants depleted of sucrose for about 7 days and exhibiting low respiration ( $2 \mu\text{M O}_2 \text{ g}^{-1} \text{FW h}^{-1}$ ),  $\text{O}_2$  uptake is stimulated by  $\text{CN}^-$  (ULLRICH-EBERIUS et al. 1983). In L. aequinoctialis the ATP level remains high in the presence of  $\text{CN}^-$ , presumably on the basis of site-I phosphorylation (LOEPPERT 1981). In sucrose-nourished L. gibba, however, cyanide reduces the ATP level in the dark by more than 50% (ULLRICH-EBERIUS et al. 1983). Corresponding to the stimulating effect of cyanide under some circumstances, DALY and BROWN (1954) found an enhancing effect of carbon monoxide on respiration in Lemna spec. (blocking of cytochrome oxidase). Other agents which lead to an increase in respiration, are mechanical damage (S. punctata: DVORAKOVA-HLADKA, 1964), certain aromatic sulphonates (anionic detergents: "Sulfapol", "Nekal"), and boron deficiency (EICHHORN and AUGSTEN 1974). The effect of "Nekal" may be caused - at least partially - by an increase of monophenolase activity which has been found in homogenates from L. minor (GODZIK 1964). Also the effect of B-deficiency has been interpreted in a similar way (see strengthening of salicylaldehyde sensitivity at B deficiency).

In photosynthetically active tissue respiration ( $\text{CO}_2$  evolution other than from photorespiration) is greatly lowered by light. Based on the oxygen-dependence of mesophyll resistance and carbon dioxide compensation concentration, FUHRER (1983) calculated the fraction of respiration not inhibited by light in L. minor. At light intensities above  $150 \mu\text{E m}^{-2} \text{sec}^{-1}$  "day" respiration amounts to 23% of dark respiration, but increases rapidly when the light intensity is decreased.

A more or less close connection between nitrogen provision, growth, and respiration exists in L. minor (WHITE and TEMPLEMAN 1937; HUMPHREY et al. 1977). When nitrate concentration of the nutrient medium is lowered to  $<1 \text{ mM}$ , frond multiplication as well as respiration decrease greatly.

A number of enzymes, including aldolase show decreased activity. Further analysis of the effect of nitrogen starvation on aldolase activity reveals that not pyridoxal phosphate (SARAWEK and DAVIES 1977a) but rather a newly produced, specific inhibitor (low molecular weight protein) is responsible for the loss of enzyme activity (SARAWEK and DAVIES 1977b). Another agent which inhibits frond multiplication as well as respiration in Lemna, is the morphactin chloroflurenol (TREICHEL 1974a).

Obviously, respiratory gas exchange comes about not only by metabolism of mitochondria but also by other energy-generating and oxidative processes. Examples are the direct oxidation of glucose through the pentose-phosphate path in chloroplasts and the cytosol, energy-generating processes presumably located at the plasmalemma (compare chapter 2.5.2.1) and the action of phenolases (see above). The key enzyme of the oxidative pentose-phosphate path, glucose-6-phosphate dehydrogenase, was investigated by EICHHORN and AUGSTEN (1977a,b). Activity of this enzyme in W. arrhiza is influenced by addition of certain carbohydrates to the nutrient medium. For example, glucose and ribose are stimulating under different growth conditions, whereas galactose, rhamnose, and especially lactose have an inhibiting effect.

A hint to respiratory gas exchange at the plasmalemma comes from the work of ZURZYCKI (1970, 1971). The author studied the effects of light on oxygen uptake in DCMU-treated leaves of L. trisulca. Blue light of very low intensity (saturation at  $10 \text{ erg/cm}^2 \text{ sec}$ ) enhances  $\text{O}_2$  uptake by 30%. With high-intensity light of short-wave range, however, the effect is diminished. When linear polarized light is used, the direction of the polarization plane has no effect in Lemna, but - to a different degree - in leaves of Elodea and two mosses. Discussing these results in connection with cell geometry of the investigated plants, ZURZYCKI comes to the conclusion that the photoreceptor for low-intensity blue-light effect on respiration may be dichroic and oriented parallel to the cell surface, that is, located in the plasmalemma. The effect of light on  $\text{O}_2$  uptake shows a close correlation with the effect of light on the arrangement of chloroplasts (chapter 2.4.1.6). A blue light effect, which decreases photosynthetic  $\text{O}_2$  evolution possibly through an enhancement of respiration, was already mentioned in chapter 2.5.1.1 (see SCHMIDT-CLAUSEN and ZIEGLER 1969).

## 2.5.2. Membrane transport

### 2.5.2.1. Membrane potential, proton extrusion, and proton co-transport processes

Electrophysiological methods have been adapted to measure the membrane potential in single subepidermal cells of Lemna fronds (LOEPPERT et al. 1978, NOVACKY et al. 1978b) as transmembrane potential changes can indicate ion-transport processes through cell membranes. Following this line much work has been done to substantiate the existence of proton pumps at the plasmalemma which extrude protons to the apparent free space, and in this way make proton co-transport processes possible for uptake of the anions nitrate, phosphate, and sulfate, as well as hexoses and amino acids. Furthermore, the energy provision for proton extrusion, the modulation of membrane potential ( $E_m$ ) by phytochrome and the effect of ABA on  $E_m$  have been investigated.

L. aequinoctialis 6746 and L. gibba G1, pre-cultured in an anorganic nutrient medium, show  $E_m$  values of -180 to -220 mV (LOEPPERT et al. 1978, LOEPPERT 1979, 1981, KANDELER et al. 1980). L. gibba G1, pre-cultured in medium with 29 mM sucrose, exhibits  $E_m$  values which are more variable (-200 to -280 mV) (NOVACKY et al. 1978a, b; ULLRICH-EBERIUS et al. 1981, 1983). After elimination of sucrose from the nutrient medium, high  $E_m$  values are maintained under continuous darkness (at least for 7 days), but not, when 7 long days are followed by 6 to 72 hours of darkness.  $E_m$  then decreases to -90 mV.

Without doubt, the high  $E_m$  values cited cannot be caused by passive diffusion of ions only. The diffusion potential, calculated for currently used potassium concentrations (as  $K^+$  is the most important ion in this connection), would amount to -80 to -125 mV. Active transport processes must contribute to the high charge difference between the cell interior (vacuole) and the surrounding medium. In fact, membrane potential can be reduced to diffusion potential when respiration is blocked by concurrent supply of cyanide and salicylhydroxamic acid (ULLRICH-EBERIUS et al. 1983). Evidence for the fact that proton extrusion plays a dominant role in maintaining high membrane potentials in Lemna, was obtained in two ways. LOEPPERT (1979) manipulated the pH at both sides of the plasmalemma and monitored the accompanying changes of  $E_m$ . Reversible depolarisation of  $E_m$  occurs after an increase of external or a decrease of inter-

nal proton concentration. These results are in agreement with the postulated  $E_m$ -generating proton pump. BOECHER et al. (1980) achieved a hyperpolarization of  $E_m$  in L. gibba by supply of fusicoccin, which was reported to stimulate a  $H^+$ -pumping ATPase at the plasmalemma.

Membrane potential of L. gibba depolarizes transiently when certain inorganic or organic nutrients are added to the bathing solution. This holds true for nitrate (NOVACKY et al. 1978a; ULLRICH and NOVACKY 1981), phosphate (ULLRICH-EBERIUS et al. 1981, 1984), sulfate (LASS and ULLRICH-EBERIUS 1984a), glucose and fructose (NOVACKY et al. 1978b), glycine and other neutral or acidic amino acids (NOVACKY et al. 1978a, FISCHER and LUETTGE 1980, JUNG and LUETTGE 1980, 1982a, JUNG et al. 1982). These  $E_m$  changes can be interpreted by the action of carriers at the plasmalemma, which facilitate uptake of certain specific substrates especially in the case that protons are co-transported. A transient lowering of the proton gradient at the plasmalemma and thus of the membrane potential should occur when an inward proton co-transport is set in motion by supply of a carrier-accepted substrate. Some lines of evidence can support this view. Firstly, NOVACKY et al. (1980) and ULLRICH-EBERIUS et al. (1980) measured extracellular pH changes on the lower surface of single L. gibba fronds with a microelectrode and found the transient  $E_m$  depolarization, occurring after supply of glucose, to be correlated with an extracellular alkalinization. Then, LUETTGE et al. (1981) pointed out the enhancement of alanine uptake by fusicoccin to be correlated with the extent of fusicoccin-triggered  $E_m$  increase. Finally, JUNG et al. (1982) calculated proton electrochemical gradients (from  $E_m$ , medium pH and assumed cytoplasmic pH) and showed a linear correlation between this proton gradient and amino acid uptake rates (at amino acid concentration of  $10^{-4}M$ ). A selective inhibition of the amino acid co-transport carrier can be achieved by pretreatment of plants with the amino acid analogue p-fluorophenylalanine (JUNG and LUETTGE 1982b). Alanine uptake and alanine-induced transient  $E_m$  depolarization are reduced, but 3-O-methylglucose uptake is only slightly, and proton pump-dependent resting potential not affected. A poison which acts on the amino acid carrier as well as the glucose carrier is  $10^{-5}M$   $HgCl_2$  (GOLLE and LUETTGE 1983). At higher concentrations of  $HgCl_2$  ( $5 \times 10^{-5}M$ ) membrane potential and passive  $Na^+/K^+$ -permeabilities are also affected (LOOS and LUETTGE 1984).

Investigating the energy provision of the proton pump ULLRICH-EBERIUS et

al. (1983) and LOEPPERT (1981, 1983) reached different conclusions. ULLRICH-EBERIUS et al. stated a similar reduction of  $E_m$  and ATP level during supply of cyanide, the ATP-level decay being more rapid than the  $E_m$  decay. In analogy of what is known from other higher and lower plants (compare NOVACKY et al. 1978b), they postulated the proton pump to be an ATPase. LOEPPERT, on the other hand, found a cancellation of the active component of  $E_m$  after supply of cyanide or after reduction of oxygen concentration below a certain value, despite a sufficient availability of ATP. Furthermore, DCCD, an inhibitor of membrane-bound ATPase, reduced the ATP level to 11%, but leaves cell potential uninfluenced. The author concluded that ATP cannot be the fuel for the electrogenic proton extrusion. A redox pump may be envisaged as an alternative. Obviously, the different results of LOEPPERT and ULLRICH-EBERIUS et al. are caused not only by the use of different plants (LOEPPERT: *L. aequinoctialis*; ULLRICH-EBERIUS et al.: *L. gibba*), but also by different pre-culture conditions. *L. aequinoctialis* was held in anorganic nutrient medium under long day, whereas *L. gibba* was fed with 1% sucrose and cultured under short day. The differing behaviour of both experimental plants in respiration (see CN<sup>-</sup> effect: chapter 2.5.1.2) and membrane potential per se (see above) has already been mentioned.

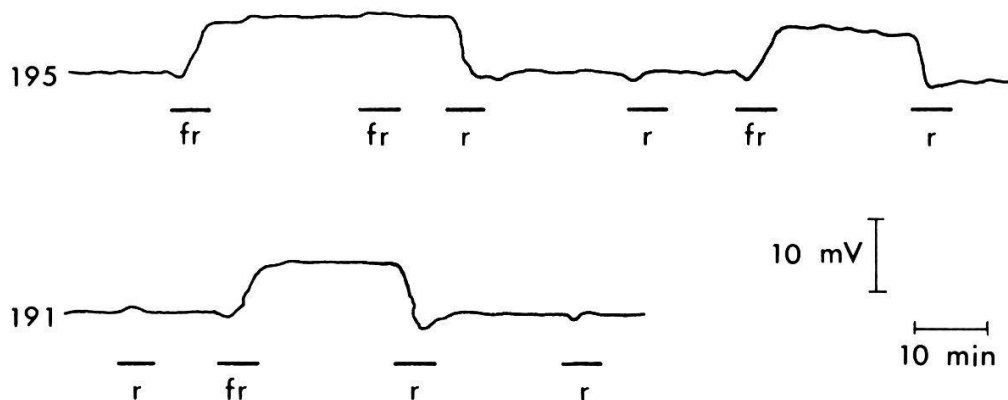


Fig. 2.39. Transmembrane potential changes in single subepidermal cells of *Lemna aequinoctialis* fronds induced by short red (r) and far-red (fr) light pulses (LOEPPERT et al. 1978). Light treatments were carried out after DCMU poisoning (for suspension of photosynthetic electron transport) and after a dark pre-treatment of 1 hour. The horizontal bars indicate the onset and duration of light treatment. The number at the beginning of each plot denotes the cell potential (inside negative); downward movement of the trace indicates depolarization.

A high concentration of ABA ( $10^{-5}$  M), which stops vegetative growth of L. minor almost completely (VAN OVERBEEK et al. 1968), exerts similar effects on membrane potential in L. gibba as fusaric acid (HARTUNG et al. 1980).  $E_m$  was increased by 85 mV in especially pre-treated (stressed) plants, which possess  $E_m$  values near the diffusion potential. Glucose and glycine uptake was stimulated, and glucose- and glycine-induced changes of  $E_m$  were diminished. It seems to be obvious to the writer that the results are brought about by an extensive ABA blocking of metabolism and energy consumption within the plant. Thus, more energy can be offered to transport processes at the plasmalemma.

Phytochrome-mediated changes of  $E_m$  have been pointed out in L. aequinoctialis (LOEPPERT et al. 1978) and L. gibba (KANDELER et al. 1980). After inhibition of photosynthesis by DCMU membrane potential is modulated by short far red and red light irradiations (fig. 2.39). Far red hyperpolarizes  $E_m$  by 5 to 9 mV within one minute. The hyperpolarization lasts after the end of irradiation and is abolished by a following red light pulse. Phytochrome seems to act on the active component of  $E_m$  as  $\text{NH}_4^+$ -treated plants show no light-mediated changes of diffusion potential.

#### 2.5.2.2. Uptake and release of electrolytes

##### 2.5.2.2.1. Alkaline cations

RATHJE (1952) demonstrated that in L. minor potassium is taken up from the medium during light and released during darkness, whereas protons show an opposite behaviour. When a high sodium concentration is present in the nutrient medium, sodium is taken up during darkness and released during light, and that to a much higher degree than potassium carries out the reverse movements (SCHEFFER et al. 1952). Potassium uptake under conditions of continuous light was investigated by YOUNG et al. (1970) and YOUNG and SIMS (1972, 1973) with exponentially growing plants of L. minor. Influx of  $^{42}\text{K}^+$  at different external K concentrations closely matches the demands of expanding tissue. At low external concentrations (i.e., below  $2 \times 10^{-4}$  M) movement is effectively unidirectional, and passive fluxes in either direction are negligible. Potassium uptake seems to be mediated by an ATPase, because ATP stimulates, but CCCP (a phosphorylation uncoupler) blocks  $\text{K}^+$  uptake. Correspondingly, LOEPPERT (1981) found a close correlation between  $\text{Rb}^+$  influx and ATP level after

treatment of L. aequinoctialis with arsenate or DCCD.

AYADI et al. (1974; see also THELLIER and AYADI 1971) studied  $K^+$  uptake in L. minor after keeping plants on distilled water for 24 h. Presence of calcium inhibits  $K^+$  absorption at moderate  $K^+$  concentrations, but activates the process for the lowest  $K^+$  concentrations. The inhibition is interpreted by structural perturbation of the catalytical  $K^+$ -uptake site and the activation by an influence on coupled (probably metabolic) processes. Addition of  $MgCl_2$  instead of  $CaCl_2$  to the KCl solution exhibits an inhibitory effect on  $K^+$  uptake, but no activating effect. When the velocity of  $K^+$  absorption is plotted against  $K^+$  concentration, the classical dual phase behaviour appears (fig. 2.40). Leaving the molecular interpretation of this behaviour open, the data are treated with an 'electrokinetic' formulation of absorption processes (THELLIER 1970, 1973), which employs linear 'flows and forces' relations when conditions are close to equilibrium, and proposes non-linear formulations through analogies with classical electrokinetics when far from equilibrium (high concentrations in the external medium).

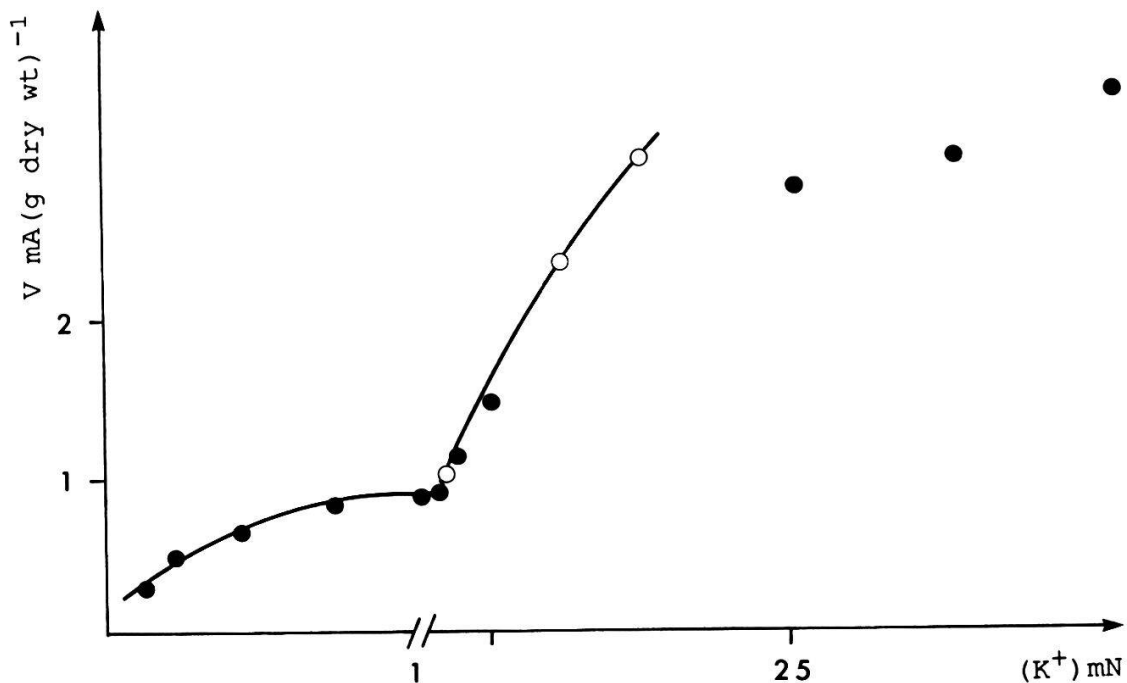


Fig. 2.40. Velocity ( $V$ ) of  $K^+$  absorption plotted against  $K^+$  concentration. Data from experiments with Lemna minor (AYADI et al. 1974)



AYADI et al. (1974) probing  $K^+$  uptake rate during different times of the day, found some variations but no diurnal oscillation. In contrast, KONDO and TSUDZUKI (1978) pointed out a circadian rhythm of potassium uptake in L. gibba, when plants were held in a weak and constantly flowing nutrient medium under continuous light (see chapter 2.5.9).

The competitive interaction between potassium and rubidium uptake was studied by AYADI et al. (1971) and LASSALLES et al. (1973). Uptake of rubidium at moderate concentrations is inhibited by calcium in a similar way as  $K^+$  uptake (AYADI and THELLIER 1970). Also caesium-137 is absorbed actively by L. minor like potassium. The concentration factor (Cs internal : Cs external) ranges from about 84 to 184 (BERGAMINI et al. 1979).

Lithium transport has been investigated in L. gibba with the help of stable isotopes,  $^6Li$  and  $^7Li$ , as tracers (BIELENSKI et al. 1984a, b). Kinetic parameters taken from influx and efflux data showed lithium to be distributed to at least three cell compartments: cell wall, cytoplasm, and vacuole. The passive permeabilities of the plasmalemma and of the tonoplast are rather low (order of  $10^{-12}$  to  $10^{-13}$  m. s $^{-1}$ ).  $Li^+$  is actively pumped out of the cytoplasm, both towards the exterior and towards the vacuole (like  $Na^+$  in other plant cells).

#### 2.5.2.2.2. Ammonium

When the nutrient medium contains ammonium as well as nitrate, ammonium is taken up preferentially (FERGUSON 1969a, FERGUSON and BOLLARD 1969, FELLER and ERISMANN 1971, GROB et al. 1973, TATKOWSKA and BUCZEK 1983, INGEMARSSON et al. 1984, ULLRICH et al. 1984b). In cultures of S. punctata without supply of glucose 80% of  $NH_4^+$  and 20% of  $NO_3^-$  are consumed from the medium within 10 days. Uptake of  $NO_3^-$  increases after practical exhaustion of  $NH_4^+$  ( $NH_4^+ = 5\%$ ). In cultures with glucose-containing medium 40% of  $NH_4^+$  and 20% of  $NO_3^-$  are used up within 3 days (GROB et al. 1973). When in sucrose-grown cultures of S. polyrrhiza one fifth of the  $NO_3^-$  is replaced by  $NH_4^+$  (1.6 mM), uptake and assimilation of  $NO_3^-$  are reduced to one half after 18 days of growth (TATKOWSKA and BUCZEK 1983). In cultures of L. gibba, which were held in a sucrose-containing medium and were adapted to limited nitrogen supply, uptake of  $NO_3^-$  was depressed as long as  $NH_4^+$  remained in the medium (INGEMARSSON et al. 1984). Similar results were obtained with L. minor

in diluted anorganic medium (FELLER and ERISMANN 1971). Even a lag-phase of about 10 hours was stated before  $\text{NO}_3^-$  uptake began after exhaustion of  $\text{NH}_4^+$ .  $\text{NH}_4^+$ , added to  $\text{NO}_3^-$ -absorbing plants of L. gibba (N-starved before the experiment), reduced the  $\text{NO}_3^-$  uptake rates to about one third until the  $\text{NH}_4^+$  was consumed. In plants provided with  $\text{NO}_3^-$ , however, the slow  $\text{NO}_3^-$  uptake was not inhibited by addition of  $\text{NH}_4^+$  (ULLRICH et al. 1984b). In all cases,  $\text{NH}_4^+$  uptake can follow the electrochemical gradient. The concentration-dependence curve of  $\text{NH}_4^+$  uptake shows a saturating as well as a linear component. Thus carrier-mediated  $\text{NH}_4^+$  uniport as well as diffusion of  $\text{NH}_3$  may contribute to the uptake of  $\text{NH}_4^+$  (ULLRICH et al. 1984b).

#### 2.5.2.2.3. Alkaline-earth cations

Absorption of calcium into L. gibba was studied by DEKOCK et al. (1973). An increase of Ca concentration in the medium from 0.05 to 2.5 mM causes a raising of internal Ca content from 1.6 to 3.5 meq/100 g dry weight. At the same time internal Mg content is lowered from 3.6 to 2.1 meq/100 g dry weight. Addition of oxalate (0.1-2.5 mM), to the medium increases Ca absorption of plants. EDTA (0.1-0.5 mM), when supplied in the presence of 2.5 mM oxalate, reduces  $^{45}\text{Ca}$  absorption as well as  $^{14}\text{C}$ -oxalate uptake. BA impedes the absorption of Ca, ABA stimulates it (DEKOCK and HALL 1981).

#### 2.5.2.2.4. Anions

At fairly low medium concentrations nitrate, phosphate, and sulfate, are all taken up by an anion/ $\text{H}^+$  co-transport (see chapter 2.5.2.1). In the case of nitrate it was demonstrated by LOEPPERT et al. (1977) that during steady-state conditions of growth the  $\text{NO}_3^-$  uptake rate parallels alkalinization of the medium with a molecular ratio of 1:1 (experiments with L. aequinoctialis). Furthermore, when the proton gradient across the plasmalemma is discharged with a permeant acid,  $\text{NO}_3^-$  uptake is inhibited completely (LOEPPERT and KRONBERGER 1979). Under such conditions even in plants well provided with  $\text{NO}_3^-$  a passive efflux of  $\text{NO}_3^-$  occurs.

The existence of a  $\text{NO}_3^-$ -carrier in Lemnaceae can be deduced from a diagram plotting  $\text{NO}_3^-$  absorption against  $\text{NO}_3^-$  concentration, which shows

a biphasic pattern for W. arrhiza (SWADER et al. 1975). In Wolffia the carrier is present also after  $\text{NO}_3^-$  starvation, but its quantity or effectivity is raised 4 to 5 hours after  $\text{NO}_3^-$  supply. In L. gibba an induction effect of low  $\text{NO}_3^-$  concentrations (0.1 mM  $\text{KNO}_3$ ) on  $\text{NO}_3^-$ -uptake rate after N-starvation has been found (ULLRICH et al. 1981). The effect appears after 1 hour, is saturated after 6 hours, and then remains constant for a fairly long time (10 to 20 hours). Also in this case formation of the nitrate carrier may be involved.

In Wolffia an increase of nitrate uptake during illumination occurs in plants with very low nitrate reduction (SWADER et al. 1975). Light via photosynthesis seems to provide energy for the uptake process. In L. aequinoctialis, on the other hand, light increases net nitrate uptake via an enhancement of metabolic  $\text{NO}_3^-$  consumption, which lowers passive nitrate efflux (LOEPPERT and KRONBERGER 1979).

Biphasic uptake kinetics have been worked out also for phosphate (L. minor: THELLIER and AYADI 1968, MONNIER and THELLIER 1970. L. gibba: NOVACKY and ULLRICH-EBERIUS 1982, ULLRICH-EBERIUS et al. 1984). NOVACKY and ULLRICH-EBERIUS (1982) suggested that the transport systems are both located at the plasmalemma and function with a single, conformational changeable phosphate/ $\text{H}^+$  carrier. Arsenate causes a competitive inhibition of phosphate uptake (MONNIER and THELLIER 1970).

Dependence on concentration of sulfate uptake was investigated in L. minor (THOIRON et al. 1969, THELLIER et al. 1971b), and in L. aequinoctialis (DATKO and MUDD 1984a). In the first two cited publications the biphasic character of kinetic curves was interpreted as being a consequence of non-equilibrium thermodynamics, and it was concluded that postulation of a double system of catalysts would not be necessary. DATKO and MUDD (1984a) obtained  $\text{SO}_4^{2-}$  uptake kinetics in L. aequinoctialis, which include a saturating and a linear component. As in other plants, molybdate competes with  $\text{SO}_4^{2-}$  for uptake by the saturating transport system. The effectivity of this transport system is regulated by the internal sulfate and cystine concentration. Between 'low- $\text{SO}_4^{2-}$ ' provided and 'high- $\text{SO}_4^{2-}$ ' provided plants a 500-fold change in effectivity can be observed. The linear, 'nonsaturating' transport system is not regulated in a similar way. It is much less affected by pH and temperature than the saturating system and may represent a diffusion-mediated uptake.

Fumigation of L. minor with 18 ppm  $\text{H}_2\text{S}$  (BRUNOLD and ERISMANN 1970) or

with 0.5 ppm  $\text{SO}_2$  (SCHAERER et al. 1975) inhibits sulfate uptake very rapidly. Both gases can be used in Lemna as an S-source (see chapter 2.5.6.6). Furthermore, the  $\text{SO}_4^{2-}$  level within the plant increases gradually during fumigation with  $\text{H}_2\text{S}$  or  $\text{SO}_2$  (BRUNOLD and ERISMANN 1970, SCHAERER et al. 1975). Thus, the inhibiting effect of  $\text{H}_2\text{S}$  and  $\text{SO}_2$  on  $\text{SO}_4^{2-}$  uptake may be caused - at least in part - by the above cited regulation of  $\text{SO}_4^{2-}$  uptake by internal cystine and sulfate concentration.

Under the conditions used by DATKO and MUDD (1984a),  $^{35}\text{SO}_4^{2-}$  efflux from equilibrium-labelled L. aequinoctialis is very low (1.1% of the total  $^{35}\text{S}$  within 22.4 hours). By contrast, radioactive  $\text{SO}_4^{2-}$  efflux from equilibrium-labelled L. minor amounts to 30% of total  $^{35}\text{S}$  within the same time (calculated by DATKO and MUDD from the data of THOIRON et al. 1981). Presumably, the rate of  $\text{SO}_4^{2-}$  metabolism (species-specific and dependent on experimental conditions) may be crucial in this respect.

Uptake of chloride and iodide has been investigated with L. minor (THELLIER et al. 1967). Within the concentration range studied (0.055 - 1 mM), chloride uptake rates reach saturation at about 0.3 mM. Iodide uptake rates, on the contrary, show a linear dependence upon external concentration (0.002 - 4 mM).

When analyzing borate uptake it must be taken into consideration that borate forms very stable borodiester complexes with cell wall constituents possessing pairs of adjacent cis-hydroxyl groups. Using the stable isotope  $^{10}\text{B}$ , which can be detected by a  $(n, \alpha)$  nuclear reaction, THELLIER et al. (1979) and DUVAL et al. (1980) carried out a compartmental analysis of borate with plants of L. minor. Evaluation of efflux and influx kinetics allowed determination of the borate capacities of the various compartments and of the borate unidirectional fluxes between the different compartments, at borate flux equilibrium. Phosphate uptake (THELLIER and AYADI 1967) and chloride uptake (THELLIER et al. 1967) are inhibited by borate, but it has no effect on sulfate uptake (THELLIER and TROMEUR 1968) and iodide uptake (THELLIER et al. 1967).

#### 2.5.2.2.5. Leakage

Release of electrolytes into the medium has been investigated in connection with the effect of several herbicides (O'BRIEN and PRENDEVILLE

1979). Dinoseb, sodium azide, linuron, prometryne, and simazine were the most active in increasing leakage in L. minor, followed by oxyfluorfen, amitrole, and 2,4-D. Glyphosate and dalapon were the least active.

#### 2.5.2.3. Uptake, transport, and release of organic substances

##### 2.5.2.3.1. Sugars and amino acids

As indicated by changes of membrane potential during uptake experiments (see chapter 2.5.2.1), hexoses as well as neutral and acidic amino acids are presumably taken up from the medium by proton co-transporters (carriers) at the plasmalemma. This view is strengthened by the fact that uptake of glucose and 3-O-methylglucose (ULLRICH-EBERIUS et al. 1978), leucine (NEWTON 1974b), and other neutral and acidic amino acids (BORSTLAP 1977a, JUNG and LUETTGE 1980, 1982a), shows saturation kinetics. Biphasic saturation was found for DL-leucine absorption by intact root tips of L. minor (NEWTON 1974b), and also for uptake of glycine, L-alanine, L-leucine, L-isoleucine, and L-valine by intact fronds of S. polyrrhiza (BORSTLAP 1977a). A common carrier of all neutral amino acids seems to exist in the low as well as in the high concentration range, because competitive uptake inhibition between these amino acids can be demonstrated (BORSTLAP 1974, 1977a, 1978). Deviating results were obtained with L. gibba by JUNG and LUETTGE (1982a). Dependence on concentration of amino acid uptake showed three-phase kinetics in the range from  $10^{-6}$  to  $10^{-2}$  M. Uptake in the lowest concentration range ( $<0.02$  mM) exhibits competitive inhibition between different neutral amino acids but only slight inhibition of L-alanine uptake by aspartate. In the concentration range of 0.02 to 0.5 mM competition between neutral amino acids and L-aspartate has been shown. This second uptake system possesses kinetic data which correspond about to the 'low-concentration' system in S. polyrrhiza. Proton co-transport works especially in this system. The third uptake system in L. gibba in the concentration range  $>0.5$  mM is assumed to come about by diffusion.

Efflux of L- $(^{14}\text{C})$  leucine has been determined from preloaded fronds of S. polyrrhiza. At a concentration of about 2.5 mM (both external and internal) the efflux was  $\leq 1\%$  of the influx (BORSTLAP 1977a). Boron deficiency has no influence on leucine uptake in L. minor (THELLIER and LE GUIEL 1967b).

Uptake of the basic amino acid arginine in L. gibba was investigated by JUNG and LUETTGE (1982a). The authors conclude from their results that arginine moves as a cation into the cells via a uniporter, driven by the negative cell potential. Recently, discrete transport systems for (a) neutral and acidic amino acids, and (b) basic amino acids have been shown to occur also in S. polyrrhiza (BORSTLAP et al. 1986) and L. aequinoctialis 6746 (DATKO and MUDD 1985). In both species and for both systems saturable uptake conforms to the sum of two Michaelis-Menten terms. In addition to the dual saturable uptake the presence of a non-saturable component has been indicated in S. polyrrhiza.

Besides the two uptake systems for amino acids (a and b) six further transport systems have been identified in L. aequinoctialis (DATKO and MUDD 1985, 1986). Reciprocal inhibition studies defined the preferred substrates for these systems as follows: (c) purine bases, (d) choline, (e) ethanolamine, (f) tyramine, (g) urea, and (h) aldohexoses. Sucrose is taken up probably only after cleavage into its hexose moieties. ULLRICH-EBERIUS et al. (1978) pointed out invertase activity to be present in the cell walls of L. gibba. Light stimulates hexose uptake in L. gibba (ULLRICH-EBERIUS et al. 1978) and W. arrhiza (BIANCHETTI 1963). Photosynthetic energy seems to contribute to preservation of the trans-membrane proton gradient which drives proton co-transport processes.

#### 2.5.2.3.2. Phytohormones and other growth substances

Auxins, gibberellins, and abscisic acid are weak acids which can penetrate cell membranes passively as undissociated molecules. Therefore, short-term uptake of such substances depends on the pH of the medium. Lowering of solution pH (from 5.1 to 4.1, for example) markedly increases the uptake of 2,4-dichlorophenoxyacetic acid (2,4-D) in L. minor (BLACKMAN et al. 1959) and S. intermedia (FERNANDEZ et al. 1972). Similar results have been obtained with 2,3,5-triiodobenzoic acid (TIBA) (BLACKMAN and SARGENT 1959). When several chlorinated derivatives are compared, uptake rate increases with progressive chlorination of phenoxyacetic acid and decreases with progressive chlorination of benzoic acid (KENNEY-WALLACE and BLACKMAN 1972). This behaviour corresponds roughly to the graduated lipophily of these substances (see transfer rates through a layer of n-octanol).

Capacity for uptake of weak acids depends on the pH within the various



cell compartments (s. ion trapping by dissociation). This may give an - at least partial - explanation for the fact that roots of L. minor accumulate much more TIBA than fronds (BLACKMAN and SARGENT 1959). Correspondingly, 2,4-D is more rapidly, and to a higher degree, taken up by roots than by fronds in S. intermedia (FERNANDEZ et al. 1972). Accumulation of gibberellin A<sub>1</sub> proceeds in young daughter fronds of L. aequinoctialis to a higher level than in mother fronds (AL-SHALAN and KANDELER 1979). Daughter fronds of L. gibba show no such dominance over the mother fronds for GA<sub>1</sub> uptake (HARTUNG and KANDELER 1976). In L. gibba the capacity for GA<sub>1</sub> uptake is relatively low in comparison to L. aequinoctialis. Only in L. gibba the GA<sub>1</sub> accumulation can be increased by a pre-treatment with far-red irradiation (10 min far red at the beginning of the daily dark period for four days).

Uptake investigations with 1- <sup>14</sup>C-indole-3-acetic acid (IAA) are made more difficult by photolytic decarboxylation of IAA in the medium and subsequent assimilation of <sup>14</sup>CO<sub>2</sub> (BHATNAGAR and TILLBERG 1982). With the α-pyrone fluorescence method for IAA determination the authors revealed a conjugation of IAA immediately after uptake in L. gibba. Also in the case of abscisic acid (ABA) transformation or destruction occurs after uptake (TILLBERG et al. 1980). Compounds containing <sup>14</sup>C other than ABA appear in the solution during long-term experiments with L. gibba.

#### 2.5.2.3.3. Further organic substances

Passive permeability of several small organic molecules has been investigated on root epidermis cells of L. minor with the deplasmolysis-time method (MARKLUND 1936). Generally, the permeability in Lemna is relatively high (but not so high as in Chara) and in accord with lipoid solubility of the respective substances. Urea and thiourea permeate faster, but methylurea and dimethylurea more slowly than in Chara (comparison on the basis of glycerol permeability).

Uptake of uridine in L. gibba was studied by NAKASHIMA and TSUDZUKI (1976, 1977). Saturation of the uptake process at a low concentration of substrate as well as competitive inhibition by compounds structurally analogous to uridine shows uridine uptake to be a carrier-mediated process. Calcium seems to be necessary for the molecular connection of transport protein(s) to the cell membrane. A treatment of plants with sodium citrate reduces the rate of uridine uptake and CaCl<sub>2</sub> is the most



efficient compound in offsetting the citrate reduction.

Ascorbic acid is a further substance taken up by L. gibba with a saturable transport system (BARBER and CALDWELL 1976).

#### 2.5.2.3.4. Leakage

Already NICKELL and FINLAY (1954; cited after HILLMAN 1961c) stated that Lemna cultures excrete organic substances into the medium. FILBIN and

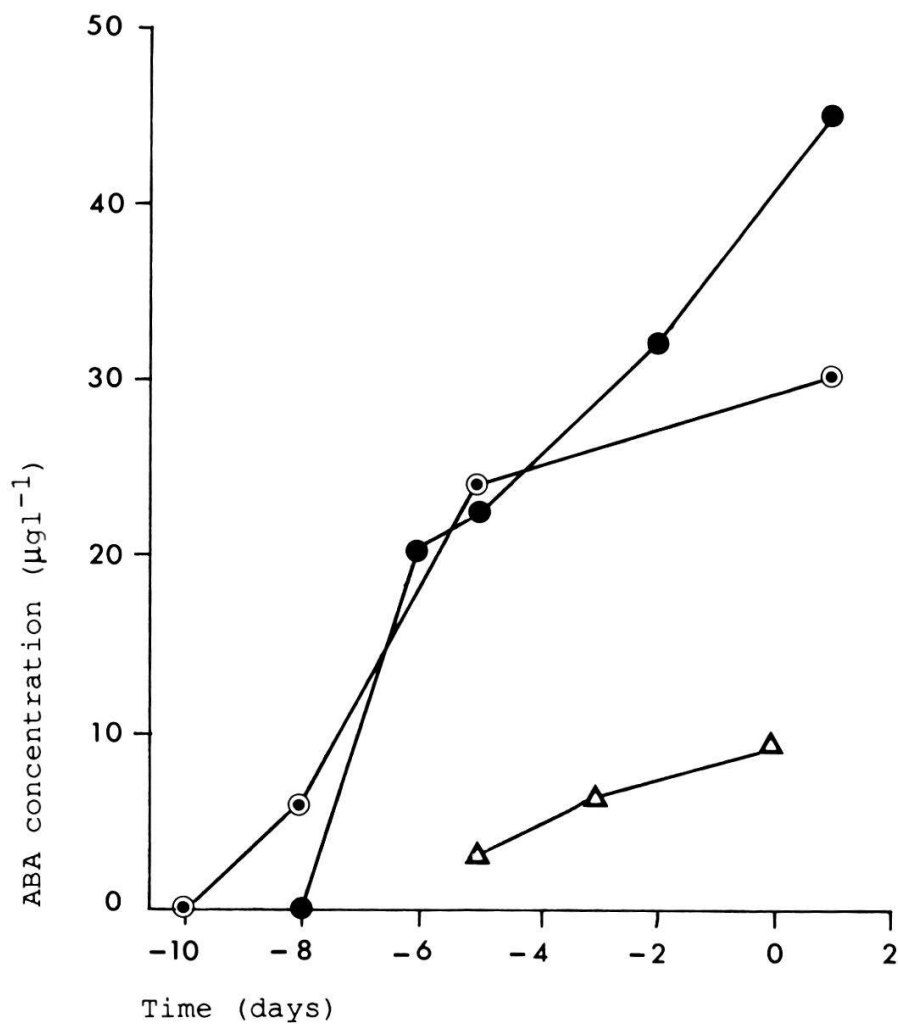


Fig. 2.41. Accumulation of released ABA in the medium of a Spirodela polyrrhiza culture. The time scale is related to the day of appearance of detached turions, which is denoted as zero. The curves represent three separate but similar experiments (SAKS et al. 1980).

HOUGH (1979) found a release of organic  $^{14}\text{C}$  into the solution after pre-labeling of L. minor plants with  $^{14}\text{CO}_2$  under photosynthetic conditions. The loss of organic material increases from 1.19 to 8.88% of recently fixed  $^{14}\text{CO}_2 \text{ h}^{-1}$ , when the concentration of  $\text{CuSO}_4$  is elevated from 2.5 to  $3.94 \text{ mg l}^{-1}$  in standard Hutner's medium (with high EDTA content). Leakage of flower-promoting material into distilled water from fronds of L. aequinoctialis was detected by HALABAN and HILLMAN (1970a, 1971). ABA is one of the compounds which are released even into a complete nutrient medium (fig. 2.41). This has been demonstrated for S. polyrrhiza (SAKS et al. 1980), L. gibba, and L. aequinoctialis (MERTEN 1979). Also WOLEK (1979) concluded from his investigations on allelopathy between S. polyrrhiza and W. arrhiza that ABA may be released into the medium by W. arrhiza. JURD et al. (1957) found a caffeic acid ester (similar to, but not identical with chlorogenic acid) to be excreted into the medium by S. punctata. Similarly, WALLACE (cited in McCLURE 1970) stated that cinnamic acids and - after several days of culture - flavonoids can be detected in the medium of axenically cultivated Lemnaceae. For the release of amino nitrogen to the medium see chapter 2.4.1.4.

### 2.5.3. Location and accumulation of minerals

#### 2.5.3.1. Location in organs and tissues

Fronds and roots of Lemna spec. contain very different amounts of nitrate (LEHMAN et al. 1981). Already in the youngest adult frond generation the root has 6 to 8 times more nitrate per dry weight than the frond. When the fronds are two generations older, the root contains 11 to 16 times more nitrate per dry weight than the frond.

The distribution of several mineral elements in roots of L. minor was investigated by ECHLIN et al. (1982a). X-ray microanalysis on fracture faces of bulk-frozen tissue has shown that  $K^+$ , P, S,  $Mg^{++}$ ,  $Cl^-$ , and  $Ca^{++}$  all are present in distinctly higher concentrations in the apical meristem than in the somewhat older tissues which have begun to differentiate. In a region approximately 90  $\mu m$  from the root tip the potassium values are more or less the same in the xylem and phloem parenchyma and in the endodermis and inner cortex, although the mean value for this element is somewhat higher in the tissues surrounding the stele than in the stele itself. In approximately 300  $\mu m$  from the root tip there appears to be a higher relative concentration of potassium in the two sectors radiating outwards from the differentiated sieve element and companion cell compared to the region between these two tissues.

#### 2.5.3.2. Location within the cell

Concentrations of  $Ca^{2+}$ ,  $K^+$ , and  $Cl^-$  in cytoplasm and vacuole of vegetative fronds and ABA-induced turions of S. polyrrhiza have been determined by SMART and TREWAVAS (1984b). Data from efflux experiments and stereological cell compartment analysis (SMART and TREWAVAS 1983b) have been used for this purpose.

From the results given in table 2.27 it can be drawn that distribution of calcium and potassium within the cells changes, when turions are produced instead of vegetative fronds. In turions the calcium content of the cytoplasm increases 2.5 times and the potassium content decreases to one-third. The differences are caused by changes in developmental processes. Turion-inducing ABA ( $10^{-7} M$ ) has no rapid effect on  $Ca^{2+}$ ,  $K^+$ , or  $Cl^-$  transport.

To some degree calcium is fixed in the Donnan free space. The cell wall

Table 2.27. Concentration (in mol m<sup>-3</sup>) of some ions in cell compartments of Spirodela polyrrhiza (after SMART and TREWAVAS 1984b)

Cytoplasm				Vacuole		
	Ca <sup>2+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	Ca <sup>2+</sup>	K <sup>+</sup>	Cl <sup>-</sup>
Vegetative fronds	16.18	61.09	15.38	3.29	188.14	41.31
Turions	39.94	20.87	3.27	0.52	246.81	3.43

contains compounds with negative charges (polyuronic acids, in the first place) and, therefore, behaves like an ionic exchanger (DEMARTY et al. 1977, 1978). MORVAN et al. (1979, 1980) titrated isolated cell walls of L. minor and showed that cell walls have at least two kinds of binding sites. One site is titratable within one hour and can be attributed to polyuronic acids. Both sites have a much greater affinity for Ca<sup>2+</sup> than for Na<sup>+</sup> and K<sup>+</sup>. In comparison to other plants Lemna has a medium capacity for Ca<sup>2+</sup> fixation (STELZ et al. 1975).

A compartmental analysis for boric acid has been made with the help of <sup>10</sup>BO<sub>3</sub><sup>3-</sup>, detectable by a (n,α) nuclear reaction (THELLIER et al. 1979). L. minor plants were grown in nutrient solution with 0.16 mM <sup>10</sup>B-boric acid and then the efflux kinetics into an <sup>11</sup>B-boric acid solution were determined. From the data borate capacities of the various compartments were calculated. The capacity of free space (including easily dissociable borate monoesters) amounts to 7.2. μmol g<sup>-1</sup> dry weight. The values for cell wall (very stable borate diesters), cytoplasm, and vacuole are 19.4, 34.6, and 32.1 μmol g<sup>-1</sup> dry weight, respectively. The values obtained from influx experiments are lower (about 1/3) but show the same result with regard to the relative distribution of borate within the cell (DUVAL et al. 1980). In comparison to other aquatic macrophytes L. minor is a strong accumulator of boron. Plants grown in a pond together with Ceratophyllum demersum show boron concentrations 10-45 times greater than Ceratophyllum throughout the growing season (GLANDON and McNABB 1978). In most determinations boron concentration in L. minor amounts to about 2-3 mg B g<sup>-1</sup> ash-free dry weight.

The significance of chemically, rather than spatially separated compart-

ments has been shown for the compartmentation of cadmium in W. gladiata (SCHREINEMAKERS 1986). Three compartments can be divided within the cytoplasm: Cytoplasmic soluble and easily exchangeable Cd, cytoplasmic weakly complexed Cd (or vacuolar Cd), and cytoplasmic strongly complexed Cd.

#### 2.5.3.3. Accumulation

Accumulation of alkaline cations in the vacuole proceeds as long as the permeability of the tonoplast is low. The drastic lowering of potassium content in L. gibba after application of a high concentration (1-10 ppm) of ABA (LIEBERT 1977) seems to be the result of a change in permeability of plasma membranes. Also the strong reduction of  $^{137}\text{Cs}$  accumulation in L. minor after a 32 krad  $\gamma$ -irradiation (LEINERTE 1969) may be explained in the same way.

As mentioned in chapter 1.1 and 1.2.4. Lemnaceae accumulate phosphate in the form of condensed anorganic phosphates and/or inositol phosphates. Without doubt, these poly-anions bind not only alkaline-earth cations, but also several heavy metals. In a number of investigations concentration factors have been determined, which are given by the ratio: content per g dry weight plant / content per ml solution (EL-SHINAWY and ABDEL-MALIK 1980; KOVACS et al. 1984; SILVEY 1967; SKLAR 1980; VERMAAK et al. 1976). Interestingly enough, especially the physiologically active micro-nutrients Fe, Mn, Zn, Cu, and Co are accumulated to a very high degree (concentration factors between  $10^4$  and  $4.6 \times 10^5$ ). Phosphate also reaches a factor of about  $10^4$ . Other elements with concentration factors in this range are Al, Ti, Sn, and Sb. Concentration factors of  $10^2$  to  $10^3$  were found for Pb, Pr, Ce, La, Cs, Mo, Nb, Zr, Y, Ni, Cr, and V. Absolute values for Mn are 1500 (L. gibba), 2800 (L. minor), 3900 (L. trisulca), and 2000 (S. polyrrhiza)  $\mu\text{g/g}$  dry weight, each (BORESCH 1935/1936).

OLSEN (1934a,b) found 500  $\mu\text{g}$  Mn/g dry weight only after culture of S. polyrrhiza in  $2.5 \text{ mg MnSO}_4 \cdot 4\text{H}_2\text{O l}^{-1}$  (The culture solution was of pH 7.0. The Mn uptake is optimum at pH 6-7). The Fe content of S. polyrrhiza was 2370  $\mu\text{g/g}$  dry weight after supply of  $7 \text{ mg Fe l}^{-1}$  as ferric citrate at pH 4, which is optimum for Fe uptake.

Another factor which contributes to accumulation of heavy metals, is the production of several chelating organic compounds (organic acids, amino

acids, peptides, nucleotides, RNA, DNA, and others). In L. minor nitrate-grown plants contain 3-4 times more iron and malate than ammonium-grown plants (FELLER and ERISMANN 1973). Correspondingly, supply of ammonium to the nutrient medium reduces the copper content in L. aequinoctialis (NASU et al. 1983).

Heavy metal accumulation in S. polyrrhiza has been investigated varying the supply of iron, magnesium, zinc, and copper (SCHREINEMAKERS 1984, SCHREINEMAKERS and DORHOUT 1985). Under most conditions, the accumulation changes more or less in parallel for pairs of elements, namely calcium and zinc, magnesium and manganese, and copper and iron.

#### 2.5.4. Water relations

Stomata are movable in Spirodela (at least S. punctata), but not in the genus Lemna (s. chapter 2.5.1.1). Therefore, transpiration proceeds in Lemna in parallel to evaporation. DEBUSK (1980) compared evapotranspiration of L. minor with evaporation of the free water surface and found a ratio evapotranspiration/evaporation = 0.9. The average water loss of plants amounted to  $4.4 \text{ l m}^{-2} \text{ d}^{-1}$ .

Duckweeds can grow on liquid media as well as on wet substrates (loamy soil or agar), but the under side of fronds is very sensitive to desiccation. Plants of W. arrhiza can survive without water (at 65% relative humidity) for 20-30 min. Plants of S. polyrrhiza, L. minor, L. gibba, and L. trisulca endure desiccation for about 2.5 hours by subsequent outgrowth of daughter fronds (WOLEK 1981).

The osmotic pressure in young, still viable stomata of L. minor fronds is stated to be equivalent to 0.25-0.3 M  $\text{CaCl}_2$  (REUTER 1948). The surrounding epidermis cells show values which are only slightly lower. In subepidermal cells of roots, however, the osmotic pressure lies in the range of 0.2-0.3 M glucose (PIRSON and SEIDEL 1950). From the tip to the basis of the root there is an osmotic gradient with a minimum at the end of the cell elongation zone. Replacement of  $\text{Ca}^{2+}$  by  $\text{K}^+$  or  $\text{Na}^+$  in the surrounding medium for 7-12 days causes a slight increase of osmotic values. A similar effect comes about by culturing plants in medium containing 0.5% glucose. Replacement of  $\text{K}^+$  by  $\text{Na}^+$ , on the other hand, decreases osmotic values somewhat, as also 4 hours of darkness. PIRSON and GOELLNER (1953) stated that reduction of nitrate supply to 1/100 or phosphate supply to 1/300 (replacement of  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  by  $\text{Cl}^-$ ) increases the osmotic pressure especially in the cell elongation zone.

The relation of osmotic quantities (osmotic pressure, turgor pressure, and suction force) to the relative cell volume of young daughter fronds (about 3 mm in length) of L. aequinoctialis was determined by TAKAOKI (1969). The increment of volume is 27%, when a frond is transferred to water from sucrose solution with a concentration identical to the incipient plasmolysis concentration (0.24 M).

Plasmolysis of L. trisulca with diverse hypertonic solutions for 30-40 min destroys all the mature cells, but meristems can grow out (KARZEL 1926). During the following 8 months (i.e., many frond generations) growth is decelerated and fronds and stalks remain smaller. A less ex-



treme form of osmotic stress comes about by application of subplasmolytic solutions. 0.2 M polyethyleneoxide, glucose or mannitol, supplied for 4 hours to L. minor plants, stops cell lengthening in the elongation zone of roots irreversibly (SCHAEFER 1958). The treatment reduces the adhesion between cytoplasm and cell wall as can be seen by subsequent plasmolysis. The plasmolysis time is shortened and the plasmolysis form is more convex.

Investigations on plasmolysis time and plasmolysis forms in root cells of L. minor have been made by several authors. STRUGGER (1934) demonstrated concave plasmolysis to occur in the elongation zone but convex plasmolysis in mature cells. Correspondingly, plasmolysis time is maximum at the end of cell elongation zone (PIRSON and SEIDEL 1950, SCHAEFER 1955). Darkening, CO<sub>2</sub>, elevated temperature (26 versus 20°C), and Ca<sup>2+</sup> deficiency are factors which shorten the plasmolysis time (PIRSON and SEIDEL 1950, PIRSON and SCHAEFER 1957, SCHAEFER 1956, 1958). Moderate N- or P-deficiency, however, extend this parameter (PIRSON and GOELLNER 1953). Investigations with the upper epidermis of L. minor and S. polyrrhiza fronds have shown that the plasmolysis form changes from the concave to the convex type with increasing senescence of the fronds (FISCHER 1949).

## 2.5.5. Carbon metabolism

### 2.5.5.1. Starch

As stated in chapter 2.5.1.1.2 Lemnaceae belong to the  $C_3$  type of photosynthetic  $CO_2$  assimilation. Products of photosynthesis which are not directly used up by cell metabolism, are stored within the chloroplast in the form of starch. Accumulation of starch depends primarily on provision of the plants with sufficient  $CO_2$ . Under continuous light, the starch content of L. minor increases with increasing  $CO_2$  supply (to the five-fold level from 100 to 9000 ppm  $CO_2$ ; MUELLER et al. 1977). At 330 ppm  $CO_2$  (normal air) starch production is adequate only when the plants are kept in the open air or are provided with a continuous air stream. Under such conditions, L. gibba shows an accumulation rate of  $16 \mu g$  starch  $mg^{-1}$  dry weight  $h^{-1}$  (light intensity 9000 lux; temperature  $26^\circ C$ ; medium pH 4.8. KOENIGSHOFER, unpublished results). During the daily light phase starch content increases continuously and decreases during the night phase. Short-period oscillations of starch level, which were found in plants cultivated in vessels with cotton stoppers (ROTTENBURG et al. 1981), cannot be ascertained in open air. Also the effect of end-of-day far red on starch content (KANDELER et al. 1980) cannot be reproduced when gas exchange is improved.

In nitrate- as well as in ammonium-grown L. minor plants sugar and starch content increases, when the pH of the nutrient medium is lowered (KOPP et al. 1974a). SATAKE and SHIMURA (1983) showed that S. polyrrhiza takes more  $CO_2$  from water at low pH than at high pH, because the dissolved  $CO_2$  content in the water is much higher. Other factors which enhance starch accumulation, are P deficiency (REID and BIELESKI 1970a),  $K^+$ -deficiency (WHITE 1939), supply of branched-chain amino acids (VAN MAZIJK 1975), and B deficiency (SCHOLZ 1962). In the first three of these cases an inhibition of growth may lead to a restricted starch utilization. B-deficiency, however, even enhances frond multiplication without a reduction of dry weight per frond. The author presumed an effect of B on metabolism of carbohydrates. Fumigation of L. minor with sublethal concentrations of  $SO_2$  (0.6 ppm) diminishes the starch content immediately by 20% and also the growth rate is reduced during the first two weeks by 25% (FANKHAUSER et al. 1974, 1976).

#### 2.5.5.2. Organic acids

In L. aequinoctialis, malate is produced as an end product of photosynthesis. As can be seen in fig. 2.42, accumulation of malate proceeds only during the light phase and is blocked by  $10^{-6}$  M DCMU (KANDELER and HELDWEIN 1979). The extent of malate synthesis seems to depend on a control mechanism, which regulates the cytoplasmic pH. When protons are consumed (especially during nitrate reduction) and cannot be delivered subsequently from outside the cell (i.e., at relatively high medium pH), then malic acid production is enhanced. This view is stressed by the finding that the inhibitor of nitrate reduction, tungstate, lowers the

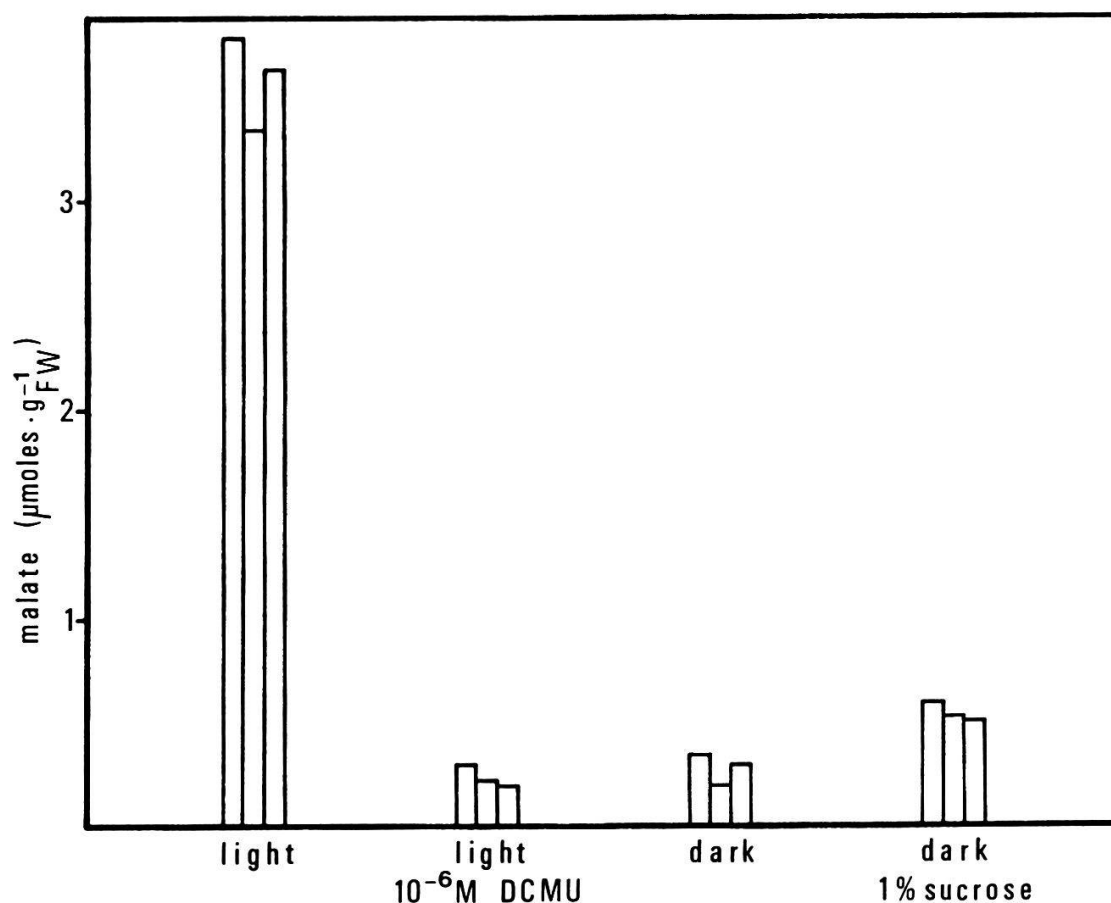


Fig. 2.42. The effects of continuous light, DCMU, and sucrose on malate accumulation in Lemna aequinoctialis 6746 (KANDELER and HELDWEIN 1979). Plants were held in Pirson-Seidel medium without nitrate at pH 6. Malate content of plants at the beginning of the 24-hour experiment was  $0.11 \mu\text{moles/g FW}$ .

malate accumulation in L. aequinoctialis and L. gibba (HELDWEIN and KANDELER 1981). Transfer of L. minor from  $\text{NH}_4^+$  to  $\text{NO}_3^-$ -containing solution raises malate content of the plants after a lag period of about 4 hours (KOPP et al. 1974b). Adaptation of plants to end-of-day irradiation with far red, which enhances malate accumulation, also seems to act through an improvement of nitrate assimilation (HELDWEIN and KANDELER 1981). Furthermore, a rise of medium pH from 4.8 to 6.0 increases malate production in L. aequinoctialis (KANDELER and HELDWEIN 1979).

Supply of ABA ( $10^{-6}$  M) is another treatment which promotes malate accumulation in L. aequinoctialis and L. gibba. This finding may explain the fact that nitrogen deficiency leads to a higher malate content in L. aequinoctialis (KANDELER and HELDWEIN 1979). Preliminary data of MERTEN (1979) have shown that endogenous ABA production is increased by N deficiency in this plant. ABA ( $3.8 \times 10^{-6}$  M) enhances the activities of PEP-carboxylase and NADP-malate dehydrogenase in L. minor (BAUER et al. 1976). NaCl as another stress factor, increasing ABA level up to 1.5 times, has no increasing effect on malate synthesis in L. minor (HUBER and SANKHLA 1979). In this case, however, the malate level of control plants lies one order of magnitude higher than that found in L. aequinoctialis and L. gibba.

Oxalate synthesis has also been considered to be a component of the biochemical "pH-stat". In correspondence with this hypothesis, BORNKAMM (1965) found a close connection between oxalate accumulation and protein content in L. minor. Plants grown with various concentrations of  $\text{NO}_3^-$ , or  $\text{H}_2\text{PO}_4^-$ , or chloramphenicol, show a parallelism between oxalate and protein content in every case. Water-soluble as well as water-insoluble oxalates are affected in the same way.

In L. minor and L. trisulca there are both forms of glycolate oxidase, the FMN-activated form as well as the glycolate-activated form (BORNKAMM 1969a). This enzyme catalyzes not only the oxidation of glycolate to glyoxylate, but also the oxidation of glyoxylate to oxalate. Thus, oxalate production in Lemna could proceed within peroxisomes in connection with photorespiration. In cell-free extracts of L. minor glyoxylate- $1\text{-}^{14}\text{C}$  is transformed to oxalate. Furthermore, in vitro oxidation of glyoxylate is inhibited by addition of 25  $\mu\text{M}$  oxalate to the cell-free system (BORNKAMM 1964, 1965). Plants grown in nutrient medium with 1 mM glyoxylate, reach a higher content of water-insoluble oxalate. Glycine, on the other hand, inhibits oxalate accumulation. NUSS and LOEWUS (1978)

stated that L-ascorbic acid can serve as another precursor of oxalic acid, but - in contrast to what is seen in several other plants - the incorporation of  $^{14}\text{C}$  from [ $1\text{-}^{14}\text{C}$ ] ascorbic acid into oxalate in intact plants of L. gibba is relatively low. Incorporation of  $^{14}\text{C}$ -labeled glycolate, glyoxylate, and ascorbate into calcium oxalate crystals has been found microautoradiographically in L. minor (FRANCESCHI 1985). The glycolate oxidase pathway of oxalate formation is operational as shown by the inhibiting effect of  $\alpha$ HPMS (2-pyridylhydroxymethane sulfonic acid) and mHBA (methylhydroxybutanoic acid). Both inhibitors are effective also during incorporation of  $^{14}\text{C}$  from ascorbate, indicating that glycolate and/or glyoxylate are intermediates of ascorbate catabolism.

Factors which inhibit oxalate synthesis in vivo to some extent, are high temperature ( $30^{\circ}\text{C}$ ; BORNKAMM 1964, 1965, 1970c), higher light intensity (4000 versus 600 lux), 1.8%  $\text{CO}_2$ , 0.5% glucose, and manganese deficiency (BORNKAMM 1964, 1965). The water-insoluble portion of oxalate is deposited in idioblasts within special membranes as the monohydrate of calcium oxalate (ARNOTT and PAUTARD 1965, AL-RAIS et al. 1971). BORNKAMM (1965) could not detect any degradation of calcium oxalate crystals (raphides) even under calcium deficiency. On the contrary, under such conditions the oxalate accumulation is not reduced, so that the portion of water-soluble oxalate increases from 7.7 to 85.4%.

#### 2.5.5.3. Myo-inositol

This compound plays a role in many metabolic processes. Two of them have been investigated with duckweeds: the production of phytic acid as a phosphate-storing substance (s. chapter 2.5.6.2), and biosynthesis of apiose, a component of pectic polysaccharides in the cell wall of Lemna-ceae (see below).

Myo-inositol is derived from glucose-6-phosphate in two steps (fig. 2.43). Firstly glucose-6-phosphate is transformed to myo-inositol-1-phosphate. The enzyme myo-inositol-1-phosphate synthase is present in L. aequinoctialis (LOEWUS and LOEWUS 1971) and has been isolated carefully from L. gibba (SCHWARCZ et al. 1974, OGUNYEMI et al. 1978). The purified enzyme has a molecular weight of  $135000 \pm 5000$  and consists of 3 subunits with identical electrophoretic behaviour. It is inhibited by EDTA and can be reactivated by  $\text{Mn}^{2+}$ . The specific activity of the enzyme is about 500 times higher than that of the animal enzymes so far investigated.

The second step of myo-inositol formation, the cleavage of myo-inositol-1-phosphate by a phosphatase, should also occur in Lemnaceae, as free myo-inositol can be handled in phytic acid metabolism (ROBERTS and LOEWUS 1968, BOLLMANN et al. 1980) as well as in biosynthesis of apiose and xylose (ROBERTS et al. 1967).

#### 2.5.5.4. Apiose and apiogalacturonans

A very characteristic feature of Lemnaceae are apiogalacturonans in the cell wall. Besides Lemnaceae apiose-containing polysaccharides have been found only in some species of Hydrocharitaceae, Potamogetonaceae, and Zannichelliaceae (see GRISEBACH 1980). Such polysaccharides with high D-apiose content are not degraded by pectinase (HART 1969, HART and KINDEL 1970b) and thus, make plants resistant to the attack of several microorganisms. When isolation of Lemna protoplasts is required during physiological investigations, an enzyme mixture must be used containing not only cellulase "Onozuka R-10" and Mazerozyme R-10, but also Driselase (LOEPPERT and KOENIGSHOFER, unpublished results).

Occurrence of apiose, a branched-chain sugar, in Lemnaceae has been ascertained several times (s. chapter 1.2.3). Biosynthesis proceeds via UDP-bound intermediates from D-glucuronic acid (fig. 2.43). UDP-glucuronic acid is oxidized with NAD to a 4-keto-intermediate. Then decarboxylation leads to a second intermediate, which undergoes a ring contraction reaction. The resulting aldehyde is reduced by NADH to UDP-D-apiose. Extensive work has been done by several working groups to elucidate this biosynthetic reaction chain in Lemna. Reviews have been given by WATSON and ORENSTEIN (1975), GRISEBACH (1980) and BECK (1982). The original papers on this topic are the following: MENDICINO and PICKEN (1965), BECK and KANDLER (1966), PICKEN and MENDICINO (1967), SANDERMANN et al. (1968), GUSTINE and KINDEL (1969), MENDICINO and HANNA (1970), SANDERMANN and GRISEBACH (1970), KELLEHER and GRISEBACH (1971), KINDEL and WATSON (1973).

The enzyme managing the reaction chain from UDP-glucuronic acid to UDP-apiose also catalyzes the formation of UDP-D-xylose from the second above-named intermediate and, therefore, has been named UDP-apiose/UDP-xylose synthase (WELLMANN and GRISEBACH 1971, GUSTINE et al. 1975). The enzyme, which has been purified more than 1000-fold, possesses a molecular weight of 110000 and a pH optimum of 7-8.5 (WELLMANN and GRISEBACH

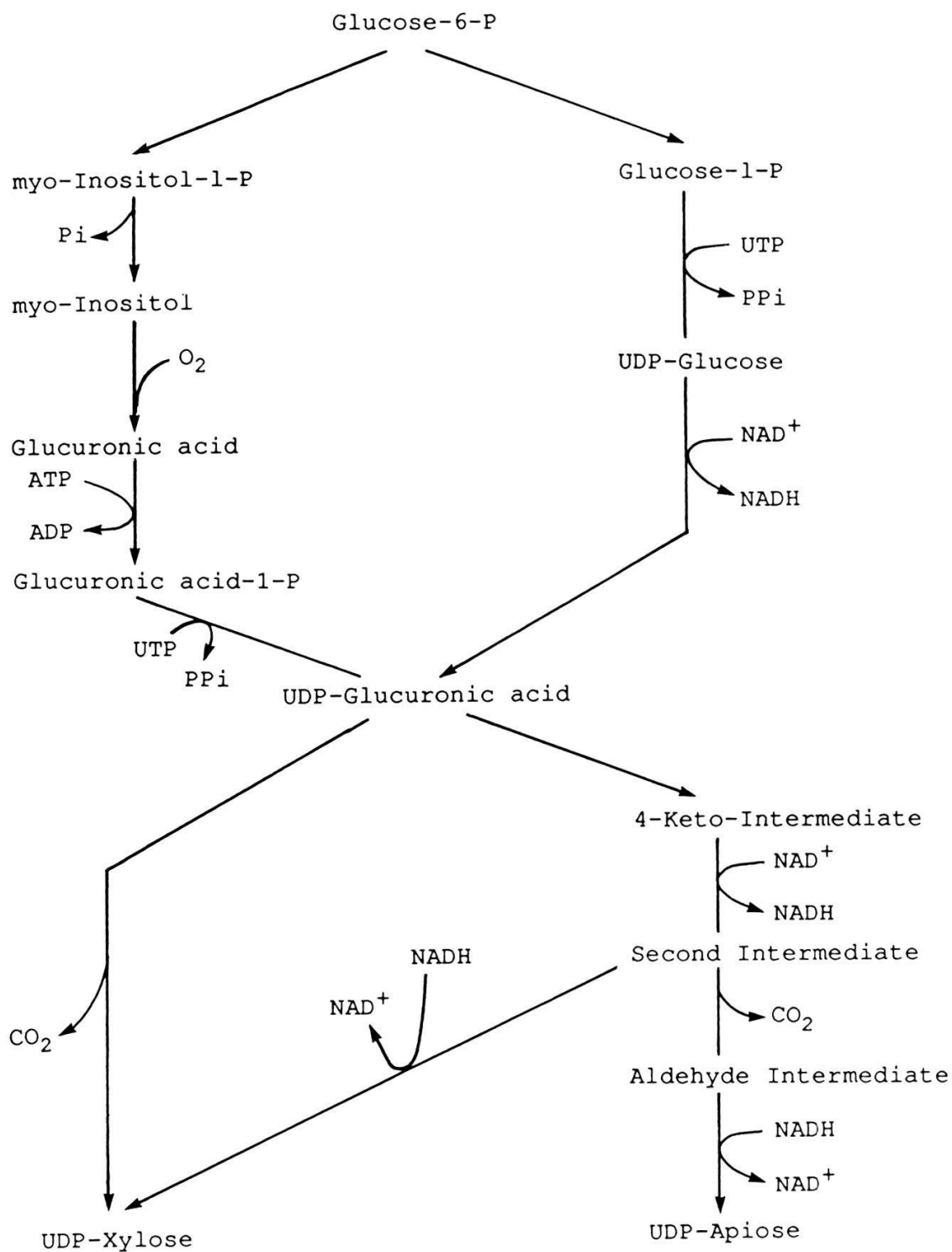


Fig. 2.43. Biosynthesis of myo-inositol, apiose, and xylose in Lemna.



1971, MENDICINO and ABOU-ISSA 1974). In addition, another enzyme (UDP-glucuronate carboxylase) exists, which catalyzes the transformation of UDP-glucuronic acid to UDP-xylose solely (GUSTINE et al. 1975).

The glucuronate units for biosynthesis of apiose are derived from glucose-6-phosphate via glucose-1-phosphate and UDP-glucose or via myo-inositol-1-phosphate and myo-inositol. ROBERTS et al. (1967) showed that myo-inositol-2-<sup>14</sup>C is incorporated into apiosyl and xylosyl units of cell wall polysaccharides in L. gibba G3.

Apiogalacturonans have been isolated from L. minor (BECK 1967). One fraction consisted solely of D-apiose and polygalacturonic acid, another one contained xylose in addition. HART and KINDEL (1970b) separated at least five polysaccharides containig D-apiose from L. minor. The apiosyl side-chains are attached to the galacturonan backbone in form of apiobiose, a disaccharide consisting of two apiose moieties (HART 1969, HART and KINDEL 1970a, MASCARO and KINDEL 1977). Transferase activity which attaches D-apiosyl- and D-xylosyl-units from UDP-D-apiose and UDP-D-xylose to unknown endogenous acceptors (apiogalacturonans), has been demonstrated in a particulate preparation of L. minor (PAN and KINDEL 1977). Also a particulate enzyme incorporating UDP-galacturonic acid into pre-existing galacturonans has been isolated (LEINBACH 1975). This D-galacturonosyl transferase is stimulated by Mn<sup>2+</sup> and possesses optimum activity at pH 6.0-6.2.

In connection with the development of intercellular air spaces Lemna should have the ability to degrade apiogalacturonans. Even an oxidation of apiose to CO<sub>2</sub> occurs when D-[U<sup>14</sup>C] apiose is supplied to sterile L. minor plants (HANNA et al. 1973).

## 2.5.6. Assimilation of phosphorus, nitrogen, and sulfur

### 2.5.6.1. Assimilation of phosphate

The use of phosphate within cell metabolism of S. punctata has been investigated by BIELESKI (1968a). After 9 days of growth in a solution containing phosphate-<sup>32</sup>P the various radioactive compounds were extracted and determined quantitatively. In the principal fractions, inorganic phosphate, RNA, phospholipids, and soluble phosphate esters, the proportion of phosphorus amounts to 71.1, 15.1, 7.5, and 4.9% of total P, respectively. Within phospholipids phosphatidyl choline (46.3%), phosphatidyl ethanolamine (22.5%), phosphatidyl glycerol (13.5%), and phosphatidyl inositol (6.3%) are the main components. In the fraction of acid-soluble phosphate esters glucose-6-phosphate (22.4%) and phosphoglyceric acid (10.2%) predominate.

In well-nourished plants inorganic phosphate is present in two pools: a metabolic (12%) and a non-metabolic pool (88%), which may be located in cytoplasm and vacuole, respectively (BIELESKI 1968b). Under conditions of phosphate deprivation, however, the non-metabolic pool is exhausted and more than 90% of the remaining inorganic phosphate is traceable in the metabolic pool. Phosphate ester content then falls from 3.5 to 0.6  $\mu$ moles/g, phospholipid content from 3.5 to 1.2  $\mu$ moles/g, and residual phosphate (mainly RNA) content from 7.5 to 2.0  $\mu$ moles/g. Relative proportions of the various phosphate esters and relative proportions of the various phospholipids, are not markedly affected by phosphate deficiency.

### 2.5.6.2. Storage of phosphate

Within minerals phosphorus is the factor which limits occurrence of Lemnaceae in the field (LUEOEND 1983). Obviously, the ability of duckweeds to store P excessively is one of the evolutionary adaptations to the habitat of these floating water plants. Besides the accumulation of orthophosphate within the vacuole, phosphate can be stored in the forms of condensed inorganic phosphates and of phytic acid. In vegetatively growing fronds of L. aequinoctialis and L. gibba linear oligophosphates with 2 to 7 residues, cyclic tri-, tetra-, penta- and hexametaphosphates, and high-molecular condensed phosphates are produced rapidly, when phos-

phate-<sup>32</sup>P is applied to the plants (NIEMEYER 1975, INHUELSEN and NIEMEYER 1975). Subsequently the condensed phosphates are degraded in connection with the synthesis of nucleic acids. Condensed phosphates, therefore, seem to function as a short-term reserve pool of inorganic phosphate. Phytic acid, on the other hand, is produced preferentially under conditions, which favour the development of turions or turion-like fronds (s. chapter 2.4.2), and can serve as a long-term P-storage compound. Nitrogen deficiency, sucrose supply, and short day in combination with low temperature (8-10°), are all factors inducing phytic acid synthesis in L. gibba. Moreover, turions of S. polyrrhiza have a very high content of phytic acid in comparison to vegetative fronds (SCHEINER et al. 1978).

Besides the inositol hexaphosphate phytic acid, also the other inositol phosphates have been found in L. minor (INHUELSEN and NIEMEYER 1978), L. gibba and Wolffiella gladiata (ROBERTS and LOEWUS 1968). A series of three enzymes is needed to convert myo-inositol to phytic acid with ATP as the phosphate donor (BOLLMANN et al. 1980). Myo-inositol kinase phosphorylates myo-inositol to myo-inositol-1-phosphate. Then myo-inositol-1-phosphate kinase catalyzes the formation of myo-inositol trisphosphate which is transformed to phytic acid by myo-inositol trisphosphate kinase.

#### 2.5.6.3. Acquisition of phosphate at P deficiency

Shortage of phosphate in the environment not only leads to consumption of endogenously stored phosphate, but also to attempts for acquisition of exogenous phosphate by enzymatic cleavage of P-containing organic compounds. At phosphate deficiency phosphatases and ribonucleases are synthesized, which either are fixed on the outer side of the plasmalemma, or are released to the medium. Induction of phosphatase synthesis by P deficiency has been demonstrated in S. polyrrhiza (JUNGNICKEL 1981), S. punctata (REID and BIELESKI 1970b, BIELESKI 1974, KNYPL and KABZINSKA 1977, KNYPL 1978), L. minor (LIEDKE and OHMANN 1969), and W. arrhiza (JUNGNICKEL and VOELKSCH 1979, JUNGNICKEL 1981). BIELESKI (1974) and KNYPL (1979a) found two alkaline phosphatases to be induced in P-starved plants, which are located (fig. 2.44) at the epidermal and subepidermal cell walls on roots and the lower side of fronds (BIELESKI and JOHNSON 1972). In addition, KNYPL (1979a; see also KNYPL and SOBOLEWSKA

1978, KNYPL 1979b) pointed out one acid phosphatase and two further alkaline phosphatases which are released to the medium at P deficiency. The two ribonucleases produced by S. punctata during P starvation, remain on the cell surface (KNYPL and KABZINSKA 1977, KNYPL 1978, 1979a). BIELESKI and JOHNSON (1972) and KNYPL (1978) have shown that inorganic phosphate appears in the medium of a P-deficient S. punctata culture,

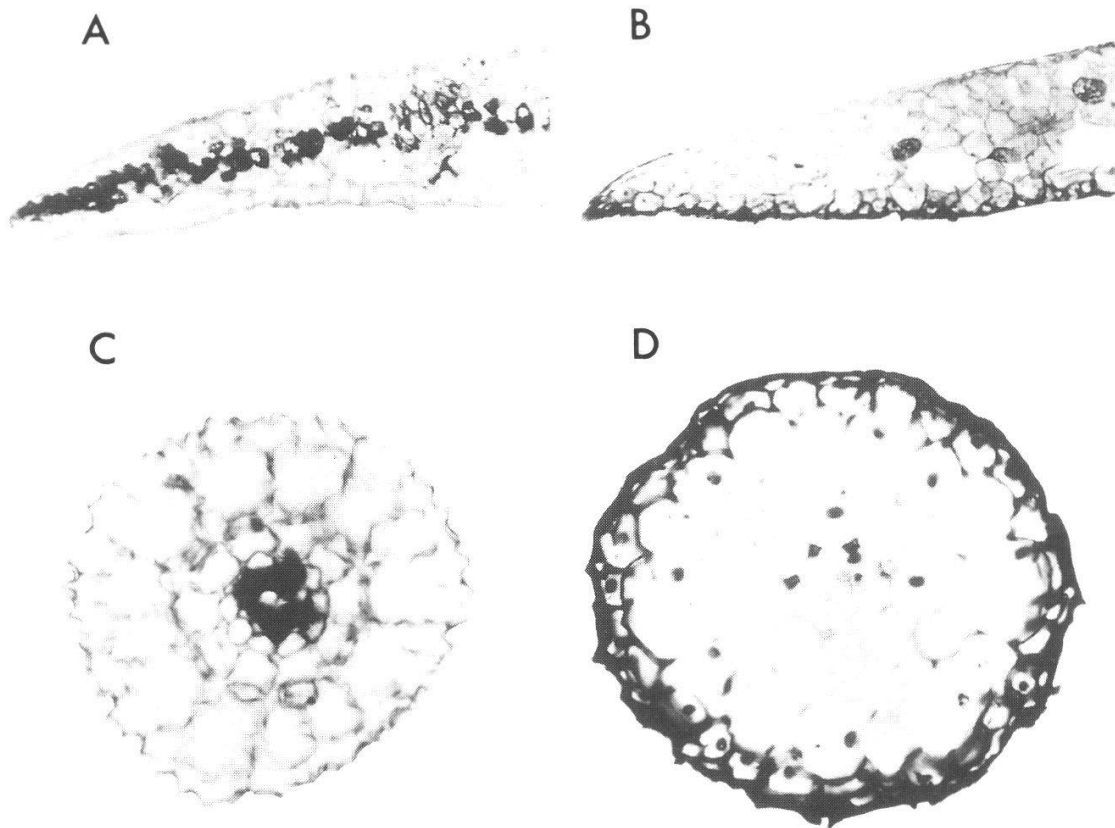


Fig. 2.44. Location of phosphatase in control (left) and phosphorus-deficient (right) Spirodela punctata plants (BIELESKI and JOHNSON 1972). Plants were fixed, stained by the procedure of Gomori, embedded, and sectioned. Note that control and P-deficient plants were stained for different lengths of time, to compensate for their different phosphatase activities.

A. Cross section of control frond (x70)

B. Cross section of P-deficient frond (x75)

C. Cross section of control root (x300)

D. Cross section of P-deficient root (x250)

Note occurrence of phosphatase (black stain) in the region of vascular tissues in control plants (left), and in the epidermis of P-deficient plants (right).

when glucose-1-phosphate or certain adenosine monophosphates (5'-AMP, 2'-AMP) are supplied. Also  $^{32}\text{P}$ -rRNA extracted from S. punctata is hydrolyzed externally by P-starved S. punctata plants (KNYPL 1980). Both oligo- and mononucleotides appear in the solution and the label is incorporated to nucleic acids and low molecular weight P compounds of the P-starved plants. Obviously, compounds containing P released from senescing plants can be re-utilized by younger neighbours. RNA-like material has been detected in the medium of senescing plants (KNYPL 1978).

Acquisition of phosphate or P-containing compounds even occurs in P-provided cultures with the help of constitutive enzymes. This fact may explain, why symptoms of P deficiency as well as induction of phosphatases and ribonucleases do not proceed, when inorganic phosphate in the solution is replaced by yeast RNA (KNYPL 1978). Inorganic P sources other than orthophosphate, such as pyrophosphate, tripolyphosphate, and shark teeth phosphorus, are accessible to L. minor (FITZGERALD 1971).

#### 2.5.6.4. Nitrate reduction

Under natural conditions, nitrate is the main N-source for duckweeds in most cases. After uptake, nitrate must be reduced to ammonia before synthesis of N-containing organic compounds can proceed. The two enzymes responsible for this reduction are NADH-specific nitrate reductase (NR) and red. ferredoxin-dependent NADH-specific nitrite reductase (NiR). Both enzymes are produced adaptively, i.e., only in the presence of their substrates. Nitrate induces synthesis of NR as well as of NiR (S. punctata: FERGUSON 1969a; L. minor: JOY 1969a, STEWART 1972b). Nitrite is less effective than nitrate for induction of NiR in both species and very little effective for induction of NR (for nitrate induction of NR in L. trisulca see MELZER and EXLER 1982, and in W. arrhiza see SWADER and STOCKING 1971). That really new synthesis of the enzymes proceeds during substrate induction, has been confirmed by experiments with inhibitors of nucleic acid and protein synthesis (JOY 1969a, STEWART 1968, 1972b, VIJAYARAGHAVAN et al. 1982). An enhancing effect of sulfate on NR induction in L. minor indicates some coordination of N- and S-metabolism (BRUNOLD and SUTER 1984a). Light and sucrose, alone as well as in combination, increase substrate induction of NADH-specific NR in S. punctata (VIJAYARAGHAVAN et al. 1982). The light effect is mediated by the photosynthetic apparatus as it is blocked by DCMU. In L. minor a NADPH-speci-

fic NR is induced after supply of sucrose to the culture medium (YOUNG 1967, cit. in SIMS et al. 1968). Iron-deficiency lowers NR activity considerably (SPILLER et al. 1973) and tungstate prevents appearance of NR activity completely (STEWART 1972b). ABA ( $2 \times 10^{-5} \text{ M}$ ) has no effect on  $\text{NO}_3^-$  induction of NR in S. polyrrhiza (STEWART and SMITH 1972). The ionic surfactant DBST (trimethylamine alkylbenzenesulfonate) distinctly lowers the activities of NR and NiR in S. polyrrhiza (BUCZEK 1984b). The non-ionic surfactant ENF (alkylphenoxypolyethoxy ethanol) inhibits only the NiR activity.

A wealth of papers has been published to answer the question of repression and/or inactivation of NR by ammonium (SIMS et al. 1968, FERGUSON 1969a, FERGUSON and BOLLARD 1969, JOY 1969a, STEWART 1972a,b, STEWART et al. 1974, OREBAMJO and STUART 1974, 1975a, 1975b, MELZER and EXLER 1982, VIJAYARAGHAVAN et al. 1982, TATKOWSKA and BUCZEK 1983). A difficulty not considered in the older works is the fact that an inhibitor of NR, tentatively characterised as a protein, seems to occur in duckweeds (EISENMEIER and SPILLER 1973, OREBAMJO 1973, cited in STEWART et al. 1974, BUCZEK 1984a) and that this inhibitor has its own dynamics (OREBAMJO and STEWART 1975a). The mode of interrelation between ammonium ions and the inactivating system is not yet known. A promotive  $\text{NH}_4^+$  effect on formation or an activation of the inhibitor protein has been discussed (OREBAMJO and STEWART 1975b).

The different results obtained for the effect of ammonium on NR induction may be caused by certain pre-culture conditions and - in consequence - by different contents of  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and the supposed inactivator. When plants were transferred to the induction ( $\text{NO}_3^-$ ) medium directly from  $\text{NH}_4^+$ - or medium containing amino acid, presence of ammonium in the induction medium strongly inhibits or prevents, the raise of NR activity (JOY 1969a, STEWART 1972a,b). A more detailed investigation of kinetics reveals, however, that under such conditions  $\text{NH}_4^+$  inhibition of NR activity originates not before 3-4 hours after the beginning of substrate induction (OREBAMJO and STEWART 1975a). This holds true also for plants which have been pre-cultured in an N-free medium for 3 days (TATKOWSKA and BUCZEK 1983). Even a promotion of the NR induction process by ammonium can be seen during the first hours (FERGUSON 1969a) or one day (VIJAYARAGHAVAN et al. 1982), when plants were N-starved for two days before the experiment.

Taking all the data together, it seems that ammonium ions inactivate NR

but do not repress this enzyme (TATKOWSKA and BUCZEK 1983). Reversibility of NR inactivation in vivo and in vitro has been shown by OREBAMJO and STEWART (1975b).

Certain amino acids (alanine and arginine, for example), asparagine and urea exert similar effects on NR induction as ammonium ions. Depending on experimental conditions a strong or moderate inhibition (SIMS et al. 1968, STEWART 1972a, 1972b, VIJAYARAGHAVAN et al. 1982) or no effect (FERGUSON 1970) has been found.

Nitrite reductase is a more stable enzyme than NR (BUCZEK 1984a) and less accessible to inhibiting effects of ammonia and amino acids (SIMS et al. 1968). Nevertheless, STEWART (1972b) obtained strong inhibitions of NiR induction by ammonia, glycine, asparagine, and urea in S. punctata. SWADER and STOCKING (1971) pointed out that  $\text{NO}_2^-$  reduction but not  $\text{NO}_3^-$  reduction is located in the chloroplasts of W. arrhiza. The providing of protons for nitrite reduction by formation of malic and oxalic acid has been reported in chapter 2.5.5.2.

#### 2.5.6.5. Assimilation of ammonia, urea, and other exogenous organic N compounds

Ammonia is taken up and utilized preferentially (s. chapter 2.5.2.2.2), when  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are both present in the nutrient medium (s. above: endproduct inhibition of nitrate reductase). However, in the case of ammonia and nitrite (FERGUSON and BOLLARD 1969) or ammonia and amino acids (FERGUSON 1970) both compounds are used up simultaneously.

BAUER et al. (1973) traced the  $^{15}\text{NH}_4^+$  incorporation in the active turnover pools of glutamic acid, alanine, and glutamine in L. minor. They concluded that glutamic acid is the most important primary assimilation product of ammonium. In a more advanced analysis RHODES et al. (1980) revealed that L. minor assimilates  $^{15}\text{NH}_4^+$  solely through the glutamine synthetase - glutamate synthase cycle when ammonium is provided at a relatively low concentration (2 mM). When activity of glutamine synthetase has fallen to zero after addition of azaserine and methionine sulfoximine, no  $^{15}\text{N}$  labelling of glutamic acid occurs. This means that in spite of the presence of active NAD-glutamate dehydrogenase  $\text{NH}_4^+$  cannot be incorporated directly into 2-oxoglutarate.

The glutamine pathway of ammonium assimilation (fig. 2.45) consists of two steps: the transformation of glutamate to glutamine with  $\text{NH}_3$  by glu-



tamine synthetase (GS), and transfer of the amide N from glutamine to 2-oxoglutarate by glutamate synthase (GOGAT). One of the two molecules of glutamate released by the second reaction is recycled to the first reaction. The GS-GOGAT cycle is located in chloroplasts (for Lemna see STEWART, cited in RHODES et al. 1979) and, therefore, connected intimately with nitrite reduction. ATP (for the first step) and reduction energy (for the second step) are provided by photosynthesis.

The glutamine pathway is the principal way of  $\text{NH}_4^+$  incorporation at least in plants grown on nitrate or relatively low concentrations of ammonium. STEWART and RHODES (1976) have shown that blocking of GS by methionine sulfoximine (MSO) causes an accumulation of ammonia and 2-oxoglutarate, and a decrease of glutamine level in nitrate-grown L. minor. Also in L. gibba MSO enhances the intracellular ammonium level (JOHANSSON and LARSSON 1986a). Furthermore, azaserine (inhibiting glutamine amide transfer of GOGAT) increases  $^{14}\text{CO}_2$  incorporation into 2-oxoglutarate associated with a decrease of incorporation into glutamate in plants grown on 5 mM ammonia (RHODES 1976, cited in RHODES et al. 1979).

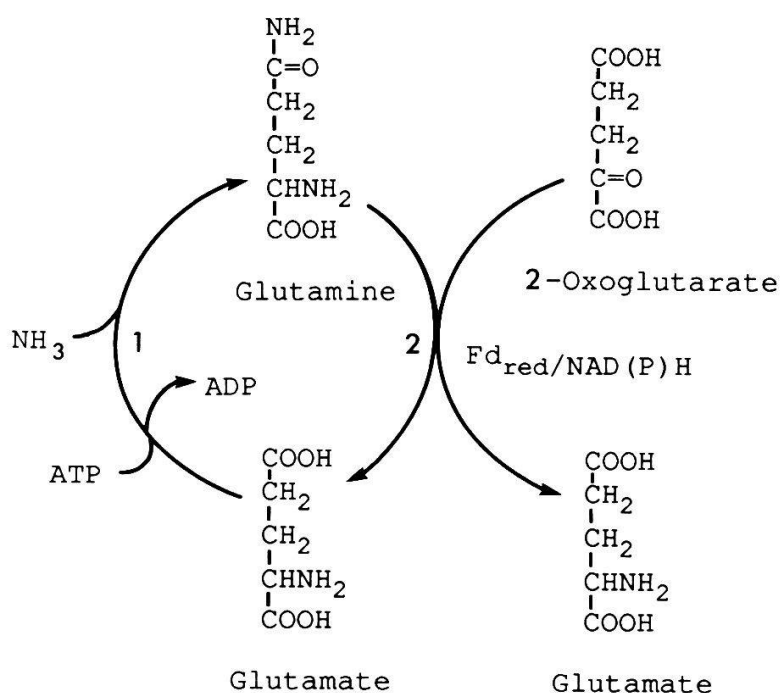


Fig. 2.45. The glutamine pathway of ammonium assimilation (from MIFLIN and LEA 1980). Enzymes: 1, glutamine synthetase; 2, glutamate synthase.

At relatively high  $\text{NH}_4^+$  concentrations, however, a partial switching over to the glutamate pathway of ammonium assimilation seems to occur. With increasing  $\text{NH}_4^+$  content of the medium (2-15 mM) the activities of GS and GOGAT decrease (RHODES et al. 1975, 1976) and the activity of glutamate dehydrogenase (GDH) increases (JOY 1969a [compare however JOY 1971], SHEPARD and THURMAN 1973, RHODES et al. 1976, EHMKE and HARTMANN 1976 [tab. 2]). The  $\text{NH}_4^+$  effect on GS activity is provable especially when ammonium treatment is combined with darkness (RHODES 1976, cited in RHODES et al. 1979).

RHODES (1976, cited in RHODES et al. 1979) and RHODES et al. (1976) pointed out that glutamine - rather than ammonia itself - may be the substance exerting negative control on GS and GOGAT and positive control on GDH. Comparison of tissue glutamine levels with enzyme activities from plants cultivated with various N-sources reveals a negative correlation between glutamine and GS, but a positive correlation between glutamine and GDH (STEWART and RHODES 1977b, cited in RHODES et al. 1979). Glutamine synthetase from L. minor has a mol. wt of about 360000 and is probably composed of eight identical sub-units. The physiologically active state needs  $\text{Mg}^{2+}$  (RHODES et al. 1979). The rapid loss of GS activity after transfer of plants to ammonium solution and darkness involves a reversible deactivation of the enzyme. A low molecular weight, heat-stable fraction has been isolated, which can promote GS deactivation and inhibit reactivation in vitro (RHODES et al. 1979). A feed-back control of GS, on the other hand, is exerted by some amino acids (alanine, glycine, serine, aspartate) and certain nucleotides. Both ADP and 5'-AMP competitively inhibit the enzyme with respect to ATP (STEWART and RHODES 1977a). The authors concluded that control of the enzyme by energy charge may be important under some conditions.

The enzyme for reductive amination of 2-oxoglutarate, glutamate dehydrogenase (GDH), has been investigated several times. The most advanced analysis was made by HARTMANN and co-workers with L. minor plants (EHMKE and HARTMANN 1976, 1978, SCHEID et al. 1980, EHMKE et al. 1980). The authors isolated a single protein (mol. wt 230000) consisting of four identical subunits. The enzyme exhibits catalytic activity preferably with NADH, but also with NADPH to some degree (ratio of activities NADH/NADPH = ca 12/1). The GDH activity was found to be located mainly in mitochondria. The complete loss of NADH-dependent activity of GDH by EDTA pretreatment as well as the moderately inhibiting action of various ade-

nylates can be fully reversed by adding  $\text{Ca}^{2+}$ . At low  $\text{Ca}^{2+}$  levels a pronounced substrate inhibition occurs, particularly with  $\text{NH}_4^+$  and NADH. Hydride transfer from NADH to the substrate takes place from the 4B position. STEWART and RHODES (1977a) confirmed that only a single form of GDH exists in L. minor plants grown in nitrate and ammonium. The 5-fold increase of GDH activity within 3 days after transfer of L. gibba plants to a high ammonium concentration (15 mM) is independent of the presence of EDTA and is due to de novo synthesis on 80 S ribosomes as shown by experiments with inhibitors of protein synthesis and with deuterium oxide-labelling technique (SHEPARD and THURMAN 1973). In L. aequinoctialis DUKE and KOUKKARI (1977) found GDH activity in the chloroplast-rich fraction to be higher than in the mitochondria-rich fraction (in control plants and - to a higher degree - in plants grown in glucose). In plants grown in sucrose GDH activity was lower especially in the chloroplast-rich fraction.

Changing over from the glutamine to the glutamate pathway of ammonium assimilation may also be induced by certain stress factors. Addition of NaCl (20-80 mM) to cultures of L. minor decreases the activities of GS, GOGAT, and nitrate reductase, but slightly increases the activity of GDH (HUBER 1982). Finally, it should be mentioned that - in principle - GDH can display NAD-dependent activity, i.e., catalysis of glutamate deamination. TEIXEIRA and DAVIES (1974) suggested that at N-starvation glycolysis is inhibited (by high pyridoxal phosphate) thereby restricting the supply of carbohydrates to the Krebs cycle. Under such conditions GDH should be blocked to prevent the loss of organic nitrogen by deamination of glutamate. In fact, the level of pyridoxal phosphate (inhibiting GDH in pea mitochondria) increases, and the level of pyridoxamine phosphate decreases in L. minor, when the nitrogen supply is restricted.

Assimilation of urea and the ureid allanthoin has been investigated with S. punctata (BOLLARD 1966, BOLLARD et al. 1968, BOLLARD and COOK 1968, COOK 1968). Under the conditions used, plants can grow with urea as the sole source of nitrogen provided that the pH of the medium is adjusted to <4.3. Correspondingly, urease activity appears after supply of urea or even at N-deficiency only in the case that the pH reaches <4.3. Urease activity has been confirmed by liberation of  $^{14}\text{CO}_2$  from  $^{14}\text{C}$  urea. The extent of  $^{14}\text{CO}_2$  evolution was proportional to the level of urease as assayed by formation of ammonia in extracts. The results obtained suffer from the fact that nickel was added to the nutrient medium not directly

but presumably only in the form of impurities of other heavy metals. Thus, the pH effect may be explained by an improvement of  $\text{Ni}^{2+}$  availability (lowering of EDTA complex stability, for example) as  $\text{Ni}^{2+}$  is known to be a constituent of urease. GORDON et al. (1978) increased the multiplication rate of L. aequinoctialis 6746 by 83-91%, when  $\text{Ni}^{2+}$  was added to the nutrient medium with urea as the sole N-source. S. polyrrhiza and W. globosa responded to  $\text{Ni}^{2+}$  and urea in qualitatively the same way as L. aequinoctialis.

More than 160 further organic N-compounds have been tested to serve as sole source of nitrogen in cultures of S. punctata (BOLLARD 1966). Positive results were obtained with some amino acids, substituted amino acids, and derivatives of amino acids, peptides such as glycyl-L-aspartic acid, putrescine, agmatine, and various urea derivatives. In the submergent L. trisulca the preferences for N-sources are different from those for emergent species of Lemnaceae (HOLST and YOPP 1979). N-sources for L. trisulca in order of effectiveness are urea, aspartic acid, nitrate, glutamic acid, arginine, ammonium, and casein hydrolysate. Urea may be of particular value for submergent plants, because  $\text{CO}_2$  released during the urease reaction can be used for photosynthesis.

#### 2.5.6.6. Assimilation of sulfur

The main anorganic source of sulfur is sulfate, but in addition also sulfide, sulfite,  $\text{H}_2\text{S}$ , and  $\text{SO}_2$  can be used when the concentration of these compounds is kept below an injuring level (ERISMANN et al. 1967, BRAENDLE and ERISMANN 1968, BRAENDLE et al. 1968, BRUNOLD and ERISMANN 1972, 1974, 1976, FANKHAUSER et al. 1976). Sulfite has been shown to be assimilated depending on sulfite resistance of plants (TAKEMOTO and NOBLE 1984, 1986, TAKEMOTO et al. 1986). Resistance to sulfite and sulfite assimilation increases with increasing growth rate caused, for example, by high light intensity. In the moderately growing L. gibba and S. punctata frond multiplication is inhibited by 5-10 mM  $\text{K}_2\text{SO}_3$ , but not in the rapidly growing L. valdiviana. The sulfite tolerance depends on the ability to form elevated levels of thiols, and to emit  $\text{H}_2\text{S}$ . Under low irradiance, the  $\text{H}_2\text{S}$  emission rate in L. valdiviana is up to four times higher than in the other species. High irradiance increases photosynthesis, thiol formation, and  $\text{H}_2\text{S}$  emission, in this way leading to enhanced sulfite detoxification.

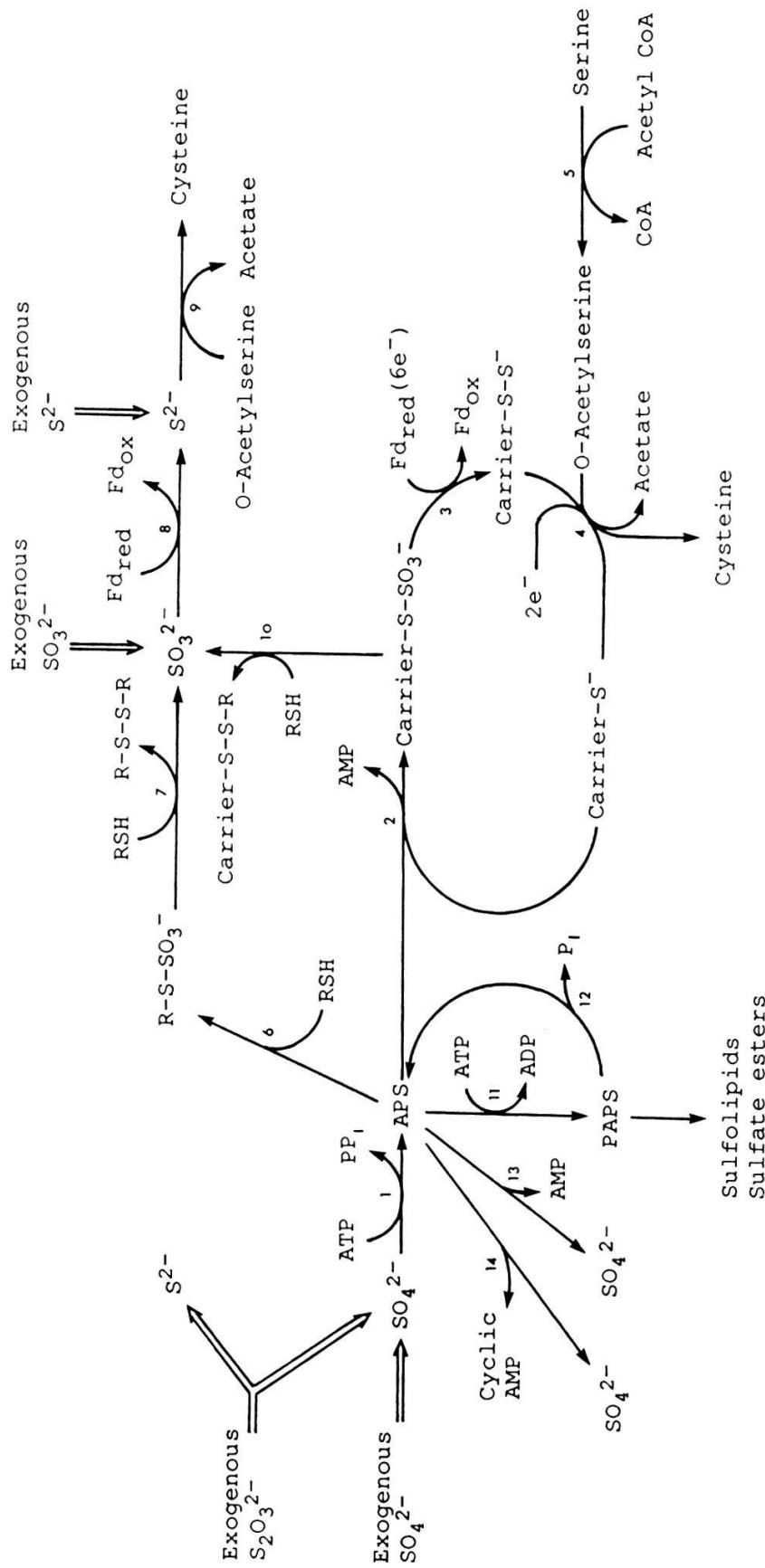


Fig. 2.46. Summary of the free and bound pathways of sulfate assimilation in plants (from ANDERSON 1980). Some related reactions and points of entry of inorganic sulfur are also shown. The reaction sequence catalyzed by (1) ATP sulfurylase, (2) APS sulfurylase, (3) thiosulfonate reductase, and (4) cysteine synthase constitutes the bound sulfate assimilation pathway. The synthesis of OAS is catalyzed by (5) serine transacetylase. The reaction sequence (1), (6)-(9) or (1), (2), (10), (8), (9) constitutes the free pathway; reactions (7) and (10) are nonenzymatic, (6) is catalyzed by APS sulfotransferase, (8) by sulfite reductase, and (9) by cysteine synthase. APS and PAPS are interrelated via (11) APS kinase and (12) NDP phosphohydrolase. APS can be hydrolyzed via (13) APS sulfolipase or (14) APS cyclase.

Organic S compounds which can be utilised by S. punctata as sole sources of sulfur in millimolar concentrations, are cysteine, cystine, and cysteic acid (FRASER 1974). At 0.1 mM L. gibba grows on cystine, cysteine, methionine, glutathione, and cysteic acid (ELLIS 1969).

DATKO et al. (1978b) determined quantitatively all the S-containing compounds which can be identified in L. aequinoctialis after labeling to radioisotopic equilibrium with  $^{35}\text{SO}_4^{2-}$ . These are inorganic sulfate, glutathione, homocyst(e)ine, cyst(e)ine, methionine, S-methylmethionine sulfonium, S-adenosylmethionine, S-adenosylhomocysteine, cystathionine, chloroform-soluble (presumed to be sulfolipid), protein cyst(e)ine, and protein methionine. The amount of each component present in plants grown over a 3000-fold range of medium sulfate (0.32-1000  $\mu\text{M}$ ) was relatively constant except for inorganic sulfate.

For assimilatory sulfate reduction possibly the "free pathway" and the "bound pathway", are both used (fig. 2.46). In each case, firstly adenosine S-phosphosulfate (APS) is formed from  $\text{SO}_4^{2-}$  and ATP, catalyzed by ATP-sulfurylase. The enzyme has been extracted from L. gibba, L. aequinoctialis, and L. minor (ELLIS 1969, WILSON and KANG 1972, 1973, REUVENY 1974, BRUNOLD and SCHMIDT 1976, SCHMUTZ and BRUNOLD 1982). In the bound pathway the sulfonyl group of APS is transferred by APS sulfotransferase to a carrier and then reduced to a thiol group. Finally, this SH-group is incorporated into o-acetylserine (OAS) by cysteine synthase to form cysteine. The presence of APS sulfotransferase in L. minor has been demonstrated by SCHMIDT (1975). Activity of this enzyme is down-regulated by  $\text{H}_2\text{S}$  (BRUNOLD and SCHMIDT 1976, VON ARB and BRUNOLD 1980) and cysteine (BRUNOLD and SCHMIDT 1978). Cysteine synthase (= o-acetylserine sulfhydrylase) has been found in L. minor (BRUNOLD and SCHMIDT 1976, VON ARB and BRUNOLD 1980) and L. aequinoctialis (THOMPSON et al. 1982a). The enzyme is not affected significantly by  $\text{H}_2\text{S}$ . Coordination of S- and N-assimilation is indicated by the fact that ammonium, arginine, asparagine, and glutamine cause a 50 to 100% increase in APS sulfotransferase activity (BRUNOLD and SUTER 1984b, VON ARB et al. 1985, SUTER et al. 1986).

APS sulfotransferase and cysteine synthase both also work in the "free" pathway of sulfate assimilation. APS sulfotransferase transfers the sulfonyl group of APS to reduced glutathione or another thiol and the product reacts nonenzymatically with a further thiol releasing free sulfite. Sulfite is reduced to sulfide and cysteine synthase then catalyzes

the reaction of sulfide and OAS to form cysteine and acetate. The existence of this pathway is indicated by direct incorporation of  $\text{H}_2\text{S}$  into cysteine without preceding oxidation of  $\text{H}_2\text{S}$  to  $\text{SO}_4^{2-}$  (BRUNOLD and ERISMANN 1975). Besides the enzyme reacting with OAS another cysteine synthase is present in L. minor which uses serine as substrate (BRUNOLD and ERISMANN 1972, 1975). Occurrence of sulfite reduction to  $\text{H}_2\text{S}$  has been demonstrated in L. valdiviana, S. punctata, L. gibba (TAKEMOTO and NOBLE 1984, TAKEMOTO et al. 1986), and L. minor (BRUNOLD and ERISMANN 1976).  $\text{H}_2\text{S}$  and  $\text{SO}_2$  are assimilated not only using the last steps of "free pathway", but also after oxidation to sulfate. Fumigation of L. minor with 6 ppm  $\text{H}_2\text{S}$  leads to a pronounced increase in internal sulfate concentration (BRUNOLD and ERISMANN 1974). Among other things sulfide may serve as an accessory electron donor in photosynthesis (KNOBLOCH 1966, ERISMANN et al. 1967). Atmospheric  $\text{SO}_2$  is oxidized very effectively to  $\text{SO}_4^{2-}$  in L. minor as indicated by isotope competition experiments (BRUNOLD and ERISMANN 1976). Under the conditions used, all the  $\text{SO}_2$  seems to be incorporated into proteins and sulfolipids following oxidation to  $\text{SO}_4^{2-}$  and subsequent reduction inspite of the presence of sulfite reductase. SCHAEERER et al. (1975) observed an increase of internal  $\text{SO}_4^{2-}$  level after fumigation with 0.3-0.6 ppm  $\text{SO}_2$ . DATKO and MUDD (1984b) mentioned that even cystine is metabolized to sulfate in L. aequinoctialis.

Selenate and selenite are also assimilated by duckweeds. S. punctata absorbs and metabolizes selenite more readily than selenate (BUTLER and PETERSON 1967). After application of  $^{75}\text{SeO}_3^{2-}$  or  $^{75}\text{SeO}_4^{2-}$  selenocystine and oxidation products predominate in the soluble fractions, but selenomethionine and its oxide predominate in the protein hydrolysate.



## **2.5.7. Amino acids, proteins, and nucleic acids**

### **2.5.7.1. Biosynthesis of amino acids**

Principally, the amino groups of amino acids are provided by transaminase reactions. Two of these enzymes have been investigated in W. arrhiza (APPENROTH et al. 1986). Under continuous light, both alanine aminotransferase and aspartate aminotransferase exhibit a distinctly higher activity under blue, than under red or even white light. A short blue light pulse (10 min), given after darkness, immediately increases the activity of both enzymes.

Pathways and regulation of amino acid biosynthesis have been investigated mainly within the aspartate family. Thus, pathways are reviewed in the following order:

- (a) Aspartate - lysine
- (b) Aspartate - homocysteine - methionine
- (c) Homoserine -threonine - isoleucine
- (d) Pyruvate - valine - leucine
- (e) Other amino acids

#### **2.5.7.1.1. Lysine**

Experiments with <sup>14</sup>C-labeled aspartic acid, acetate, and alanine revealed that biosynthesis of lysine proceeds via aspartate (VOGEL 1959). The first step of this pathway, the phosphorylation of aspartate, is catalyzed by aspartate kinase. The enzyme exhibiting feedback inhibition by lysine and higher concentrations of threonine (but not homoserine, methionine, or isoleucine) has been isolated from L. minor (WONG and DENNIS 1973). Through the pathway from 3-aspartylphosphate to lysine meso- $\alpha,\epsilon$ -diaminopimelic acid is the last intermediate. Decarboxylation of this compound is exerted by diaminopimelate decarboxylase, which has been extracted and characterized from L. aequinoctialis (SHIMURA and VOGEL 1966) and S. punctata (VOGEL and HIRVONEN 1971).

#### **2.5.7.1.2. Homocysteine, methionine**

The pathway from aspartate to methionine includes the following steps:  
aspartate → 3-aspartylphosphate → 3-aspartate semialdehyde → homo-

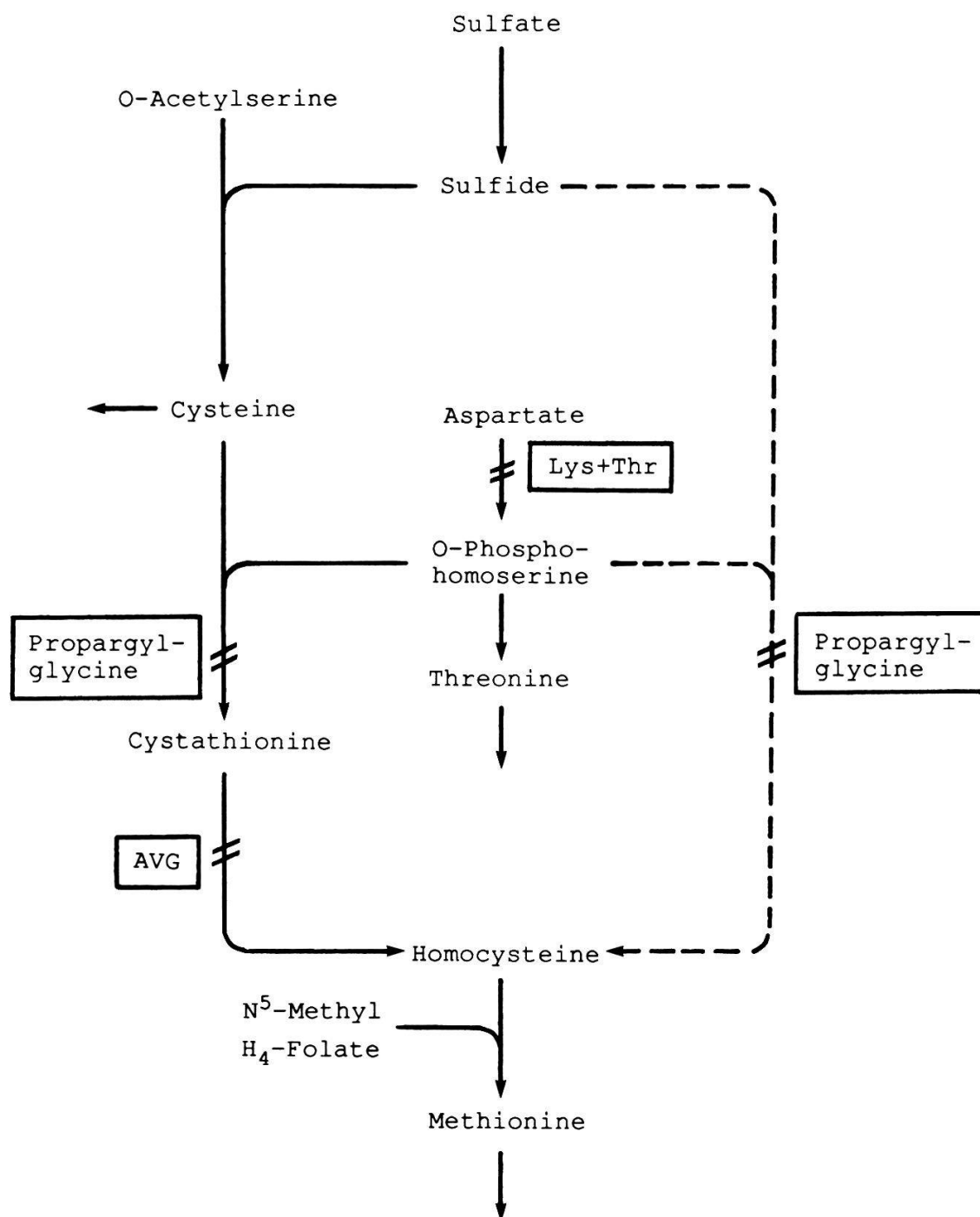


Fig. 2.47. Methionine biosynthesis in Lemna (after THOMPSON et al. 1982a). The solid arrows represent the major pathway. The dashed arrows represent an alternative pathway which, however, seems not to be active in vivo (see text). Also indicated are the metabolic sites where lysine plus threonine (Lys+Thr), AVG, and propargylglycine block methionine formation.

serine → o-phosphohomoserine (OPH) → cystathionine → homocysteine → methionine. Corresponding to this line  $^{14}\text{C}$  aspartate and  $^{14}\text{C}$  homoserine are incorporated into methionine in S. polyrrhiza (MALEK and COSSINS 1982). In L. aequinoctialis, inhibition of aspartate kinase by the combined supply of lysine and threonine prevents biosynthesis of OPH (fig. 2.47) and consequently the reaction of OPH with cysteine to cystathionine (DATKO and MUDD 1982). The step of OPH + cysteine to cystathionine is catalyzed by cystathionine-γ-synthase, an enzyme which is down-regulated in vivo by methionine (THOMPSON et al. 1982a, GIOVANELLI et al. 1985a) and inactivated irreversibly in vivo and in vitro by propargylglycine (PAG), vinylglycine, and cysteine (THOMPSON et al. 1982b). The inactivation is prevented (PAG, vinylglycine) or reduced (cysteine) by 40 mM OPH or o-succinyl-L-homoserine. Experiments with the inactivator PAG have shown that enzyme activity can be reduced to 16% without an effect on growth and methionine biosynthesis. When the cystathionine-γ-synthase activity is decreased to 9% by PAG treatment, the sublethal methionine deprivation which appears in the plants is abolished within two days on the grounds of an adaptation process (THOMPSON et al. 1983). Cystathionine-γ-synthase is located in the chloroplasts. The effect of inorganic phosphate in this cell organelle, which inhibits the enzyme in vitro, may be diminished by OPH (GIOVANELLI et al. 1986).

o-phosphohomoserine sulfhydrylase, which combines OPH with sulfide directly to homocysteine (fig. 2.47), shows the same dependence on methionine and inhibitors as cystathionine-γ-synthase. Both activities are probably caused by one enzyme (THOMPSON et al. 1982a, 1982b). The affinity of OPH sulfhydrylase for sulfide is relatively low in comparison to the  $\text{S}^{2-}$ -affinity of o-acetylserine sulfhydrylase, i.e., cysteine synthase. Physiologically, the OPH sulfhydrylase activity seems to be of no importance. Short term labeling experiments with  $^{35}\text{SO}_4^{2-}$  revealed that radioactivity is incorporated predominantly in cystathionine (MACNICOL et al. 1981). The authors concluded that 85-100% of homocysteine biosynthesis proceeds through the transsulfuration pathway (correcting an earlier statement of DATKO et al. 1977).

The next step in methionine biosynthesis, the cleavage of cystathionine into homocysteine and pyruvate +  $\text{NH}_3$ , is impaired by L-aminoethoxyvinylglycine (AVG). [ $^{35}\text{S}$ ] cystathionine accumulates in L. aequinoctialis, when the medium contains  $^{35}\text{SO}_4^{2-}$  and AVG (DATKO and MUDD 1982). During the final step of methionine biosynthesis methyl tetrahydrofolate is

used for methylation of homocysteine. The precursor of methyl tetrahydrofolate, 5,10-methylenetetrahydrofolate, is provided by serine hydroxymethyltransferase. Activity of this enzyme is partially inhibited in L. minor, when lysine is added to the culture medium (WONG and COSSINS 1976).

#### 2.5.7.1.3. Threonine, isoleucine

Biosynthesis of threonine from aspartate follows the methionine pathway till OPH. Then OPH reacts with  $H_2O$  catalyzed by threonine synthase. Five further steps are needed to transform threonine into isoleucine. Validity of this pathway in S. polyrrhiza has been shown by  $^{14}C$  aspartate- and  $^{14}C$  homoserine-incorporation into threonine and isoleucine (MALEK and COSSINS 1982). Threonine synthase has been isolated from L. minor (SCHNYDER et al. 1975) and L. aequinoctialis (VELUTHAMBI et al. 1983, GIOVANELLI et al. 1984). The investigations with L. aequinoctialis revealed a reversible stimulation (over 30-fold) of threonine synthase by S-adenosylmethionine (SAM) and a down-regulation of the enzyme by methionine. As SAM is the first subsequent product of methionine in the methionine cycle (see chapter 2.5.7.2), it could be the signal of methionine overproduction which switches the pathway from methionine to threonine production. Methionine, on the other hand, may help to limit the overproduction of threonine. Threonine and also isoleucine have no feedback or repressing effect on threonine synthase. Inorganic phosphate and AMP are further inhibitors of threonine synthase, but OPH counteracts these effects (GIOVANELLI et al. 1986). If total OPH is restricted to the chloroplasts, in which threonine synthase is located, then the  $P_i$  concentration in this organelle does not limit the production of threonine.

The second step of the pathway from threonine to isoleucine, the transformation of 2-oxobutyrate into 2-acetohydroxybutyrate, depends on the activity of acetohydroxy acid synthase (fig. 2.48). This enzyme as well as the enzymes catalyzing the subsequent steps of isoleucine biosynthesis also work in valine-leucine biosynthesis. Therefore, it does not seem to be surprising that valine and leucine exert an inhibition of acetohydroxy acid synthase. The end-product inhibition has been deduced from the fact that 2-aminobutyrate, a side-chain product of 2-oxobutyrate, is accumulated in S. polyrrhiza after addition of valine to the

nutrient medium (BORSTLAP 1972). The content of isoleucine decreases under these conditions as is the case after leucine supply.

#### 2.5.7.1.4. Valine, leucine

Acetohydroxy acid synthase catalyzes, in addition to the above cited reaction, the transformation of pyruvate to 2-acetolactate, the first intermediate of valine-leucine biosynthesis (fig. 2.48). The effectivity of in vivo control of this enzyme has been investigated by isotope dilution experiments (BORSTLAP and VERNOOY-GERRITSEN 1985). An increase in the internal valine pool of S. polyrrhiza from 3.2 to 6.6 nmol mg<sup>-1</sup> dry weight reduced valine synthesis by 70%, but not isoleucine synthesis. From these and other results it was concluded that feedback inhibition of acetohydroxy acid synthase by valine and leucine can be exerted in such a way that synthesis of 2-acetolactate is appreciably reduced, whereas synthesis of 2-acetohydroxybutyrate (the isoleucine precursor) is not inhibited.

Another control point in the valine-leucine pathway is 2-isopropylmalate synthase, which switches the final intermediate of valine synthesis to the leucine pathway. BORSTLAP (1972, 1981) showed that activity of this enzyme is inhibited by isoleucine. The content of leucine in S. polyrrhiza decreases, but the content of valine increases, when isoleucine is added to the culture solution. Growth inhibitions caused by valine, leucine, isoleucine, valine + leucine, or leucine + isoleucine (BORSTLAP 1970) can be interpreted by deprivation of one or two of the afore-named branched-chain amino acids via inhibition of acetohydroxy acid synthase and/or isopropylmalate synthase (BORSTLAP 1981).

Experiments with <sup>14</sup>C-labeled valine have shown that plants can convert valine to leucine (BORSTLAP 1975). Thus the last step of valine synthesis is reversible. Growth-inhibiting concentrations of valine increase the level of free leucine in the plant even thirty-fold (BORSTLAP 1972).

#### 2.5.7.1.5. Other amino acids

As known for other plants, arginine metabolism is blocked by canavanine in L. minor (NICKELL 1956). The growth inhibition caused by 10 ppm canavanine can be overcome by 50 ppm arginine or 100 ppm citrulline. Biosynthesis of aromatic amino acids is inhibited by the herbicide glyphosate

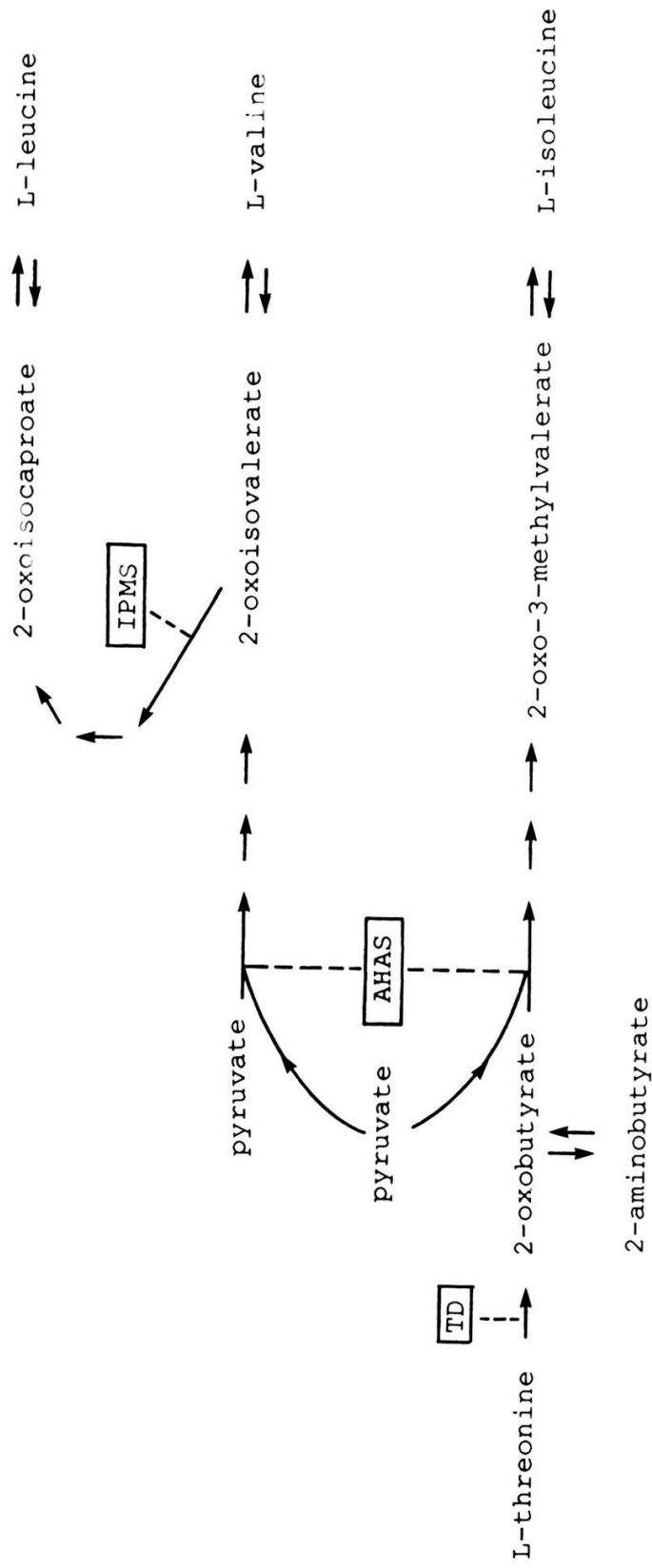


Fig. 2.48. Biosynthetic pathway of branched-chain amino acids (after BORSTLAP and VERNOOY-GERRITSEN 1985).

TD = threonine deaminase; AHAS = acetohydroxy acid synthetase; IPMS = 2-isopropylmalate synthetase.

(N-phosphonomethylglycine). The growth inhibition of *L. gibba* in the presence of this herbicide can be abolished completely by the combined supply of phenylalanine, tyrosine, and tryptophane (JAWORSKI 1972).

#### 2.5.7.2. Methionine metabolism

Methionine not only serves as one of the amino acids in protein synthesis, but also as an intermediate in methyl group transfer, polyamine biosynthesis, and ethylene formation. The pathways from methionine are summarized in fig. 2.49. Details of the SAM-SA-HC cycle (recycling of the homocysteinyl moiety of methionine) and the SAM-MTA-MTR cycle (recycling of the methylthio moiety of methionine) were worked out by GIOVANELLI et al. (1980b, 1981, 1983, 1985b). GIOVANELLI et al. (1985b) stated that in *L. aequinoctialis* 6746 synthesis of S-adenosylmethionine (SAM) is the major pathway for methionine metabolism, with over 4 times as much methionine metabolized by this route as accumulates in protein. More than 90% of SAM is used for transmethylation. Methyl groups of cho-

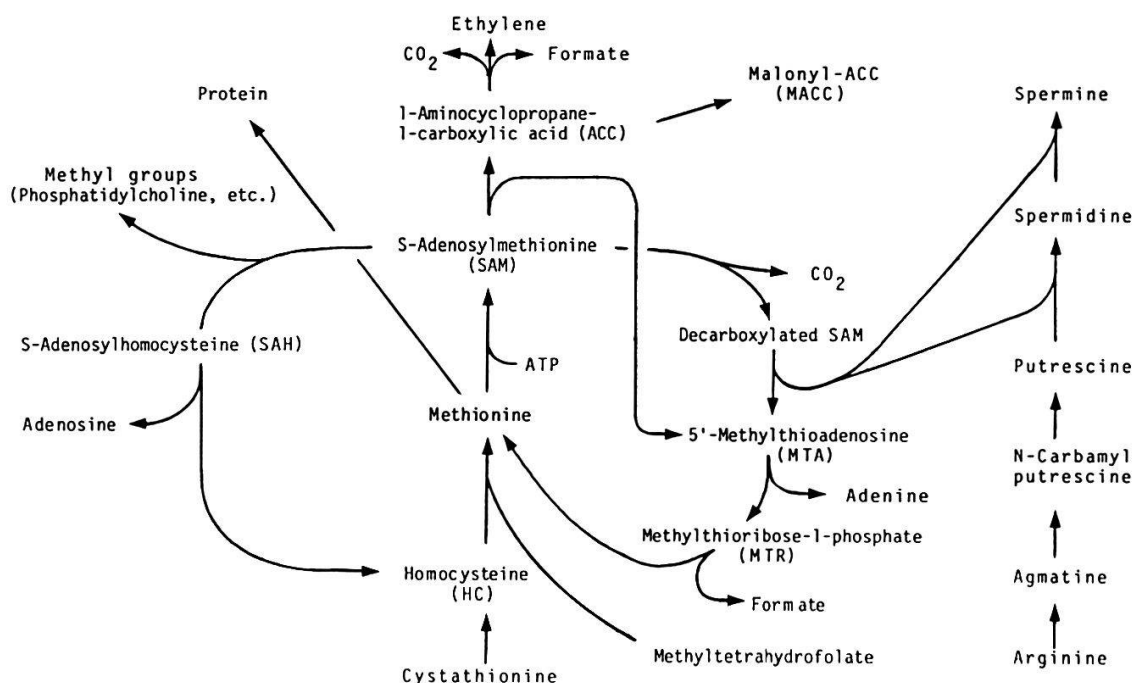


Fig. 2.49. Methionine metabolism (derived from a scheme of GIOVANELLI et al. 1985b).



line, phosphatidylcholine, and phosphorylcholine are major end products of this pathway. Flux through methylthio recycling is about one-third the amount of methionine which accumulates in protein. Spermidine synthesis accounts for at least 60% of the flux through methylthio recycling. Supply of methionine to the nutrient solution has no significant effect on transmethylation or methylthio recycling.

In a further publication, MUDD and DATKO (1986a) have given a more detailed analysis of methionine methyl group metabolism. When radioactive methyl-labeled methionine is supplied to plants, about 19% of the label accumulates in protein, 46% in methylated ethanolamine derivatives, 15% in pectin methyl esters, 8% in chlorophyll methyl esters, 6% in unidentified neutral lipids, 2-5% in nucleic acid derivatives, and 2% in methylated basic amino acids.

The biosynthesis of spermidine from putrescine consumes the  $\alpha$ -aminopropyl moiety of decarboxylated SAM, which is produced during the SAM-MTA-MTR cycle. Also the step from spermidine to spermine needs the  $\alpha$ -aminopropyl moiety. In L. gibba G3 spermidine, spermine, and other polyamines have been analysed by high performance liquid chromatography (FLORES and GALSTON 1982). Mainly agmatine and spermidine are present in both vegetative and flowering fronds. A quantifiable amount of spermine occurs only in flowering material. In vegetative plants of L. aequinoctialis 6746 spermidine, but not spermine has been detected (GIOVANELLI et al. 1981).

The third pathway originating from S-adenosyl methionine is the production of ethylene via 1-aminocyclopropane-1-carboxylic acid (ACC). As a sideway of this path a transformation of ACC to MACC [1(malonylamino)cyclopropane-1-carboxylic acid] can occur. Both substances, ACC and MACC, have been detected in S. polyrrhiza Pl43 and L. aequinoctialis 6746 (FAERBER et al. 1986). In freely floating plants of strain 6746 the ACC content (0.06 nmol/gFW) and the ethylene formation (0.25-0.5 nl/gFW/h) are very low. The same holds true for the ethylene production of L. gibba Gl. In S. polyrrhiza, however, ethylene formation ranges between 1.5 and 2.0 nl/gFW/h (ACC content 0.56 nmol/gFW).

A dramatic change of ethylene evolution occurs in S. polyrrhiza, when the freely floating plants are pushed together (FAERBER et al. 1986). In the crowded state ethylene emanation increases immediately, reaching a first maximum after 2-3 hours and a second between 18-20 hours. The "overcrowding-signal" also works in L. aequinoctialis 6746 and L. gibba

G1, but strain 6746 exhibits only the first of the two peaks of ethylene evolution, and strain G1 only the second one.

Exogenous ACC is taken up by L. minor in the dark with no saturation (FUHRER 1985). ACC-dependent ethylene production, on the other hand, is saturated above 10  $\mu\text{M}$  ACC (equivalent to 32  $\mu\text{M}$  internal ACC). Only when 1  $\mu\text{M}$  or more ACC is supplied to light-grown plants, increasing  $\text{CO}_2$  concentrations elevate the ethylene emanation. This effect seems to be based on a  $\text{CO}_2$  inhibition of ethylene metabolization, as internal levels of ACC and MACC are not influenced by the  $\text{CO}_2$  treatment.

When toxic concentrations of copper ( $10^{-4}$ - $10^{-3}$  M) cause a damage of intracellular membranes in S. punctata, not ACC but other sources are responsible for the increase in ethylene evolution (MATTOO et al. 1984c, 1986a). The  $^1\text{O}_2$  quenchers and inhibitors of lipid peroxidation, viz. propyl gallate, 1,4-diazobicyclo [2,2,2] octane and selenomethionine, all markedly inhibit the Cu(II)-induced ethylene production. Selenomethionine also suppresses the membrane damage in Cu(II)-treated plants. The authors suggest that copper stimulates lipid oxidation by reacting with lipid peroxides to form new radical species. Both linoleic acid and methionine seem to act as precursors of ethylene after copper poisoning.

#### 2.5.7.3. Biosynthesis and turnover of proteins

##### 2.5.7.3.1. Dependence on light

The ability of several Lemnaceae to grow in complete darkness when provided with sucrose (see chapter 2.3.5.4) demonstrates that light is not needed for biosynthesis of at least most proteins. As early as 1896 and 1899 HANSTEEN found protein accumulation in darkness, when plants of L. minor obtained a certain combination of N source and carbohydrate. Asparagine, urea, or ammonium salt were effective as N source in combination with glucose, and urea or glycine were effective in combination with sucrose. A special case, however, are proteins of the photosynthetic apparatus. For economy, development of chloroplasts is completed only in the presence of light (see chapter 2.4.1.5). The bulk proteins of this cell organelle are especially affected. When L. gibba plants are transferred to darkness 19-21 hours, a dramatic decrease in biosynthesis of chlorophyll a/b-binding protein and the large and small subunits of ribulose-1,5-diphosphate carboxylase (RuDPCase) occurs (TOBIN 1978, 1981a, 1981c,

TOBIN and SUTTIE 1980). Re-lightening causes a strong increase in synthesis of RuDPCase subunits within 1-3 hours. In L. minor light stimulated amino acid incorporation into large subunit of Fraction I protein (=RuDPCase) and two plastid membrane proteins (BLACKWOOD and LEAVER 1977). In addition the assembly of large and small subunits of Fraction I protein is promoted by light. In S. punctata, <sup>35</sup>S methionine incorporation into soluble and membrane proteins (including a 32kDa protein) is enhanced especially 3 hours after dark-light transition (REISFELD et al. 1978a). Synthesis of a 33.5 kDa precursor of 32 kDa protein and its conversion are also light-dependent (EDELMAN and REISFELD 1978, 1980, MATTOO et al. 1980). Further proteins greatly increasing during greening of etiolated plants are the chlorophyll protein complex I, subunits of the ATPase complex, cytochrome f, and a putative cytochrome b<sub>6</sub> subunit (DE HEIJ et al. 1984).

In all cases investigated so far, the light effect is exerted primarily through increased amounts of translatable messenger RNAs. This holds true for the small subunit precursor of RuDPCase (TOBIN 1978, TOBIN and SUTTIE 1980), a 32kDa protein being the precursor of light-harvesting chlorophyll a/b-protein (TOBIN 1981a, 1981c), and another 32 kDa protein (REISFELD et al. 1978a) identified later on as shield protein for the electron acceptor II of photosynthesis (see chapter 2.7.1.1). In the case of small subunit of RuDPCase and the light-harvesting chlorophyll a/b-protein the light effect on mRNA content of plants is mediated by phytochrome (TOBIN 1981a, 1981b, STIEKEMA et al. 1983a). This result should be related to the fact that both proteins are encoded by nuclear genes and synthesized from polyadenylated RNA on cytoplasmic ribosomes as precursor polypeptides which are transported into the chloroplasts (TOBIN 1978, STIEKEMA et al. 1983a). The phytochrome effect on both messenger RNAs can be imitated or magnified by benzyladenine (FLORES and TOBIN 1986b). On ground of their experiments the authors suggest that BA exerts this effect at a post-transcriptional level, possibly by affecting the stability of the RNAs.

In the case of 32 kDa shield protein blue light is >5 times as effective as red light in stimulating the appearance of competent mRNA (GRESSEL 1978, MATTOO et al. 1980). Phytochrome is not involved in this light effect. It is interesting to note that the mRNA ( $5 \times 10^5$  dalton) was isolated from plastids and does not contain poly(A) sequences (ROSNER et al. 1975a).

In addition to the light effects on mRNA content a light-dependence of translation has been found for the chlorophyll a/b-apoproteins (SLOVIN and TOBIN 1982). Under conditions of heterotrophic growth with intermittent red light, formation of mRNA and protein synthesis are uncoupled. Whereas the level of translatable mRNA is essentially the same as that found in plants grown in white light (TOBIN 1981b), the incorporation of [<sup>35</sup>S] methionine into the apoproteins is very low. Possibly, the necessity of such an additional light effect on translation or processing also holds true for RuDPCase (TOBIN unpubl., cited in SLOVIN and TOBIN 1980).

#### 2.5.7.3.2. Proteins of the chloroplast

Major protein components of thylakoid membranes are the two apoproteins of the light-harvesting chlorophyll a/b-protein complex. In L. gibba molecular weights of these apoproteins are 28000 and 28500, respectively (TOBIN 1981a, 1981c). The precursor of the apoproteins possesses a molecular weight of 32000 and is synthesized on cytoplasmic ribosomes with a poly(A) mRNA (TOBIN 1981a, 1981c, SLOVIN and TOBIN 1982). Chlorophyll a/b-protein is quite stable, as it is not degraded to an appreciable amount during a four-day dark treatment (TOBIN 1981c). The mRNA for the 32 kDa precursor, however, declines sharply during the first two days of darkness. Kinetin ( $3 \times 10^{-6}$  M) can retard the degradation to some extent (TOBIN 1981a, TOBIN and TURKALY 1982). In the presence of glyphosine [N,N-bis(phosphonomethyl)glycine] synthesis of chlorophyll a/b apoprotein is extremely low compared to control plants, whereas the large subunit of RuDPCase is synthesized further (SLOVIN and TOBIN 1981).

A reversible phosphorylation of the light-harvesting pigment-protein complex of photosystem II (PS II) has been found in L. aequinoctialis (STEINBACK and WATSON 1981). Excitation of PS II by red light in vivo causes a phosphorylation of PS II and in this way an increased energy transfer to PS I at the expense of PS II. Excitation of PS I by far red results in a rapid dephosphorylation ( $T_{1/2} = 4$  min) of PS II. Such a regulation of energy distribution between the photosystems via protein phosphorylation may be of ecological relevance in plants, shaded variously by leaves.

Another membrane protein of chloroplasts is the 32 kDa shield protein regulating electron transport (see chapter 2.5.1.1.2). Occurrence of

this protein has been demonstrated for S. punctata, several other angiosperms, and the alga Chlamydomonas (HOFFMANN-FALK et al. 1982). The shield protein contains no lysine (EDELMAN and REISFELD 1978, 1980, REISFELD et al. 1982) and is tightly bound to the thylakoids (EDELMAN and REISFELD 1978, 1980). Its half-life is relatively short. Decay proceeds at least one order of magnitude more rapidly than that of other major plastid polypeptides (EDELMAN and REISFELD 1980). Synthesis and processing take place within the chloroplast. Three hours after illumination with white light there is a burst in the synthesis of plastid mRNA coding for the 33.5 kDa precursor of shield protein (REISFELD et al. 1978a,b, EDELMAN and REISFELD 1980). The mRNA is characterized by a molecular weight of 500000 and the lack of poly(A). Translation on chloroplast ribosomes is confirmed by suppressing the process with low concentrations of D-threo-chloramphenicol (WEINBAUM et al. 1979a,b). Transformation of the 33.5 kDa precursor into the 32 kDa protein commences only after completion of the precursor polypeptide chain and insertion into the thylakoids (REISFELD et al. 1982). Processing of the 33.5 kDa protein, like synthesis, is dependent on light and takes place with a  $t_{1/2}$  of ~3 min (EDELMAN and REISFELD 1980, MATTOO et al. 1980). Light is effective only in green plants, i.e., in the presence of differentiated thylakoids. In etiolated plants shield protein does not appear before 2 or 3 days of greening (MATTOO et al. 1980).

The photosystem I reaction center isolated from S. punctata contains seven different subunits (NECHUSHTAI et al. 1981). Inhibitor experiments with D-threo-chloramphenicol and cycloheximide revealed that subunit II is synthesized on cytoplasmic ribosomes, whilst subunits I, V, and VIb are translated within the chloroplast. In the same way the origin of the 8 subunits of the proton-ATPase complex were investigated. Subunits  $\gamma$  and  $\delta$  of CF<sub>1</sub> and subunit II of CF<sub>0</sub> are products of the cytoplasmic translation system. Subunits  $\alpha$ ,  $\beta$ , and  $\epsilon$  of CF<sub>1</sub>, as well as subunit III of CF<sub>0</sub> are synthesized on the chloroplast translation system. Protein synthesis at the two cellular locations is strictly coordinated, as the molar ratio of subunits produced (see CF<sub>1</sub>- $\beta$  and CF<sub>1</sub>- $\gamma$ ) resembles the stoichiometrical amounts in the native protein complex (DE HEIJ et al. 1984).

The main polypeptides in the soluble part of chloroplasts are the large and small subunits (LS and SS) of ribulose-1,5-diphosphate carboxylase. Within Lemnaceae both subunits show some variation in the polypeptide

composition (CHEN and WILDMAN 1981). After electrofocussing four different types of the LS polypeptide clusters and eight individual SS polypeptides have been found. Each of the 11 investigated species (representing the four genera) contains one type of LS and one, two, or four types of SS polypeptide. In L. gibba LS and SS are quite stable, as they do not show any decline during 4 days in the dark (TOBIN and SUTTIE 1980).

Messenger RNA for LS-RuDPCase is located in the chloroplast, contains no poly(A) sequences, and has a molecular weight range of  $0.56-0.7 \times 10^6$  (ROSNER et al. 1976, REISFELD et al. 1978b,c). The poly(A) mRNA for the precursor of SS-RuDPCase has been isolated from cytoplasmic polysomes (TOBIN 1978). The rapid decline of this mRNA in L. gibba during four days in the dark can be diminished somewhat by supply of kinetin (TOBIN and TURKALY 1982).

#### 2.5.7.3.3. Other proteins

Protein phosphorylation occurs not only in the photosystem II (see above), but also in ribosomes and chromatin. TREWAVAS (1973) labeled L. minor plants with  $^{32}\text{PO}_4^{3-}$  and found radioactivity in the small subunit of ribosomes. After acid hydrolysis of the ribosomal protein serine phosphate appeared. The P-content per ribosome amounts to 0.75 atom in control plants, but only 0.36 atom of phosphorus in plants treated for 24 hours with 5  $\mu\text{M}$  ABA. The enzyme catalyzing the phosphorylation of ribosomal proteins, protein kinase (ATP: protein phosphokinase), sediments with ribosomes during sucrose density gradient centrifugation. Seventy per cent of the enzymic activity dissociates from the ribosomes in 0.3M KCl, but the remaining protein kinase remains firmly bound in 0.7 KCl (KEATES and TREWAVAS 1974).

Phosphorylation of chromatin-associated proteins has been investigated in L. aequinoctialis (VAN LOON et al. 1975, CHAPMAN et al. 1975). After labeling of plants with inorganic  $^{32}\text{P}$ , chromatin were prepared and then the associated proteins separated by gel electrophoresis. At least 15-20 proteins (with molecular weights ranging from 10000 to 100000) were found to be phosphorylated. Most of them are acidic proteins, but 25-30% may be histone material. Incubation of isolated nuclei with  $^{32}\text{P}$  - ATP resulted also in labeling of chromatin proteins indicating the presence of an endogenous kinase. Partial acid hydrolysis of labeled proteins



yielded serine phosphate and traces of threonine phosphate. Pretreatment of plants with ABA (10 mg/l) causes substantial alterations in the labeling of three protein bands. There is an almost total loss of one protein band and an increase in labeling of two other protein bands.

Three soluble protein kinases (P I, P II, and P III) phosphorylating histones in vitro have been isolated from L. aequinoctialis by KATO et al. (1983, 1984). Activity of the kinases (with molecular weights of 165000, 85000, and 145000, respectively) is dependent on cyclic nucleotides in a different manner. P I is partially inhibited by cAMP, cGMP, or cIMP, while the P II enzyme is activated by these nucleotides. P III is not influenced by cAMP, but slightly inhibited by cCMP and cUMP. The three protein kinases are Mg-dependent. The P II enzyme, however, is stimulated more effectively by high concentrations of  $\text{Co}^{2+}$ , and the P III activity is maximum with  $\text{Mn}^{2+}$ . Cytokinins exert some inhibition in P III activity. At present, the in vivo functions of the three protein kinases are not known. The same holds true for a membrane-associated protein kinase, which has been isolated from endoplasmic reticulum-rich fractions (KATO and FUJII 1985). The enzyme (molecular weight 220000) shows optimum activity at pH 9.0-9.5 and is stimulated by  $\text{Co}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Mn}^{2+}$ . Cytokinins and cyclic nucleotides do not affect the activity.

Glycoproteins have been claimed to occur in L. minor, as the amino sugar D-[1- $^{14}\text{C}$ ] glucosamine is incorporated into compounds of high molecular weight that resemble proteins in their physical properties (ROBERTS et al. 1971).

#### 2.5.7.3.4. Protein metabolism in general

Growth and development are based mainly on synthesis and degradation of proteins. Thus it is not surprising that growth-regulating factors influence the overall protein metabolism. Addition of glucose to cultures of S. punctata, for example, accelerates the production of water-soluble and water-insoluble proteins (GROB et al. 1973), doubtlessly in parallel to the increased growth rate. Ammonium, more specifically, increases the protein content of L. minor plants per dry weight, reversibly. In transition experiments from nitrate to ammonium the total protein rises by 25-30% within 40 hours. Re-transfer of plants to nitrate restores the original "steady state" value of proteins within 30-40 hours (DICHT et



al. 1976). The ammonium effect may come about through an enhancement of the endogenous cytokinin and auxin level. An increase in endogenous cytokinins after transition of plants to ammonium was found in L. aequinoctialis by GRUNTZEL (1982). Supply of kinetin or IAA, on the other hand, to cultures of L. minor increases the protein content per dry weight (KRZECZOWSKA and ZIMNA 1972, MACIEJEWSKA-POTAPCZYK et al. 1976). With kinetin especially the high molecular proteins are promoted whereas the level of mean and low molecular proteins decreases. IAA slightly decreases the level of high molecular proteins and increases the content of mean and low molecular proteins. A differential effect on protein synthesis has been demonstrated in detail for ABA (SMART and TREWAVAS 1984a). In connection with the change in developmental processes (turion formation) ABA changes the pattern of proteins seriously. Two-dimensional polyacrylamide gel electrophoresis of in vivo  $^{35}\text{S}$ -methionine-labeled soluble proteins revealed that the majority of proteins decreases or disappears during the development of the turion, whilst some show increased labeling and others appear de novo. Also in the turion-producing mother frond certain changes of protein pattern are induced. ABA seems to exert its effects on the transcriptional as well as on the translational level. The rapid general inhibition of protein synthesis during the first two days of ABA action should be initiated at the translational level, as no decrease in translatable RNA occurs during this time. Induction of de novo synthesis of new proteins, however, could come about by changes in gene expression.

Evaluation of the rates of overall protein synthesis and degradation with labeled amino acids are complicated by the fact that at least two pools of amino acids exist in the plant, only one of which contributes to the synthesis of protein. TREWAVAS (1972a) used the tRNA-bound  $^3\text{H}$ -methyl-methionine for calculation of the flux rates of methionine into and out of protein and reached the following results: plants of L. minor cultivated under certain light and temperature conditions exhibit rate constants for protein synthesis and degradation of 0.48 and 0.09 days<sup>-1</sup>, respectively, when grown in complete nutrient medium. Reduction of the  $\text{MgSO}_4$  content of medium to 1/10 results in a decrease of protein synthesis and an increase in protein degradation. The rate constants for both processes then are 0.32 and 0.13 days<sup>-1</sup>, respectively. Rate constants for fresh weight increase in these experiments (0.37 days<sup>-1</sup> for full-strength medium, and 0.18 days<sup>-1</sup> for 1/10  $\text{MgSO}_4$  medium) are in close re-

lation to the net production of proteins. Similar investigations with S. polyrrhiza have been carried out by MALEK and COSSINS (1983b). In this case, lowering of nitrate concentration to 1/20 does not affect the rate of protein synthesis, but increases the rate constant of protein degradation. Further work on protein turnover has already been reviewed in connection with the physiology of senescence and rejuvenation (see chapter 2.4.1.4.). Especially the effects of several stress factors and of plant growth regulators have been represented in some detail. The question which properties of proteins may be of significance for a selective protein degradation have been investigated in L. minor (COATES and DAVIES 1983). The authors found only a weak correlation between SH content of proteins and degradation, and little or no correlation between molecular weight or charge of proteins and degradation. They suggested that half-life of proteins is determined by the sum of many physical properties rather than by a single "signal" property.

#### **2.5.7.4. RNA metabolism**

##### **2.5.7.4.1. Ribosomal RNA**

Nucleic acid fractionations from duckweed material were done by HEMLEBEN (1972). After short labeling of L. aequinoctialis plants with [<sup>3</sup>H] uridine, nucleic acids were separated using MAK-column chromatography or polyacrylamide-gel electrophoresis. Fraction I (4 S tRNA) and fraction II (5 and 5.8 S) contained low-molecular RNA components, fraction III was DNA, fractions IV and V were high-molecular ribosomal (r) RNAs, and fraction VI contained components being presumably precursors of rRNA. Fraction IV was the 16 S rRNA of chloroplast and the 18 S rRNA of cytoplasm (with a molecular weight of  $0.7 \times 10^6$ ). Fraction V held the 23 S rRNA of chloroplasts and the 25 S rRNA of cytoplasm (MW of  $1.3 \times 10^6$ ). The components of fraction VI were separated further and had sedimentation constants (molecular weights) of 33-34 S (2.4 M), 29 S (1.7 M), and 27 S (1.4 M). Rates of [<sup>3</sup>H] uridine incorporation into single RNA fractions could be altered by certain environmental factors (photoperiod, blue light, supply of sucrose, or phosphate deficiency). Most interestingly, transfer of plants from continuous light to short day caused - besides other reactions - an increase in the ratio of fractions VI/IV+V especially in the first half of the dark phase. Interruption of the dark phase

by light (30 min, 8 hours after the beginning of darkness) prevented this increase.

TREWAVAS (1970) determined rate constants of synthesis and degradation of cytoplasmic rRNA (25 S and 18 S) and chloroplast rRNA (23 + 13.7 S and 16 S) in mixotrophic cultures of L. minor. Half-life of both cytoplasmic rRNA components amounts to 4.3 days. Half-life of the chloroplast rRNA components is considerably longer and amounts to 15.5 days. The degradation rate for total rRNA increases after omission of either nitrate, phosphate, calcium, or magnesium. Transfer of plants to water causes an increase of rRNA degradation and a decrease of rRNA synthesis, but ABA reduces only rRNA synthesis.

Processing of presumptive precursors into mature rRNA has been followed in S. punctata by the time-course of [<sup>3</sup>H] uridine incorporation into different RNA fractions and by inhibition of certain steps with darkness, transfer of plants to distilled water, or chloramphenicol (ROSNER et al. 1973, POSNER and ROSNER 1975, ROSNER et al. 1977a). After short labeling of plants two cytoplasmic RNAs with high molecular weight (2.8 M and 2.3 M) appear. The 2.8 M species seems to be cleaved to form 2.3 M RNA and "excess" RNA with a molecular weight of 0.5 M. The "excess" RNA is not methylated and may be discarded during the further metabolism (POSNER et al. 1974). The 2.3 M RNA then is processed to a 1.4 M intermediate and finally the light rRNA (0.7 M) and the heavy rRNA (1.3 M) are formed. Darkening of plants or incubation of plants on distilled water lead to an accumulation of the 2.3 M precursor. During prolonged darkening, however, a reduction of transcription and therefore a re-adjustment of transcription and processing takes place in the newly-produced, colorless fronds.

In chloroplasts precursors with 2.7 M, 1.2 M, 0.7-0.75 M and 0.5 M are labeled more rapidly than the mature rRNAs. The mature species with 1.05-1.1 M and 0.56 M are detectable only after labeling for more than two hours. Chloramphenicol treatment of plants inhibits processing of chloroplast rRNA, as incorporation of radioactivity into the mature rRNAs is inhibited, but the labeling of the above named precursors is enhanced. Darkening of plants also seriously retards processing of chloroplast rRNA. In such plants especially the 1.2 M, 0.75 M, and 0.5 M species accumulate. Supply of sucrose to the cultures can diminish not prevent the effect of darkening (on chloroplast rRNAs as well as on cytoplasmic rRNAs). The ability to synthesize high-molecular-weight rRNA

is retained to some degree in isolated chloroplasts of W. arrhiza (EICH-HORN and AUGSTEN 1984).

In addition to the high-molecular-weight rRNA species described above, ribosomes contain low-molecular-weight rRNAs, which are a further part of the large ribosomal subunit. Cytoplasmic ribosomes possess rRNA species of 5 S and 5.8 S, chloroplast ribosomes have rRNAs of 5 S and 4.5 S. Using L. minor as plant material complete nucleotide sequences were elaborated for the two 5 S rRNA species (DYER and BOWMAN 1976, 1979). The cytoplasmic 5 S rRNA has 118 nucleotides and a mol. wt. of 37800, the chloroplast 5 S rRNA contains 121 nucleotides and has a mol. wt. of 39300. Only few deviations in nucleotide positions were found when the sequences were compared with the respective 5 S rRNAs from other flowering plants. Comparison one with another, however, revealed that about 50% of the residues are different at equivalent positions in the two. There is a remarkably greater similarity between the chloroplast 5 S rRNA of Lemna and that of the prokaryote Anacystis nidulans. A detailed discussion of the results yielded a strong support of the endosymbiont hypothesis of chloroplast origin. The possible secondary structure of chloroplast 5 S rRNA is shown in fig. 2.50. The cytosol 5 S rRNA, although dissimilar in sequence from that of the chloroplast, may also be folded in the same way. The shape of both molecules seems to be essential for their function.

The chloroplast 4.5 S rRNA from L. minor has been fractionated by gel

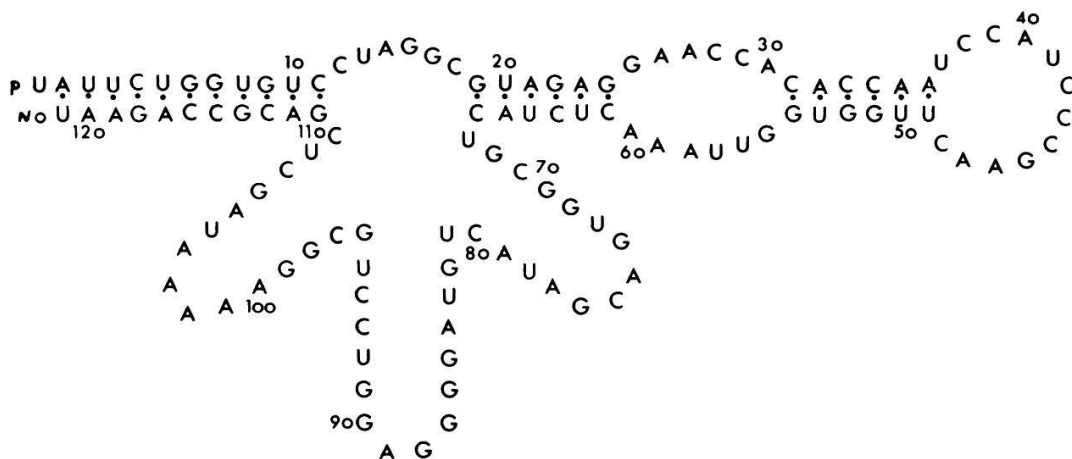


Fig. 2.50. Possible secondary structure of Lemna minor chloroplast 5 S rRNA (DYER and BOWMAN 1979).

electrophoresis into three variants (BOWMAN and DYER 1979). The major component (70-80% of the total yield) contains 103 nucleotides and has a molecular weight of 33600. The two smaller, less-prevalent, variants contain 96 and about 63 nucleotides respectively. All the 4.5 S rRNA species have the same A-A-C<sub>OH</sub> sequence at the 3'-end of the molecule; the 5'-end is not phosphorylated and apparently none contains any modified nucleotides. The 4.5 S rRNA seems to be synthesized as a segment of the 2.7 M precursor. It has been hypothesized that this rRNA may represent transcribed spacer RNA with a possible function in assembling of ribosomal components.

Density of the distribution of chloroplast and cytoplasmic ribosomes has been evaluated by electron microscopy in S. punctata (ROSNER et al. 1974a). In principle, density is considerably higher in light-grown than in dark-grown plants, but chloroplast ribosomes disappeared from the stroma in adult fronds. As the relative amount of chloroplast rRNA increases in adult fronds, chloroplast ribosomes seem to move from the stroma to the thylakoid lamellae. At this stage the heavy chloroplast rRNA loses its integrity and shows thermal instability. Cytoplasmic polyribosomes have sizes up to octamers (free polysomes) or heptamers (membrane-bound polysomes) in light-grown plants of S. punctata (VAN EE and PLANTA 1982). In dark-grown plants pentamers (free polysomes) and trimers (membrane-bound polysomes) are the largest polyribosomes.

#### 2.5.7.4.2. Poly(A) RNA

COVEY and GRIERSON (1976) detected poly(A) sequences in the RNA of L. minor by hybridisation with [<sup>3</sup>H] polyuridylic acid. The poly(A) content of total RNA determined in this way amounted to 50 ng/100 µg RNA. Isolation of polyadenylated RNA from total RNA was achieved by affinity chromatography on oligo(dT)-cellulose or poly(U)-sepharose 4 B (TOBIN and KLEIN 1975, EICHHORN and AUGSTEN 1983b). The messenger RNA capacity of the separated poly(A) RNA from L. gibba was confirmed by its ability to serve as template in a cell-free translation system derived from wheat germ (TOBIN and KLEIN 1975). Poly(A) RNA from W. arrhiza amounts to 5.0% of total RNA, when plants were cultivated under mixotrophic conditions in a chemostat and illuminated with continuous white light. Reduction of growth rate by stronger P limitation has no influence on the poly(A) RNA portion. Under continuous blue or red light the plants contain only 3.5

and 1.5% poly(A) RNA, respectively. Darkening of plants for 24 hours diminishes the poly(A) RNA content to 1.5% after white or blue light, and 0% after red light. Two bulk proteins of chloroplasts, which are synthesized on cytoplasmic ribosomes with light-dependent poly(A) RNA, the light-harvesting chlorophyll a/b-protein and the small subunit of ribulose-1,5-diphosphate carboxylase, have been reviewed in the foregoing chapter (2.5.7.3).

#### 2.5.7.4.3. Inhibitor studies

Addition of  $8 \times 10^{-5}$  M 2-thiouracil (TU) to the culture medium inhibits frond elongation and differentiation of aerenchyma and vascular bundles in S. punctata (RIMON and GALUN 1967). The effect of TU is completely counteracted by uridine or uracil. TU works through an incorporation into all RNA species, in this way preventing uracil incorporation (RIMON et al. 1969). RNA synthesis is not suppressed, even after 20 h of TU treatment, but synthesis of proteins and DNA are reduced after TU incorporation into RNA. Another inhibitor which is incorporated into all RNA species is 5-fluorouracil (FU). This substance, however, inhibits not only frond elongation and differentiation, but also cell division, and these effects cannot be counteracted by either uridine, uracil, thymidine, or simultaneous addition of uracil and thymidine. A certain conversion of FU to 5-fluorodeoxyuridine (a DNA synthesis blocker) therefore may explain the FU action on development only partially. Extremely toxic to the growth of L. minor are the non-protein amino acids L-canavanine, L-canaline, and o-ureido-L-homoserine (ROSENTHAL et al. 1975). They are structural analogues of the ornithine-urea cycle amino acids, and their effect on growth can be abolished to some degree by arginine, ornithine, and citrulline. The substances inhibit [ $^3$ H] uridine incorporation into RNA more than the [ $^3$ H] thymidine incorporation into DNA (GULATI et al. 1977). The possible involvement of polyamines, which are derived from the ornithine-urea cycle, may be considered in this context, as they inhibit RNase activity and induce DNA synthesis in other organisms.

#### 2.5.7.4.4. Ribonucleases

RNA degrading enzymes have been isolated from S. punctata. From plants

grown under standard conditions three ribonucleases (RNases) were separated by DEAE-Sephadex A 25 column chromatography (LASSOCINSKI 1982). The pH optima of enzyme activity were found to be 7.9, 7.5, and 7.2, respectively. All three enzymes have an endonucleolytic mode of action and produce, during poly(A) digestion, cyclic adenosine-2':3'-phosphate, which then is hydrolysed to adenosine-3'-phosphate. One of the RNases has a preference for poly(C) compared with poly(A), the other two RNases show the opposite behaviour.

With polyacrylamide gel electrophoresis KNYPL (1977b) revealed the presence of 10 RNase bands in extracts of S. punctata. When plants were treated with growth-inhibiting doses of N,N-dimethylmorpholinium chloride (DMMC) or CCC for 3-6 days before analysis, three of the RNase bands were markedly intensified, and three new isoenzymes appeared. Additional supply of gibberellin A<sub>3</sub> or benzyladenine reduces the increase in RNase activity. The DMMC-induced RNase has a pH optimum of 6.2-6.3, is activated by citrate, and has a molecular weight of about 31500. The growth retardant alden is another agent which increases the activity of RNase in S. punctata (KNYPL 1977a). Fusicoccin increasing fresh weight of S. punctata by 50%, enhances activity of RNases by more than 100% (KNYPL and JANAS 1980). Ribonucleases which are produced at P-deficiency and are located at the cell surface for acquisition of phosphate, have been mentioned in chapter 2.5.6.3.

#### 2.5.7.5. DNA structure and function

##### 2.5.7.5.1. Chloroplast DNA

S. punctata is the organism which has been used within the Lemnaceae for characterization of chloroplast DNA (cpDNA), for physical mapping, and for sequencing of some regions. The cpDNA isolated by CsCl gradient centrifugation is circular in shape, has a buoyant density of  $1.698 \pm 0.002$  g cm<sup>-3</sup>, a G+C content of 37%, and an at least bimodal melting behaviour, resembling features of many other cpDNA species. Length measurements on electron micrographs, however, revealed a contour length of  $54.1 \pm 1.8$   $\mu$ m (corresponding to a molecular weight of 115 to 120x10<sup>6</sup>), which is about 10  $\mu$ m longer than the length of most other cpDNA species (VAN EE et al. 1980a). Analysing the digestion products of cpDNA after treatment with restriction endonucleases the order of cpDNA fragments within the



whole molecule could be determined (VAN EE et al. 1980b, 1982). The cpDNA is composed of two invertedly repeated DNA segments of 18 Md, separated by a small single-copy region of 19 Md and a large single-copy region of 67 Md. The surplus segment, which is responsible for the greater length in comparison to other cpDNAs, lies in the large single-

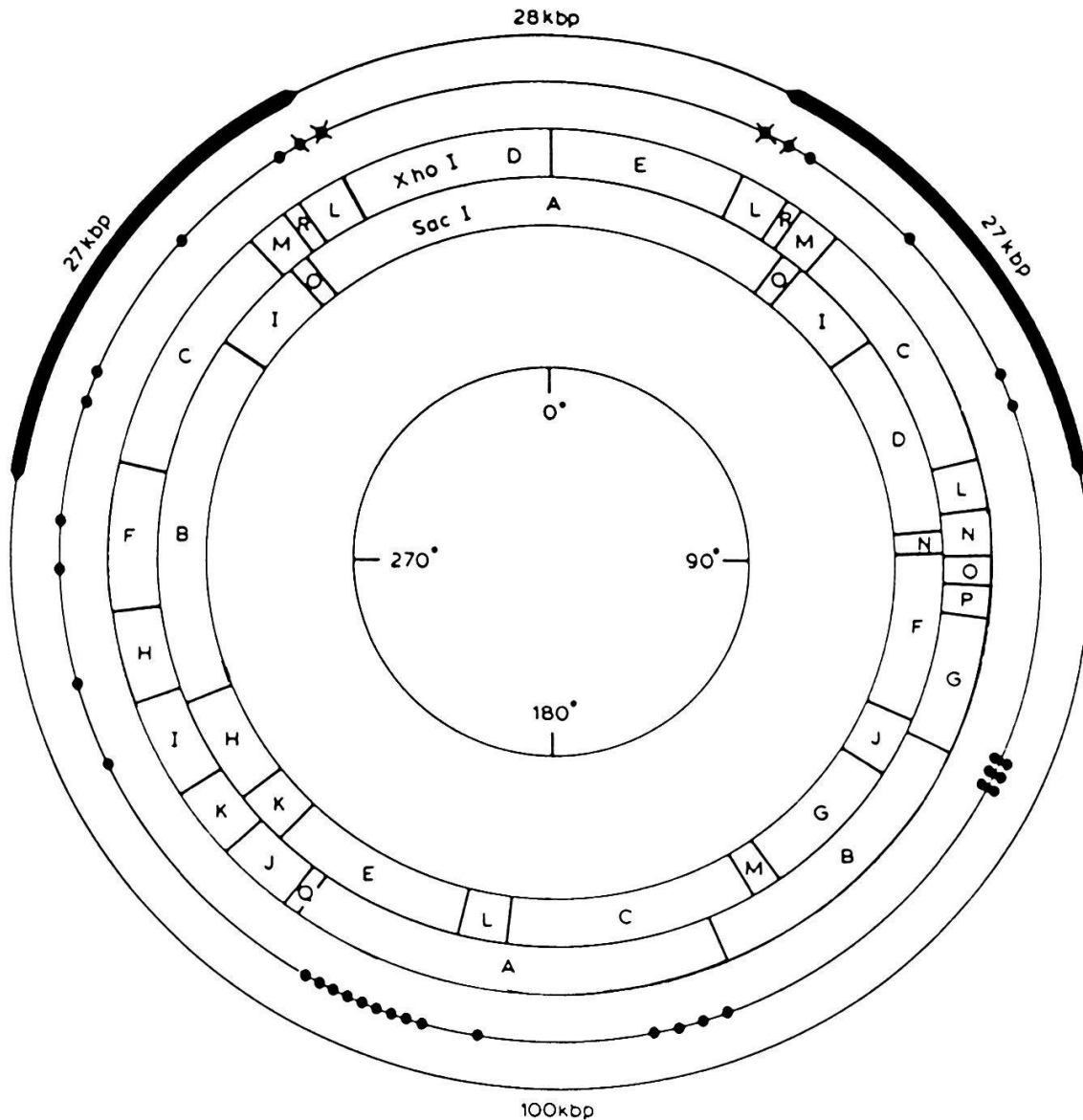


Fig. 2.51. Restriction fragment map of *Spirodela punctata* chloroplast DNA, elaborated with restriction endonucleases Xho I and Sac I, and the positions of low-molecular-weight rRNA genes (★ = 5S RNA, ● = 4.5S RNA) and tRNA genes (○ = 4S RNA). From GROOT and VAN HARTEN-LOOSBROEK, 1981 (see text).

copy region. Using hybridization techniques the positions of the low-molecular-weight rRNA genes and the tRNA genes within the restriction fragment map of cpDNA were determined (GROOT and VAN HARTEN-LOOSBROEK 1981). As can be seen from figure 2.51, the genes for 5 S rRNA and 4.5 S rRNA are located in the two sequence repeats of 17 Md. Three clusters of 4 S tRNA genes are situated at 120°, 170°, and 210° within the large single-copy region, and further 4 S RNA genes are placed around map position 270° and in the inverted repeat region. 35-40 4 S RNA genes have been calculated to be present in the cpDNA of Spirodela. The overall distribution of these genes is very similar to the distribution of tRNAs on spinach and maize cpDNA. Further investigations on cpDNA gene mapping in S. punctata have been made by VAN EE et al. (1982). Each of the two repeat units contains one set of rRNA genes in the order: 16 S rRNA gene-spacer - 23 rRNA gene - 5 rRNA gene. Within the large single copy region genes for a 32Kd protein, a 55Kd protein, and the large sub-unit of ribulose 1,5-diphosphate carboxylase have been localized. The genes for 4.5 S rRNA and 5 S rRNA, their flanking regions and the spacer between the two genes have been sequenced by KEUS et al. (1983b). The results for the 5 S rRNA gene are in agreement with the sequence analysis for 5 S rRNA in L. minor of DYER and BOWMAN (1979). At the 3'end of both rRNA coding regions a prokaryotic termination signal (containing a palindrome of usual length followed by an AT rich region) has been found. A possible promoter site at the 5'end of the 5 S rRNA gene has been localized, too. In a further paper KEUS et al. (1983a) have communicated the nucleotide sequences of flanking regions of the genes coding for high-molecular-weight chloroplast rRNAs (23 S and 16 S), which also are located in the 17 Md repeats of cpDNA. The region 5'-proximal to the 16 S rRNA gene contains three prokaryotic promoter motifs and - between them - a gene coding for valine tRNA. Oligonucleotide sequences in the 16 S rRNA have been determined for Lemna (see STACKE-BRANDT and WOESE 1981) and compared with that of several other plants (SEEWALDT and STACKE-BRANDT 1982). There is no specific relationship between the 16 S rRNAs of Lemna and Prochloron, a unicellular prokaryote containing chlorophylls a and b and lacking phycobiliproteins.

#### 2.5.7.5.2. Mitochondrial DNA

The mitochondrial genome of S. punctata has a size of at least 250 kilo

basepairs as revealed by restriction enzyme analysis (DE HEIJ et al. 1985). Certain restriction fragments of the mitochondrial DNA (mtDNA) were cloned. Then inserts of these clones were isolated, labeled by nick-translation, and used for back-hybridization. Each insert hybridizes to nearly all restriction fragments. The authors concluded from their results that the mtDNA contains a sequence which is reiterated about 100-fold.

#### **2.5.7.5.3. Nuclear genes**

The nucleotide sequence encoding the small subunit of ribulose 1,5-diphosphate carboxylase has been elaborated from L. gibba (STIEKEMA et al. 1983b). A cDNA clone has been used which was isolated from a poly(A) RNA-derived cDNA library (WIMPEE and TOBIN 1982, STIEKEMA et al. 1983a). The clone encodes 53 amino acids of the precursor transit peptide, the entire 120 amino acids of the mature small subunit polypeptide, and 241 nucleotides representing the 3' non-coding region of the mRNA. The amino acid sequence of the mature small subunit RuDPCase shows 70-75% homology to the reported sequences of other species.

#### **2.5.7.5.4. In vitro transcription, DNA repair, plasmid uptake**

Isolated nuclei from L. gibba have been used to investigate nuclear RNA synthesis and RNA polymerase activity. Incorporation rate of [<sup>3</sup>H]-uridine into RNA as well as RNA polymerase I activity depend on the nucleus isolation time during the day. Highest values for both parameters are reached during the night phase and a diurnal rhythm is exhibited with nuclei from plants held under continuous light. By contrast, activity of RNA polymerase II remains constant throughout the day (NAKASHIMA 1978, 1979a). Magnesium has three different functions on maintenance of RNA synthetic activity in isolated nuclei. Magnesium is a co-factor of RNA polymerase, it is a stabilizer for the binding of RNA polymerase I to nuclei, and for the binding of stimulating factors to the nuclei (NAKASHIMA 1979b).

Genetic information of chloroplast DNA from S. punctata has been expressed in vitro with a coupled transcription-translation system extracted from Escherichia coli (DE HEIJ and GROOT 1981). Within the products the  $\alpha$ - and  $\beta$ -subunits of ATPase were identified by immunoprecipitation.

tation with heterologous antisera raised against spinach  $\alpha$ - and  $\beta$ -subunits.

UV-induced DNA damage and repair have been investigated with W. microscopica and S. polyrrhiza (DEGANI et al. 1980). UV irradiation ( $250 \text{ Jm}^{-2}$ ) of plants induces the formation of cytosine-thymine and thymine-thymine dimers which are incorporated into the DNA. The capacity of plants to excise the dimers from their DNA has been examined by analysing DNA hydrolysates at different times after irradiation with UV. The ratio of thymine dimers to thymine in purified DNA amounts to 0.2% directly after irradiation. About 60% of dimers are removed during the following 7 hours of darkness and a total of 80% is removed after two days. In the light the disappearance of dimers from DNA proceeds faster and is complete within 3 hours (see 'photoreactivation').

Uptake of plasmid DNA into intact L. aequinoctialis has been tried by FREY et al. (1980). The plants were incubated with Escherichia coli plasmids pMB9 or pBR325 for 20-22 hours, then washed and treated with DNase I and proteinase K. The DNA isolated subsequently was tested for transformants by a bioassay (E. coli transformation to tetracycline resistance). The authors concluded from their results that in 7 out of 15 cases stable transformants were produced and that about 100-900 intact plasmid molecules were taken up by one Lemna plant.

## 2.5.8. Further compounds

### 2.5.8.1. Phospholipids and galactolipids

Metabolism of phospholipids has been followed in S. punctata after supplying plants with a 15-min pulse of  $^{32}\text{P}$ -phosphate (BIELESKI 1972). The first phospholipid to become fully labeled is phosphatidic acid. Then phosphatidyl glycerol and phosphatidyl inositol become labeled more rapidly than phosphatidyl choline and phosphatidyl ethanolamine. Phosphatidyl serine is the slowest to label and comprises only about 1% of the  $^{32}\text{P}$ -lipid. Synthesis of phosphatidyl glycerol is more sensitive to phosphorus deficiency than the other phospholipids. As phosphatidyl glycerol is located mainly in the chloroplast, the composition of lipids in the chloroplast changes. The phosphorus-deficient chloroplast has a 60% lower phospholipid content. Zeatin reduces the effect of P-deficiency on phospholipid synthesis.

The high percentage of galactolipids and unsaturated fatty acids is typical for the lipids of normal chloroplasts. In cultures of S. polyrrhiza provided with 1% sucrose not only the chlorophyll content per mg protein but also the galactolipid (especially digalactosyl diglyceride) content increase drastically (BAHL et al. 1971, LECHEVALLIER et al. 1971). High concentrations of benzyladenine (2-5 mg/l), which increase or slightly decrease the chlorophyll content in L. minor, lower the proportion of galactolipids as compared with phospholipids (BERUBE et al. 1982).

Sublethal doses of the herbicide atrazine increase the number of grana per chloroplast in L. minor (BEAUMONT et al. 1980) respectively the number and length of grana lamellae in S. punctata (MATTOO et al. 1983b). Correspondingly, the percentage of diacylgalactosyl glycerol (compared with total phospholipids and total neutral lipids) increases in L. minor on ground of the atrazine treatment (GRENIER et al. 1979). In the presence of atrazine, labeled acetate is incorporated more strongly in total lipids, diacylgalactosylglycerol, diacyldigalactosylglycerol, phosphatidylglycerol, and diacylglycerol (GRENIER and BEAUMONT 1983). Furthermore, atrazine stimulates the desaturation of fatty acids. The specific radioactivity after acetate incorporation increases under the influence of atrazine in linoleic and linolenic acid in nearly all investigated lipids (GRENIER and BEAUMONT 1983). The ratio of linolenic to linoleic acid in galactolipids and phospholipids is raised by atrazine



sterol content and increases the ratio of stigmasterol to campesterol (D'HARLINGUE et al. 1976).

Within carotinoids (see chapter 1.2.6) the colorless lycopersene (a C<sub>40</sub> analogue of squalene) occurs in L. minor (PREVITERA and MONACO 1984). The well-known effect of Sandoz 6706 [4-chloro-5(dimethylamino)-2-(tri-fluoro-m-tolyl)-3(2H)pyridotinone] to eliminate the synthesis of carotinoids, has been confirmed for S. punctata and L. minor (BIGGS and KOS-SUTH 1980). Experiments with plants treated with Sandoz 6706 revealed that carotinoids play an important role as protectant against ultraviolet-B radiation. Light effects on the carotinoid content in Lemnaceae have been investigated by MONEGER (1968d,f), MONEGER and JACQUES (1968), LECHEVALLIER et al. (1976), and ROMBACH (1976). Under prolonged illumination the action spectra for carotinoid production and photosynthetic activity (CO<sub>2</sub> fixation) are very similar. With short illumination, however, the action spectrum for accumulation of carotinoids in etiolated plants shows 3 maxima: in the blue, green, and red range (MONEGER 1968d, 1968f, MONEGER and JACQUES 1968).

Gibberellin A<sub>3</sub> (10<sup>-5</sup> M) decreases the carotinoid content somewhat in light-grown S. polyrrhiza (D'HARLINGUE 1976). Kinetin modifies the <sup>14</sup>C-acetate incorporation into carotinoids mainly during a prolonged dark phase. The radioactivity incorporated into violaxanthin, for example, is diminished by 10<sup>-7</sup> M kinetin, but enhanced by 3.3x10<sup>-6</sup> M kinetin, and strongly lowered by 3.3x10<sup>-5</sup> M kinetin (ADABRA-MICHANOL et al. 1975). The last-named kinetin concentration inhibits the production of chlorophylls a and b as well as of  $\beta$ -carotene, lutein, violaxanthin, and neoxanthin. In etiolated fronds of S. polyrrhiza, puromycin stimulates carotinoid synthesis and accumulation in the dark (MONEGER 1968d).

#### 2.5.8.3. Chlorophyll

Very different amounts of chlorophyll have been found in Lemnaceae depending on species and culture conditions (see chapter 1.2.6). The precursor of chlorophylls, chlorophyllide, is accumulated to a determinable degree in L. trisulca, but not in S. polyrrhiza and L. minor (ANTONIELLI and CAGIOTTI 1976).

Chlorophyll synthesis takes place in intimate correlation with chloroplast development. Several factors which inhibit the development of chloroplasts have a bleaching effect on developing duckweed fronds.



These investigations have been reported in detail in chapter 2.4.1.5. Destruction of chlorophyll occurs in L. trisulca at light intensities higher than 50000 lux, i.e., in light of an intensity 5 times higher than at the saturation point of photosynthesis (ZURZYCKI 1957a). Ultra-violet radiation, which leads to a rapid destruction of chlorophyll in L. minor, exerts its effect largely through the formation of ozone (WANGERMANN and LACEY 1952). In these and other cases not only the chlorophyll itself but also the thylakoid structure of chloroplasts may be affected. The herbicide nitrofen (2,4-dichlorophenyl-4-nitrophenylether) reduces not only the content of chlorophyll and carotinoids in L. gibba (WEJNAR and TAIS 1983), but also destabilizes the chloroplast structure (WEJNAR and MICHEL 1983). Stress factors such as polyethylene glycol (LECHEVALLIER 1977b) and senescence-promoting factors such as exhaustion of minerals, especially in cultures supplied with sucrose or gibberellin A<sub>3</sub> (D'HARLINGUE 1976, D'HARLINGUE et al. 1976), are further agents which lead to degradation of pigments and structures in chloroplasts (compare chapters 1.2.6 and 2.4.1.5).

Sucrose increases the chlorophyll content in S. polyrrhiza very greatly, when minerals are not limiting growth (BAHL et al. 1971). The positive effect of gibberellin A<sub>3</sub> on chlorophyll content in L. trisulca comes about by inhibition of senescence processes (BATA and NESKOVIC 1974). Growth-inhibiting agents can increase or decrease chlorophyll accumulation. Supply of  $10^{-6}$  M ABA (ALBANELL et al. 1985) or  $>0.6 \times 10^{-6}$  M CCC (SUPNIEWSKA 1963) to L. minor cultures causes a rise in chlorophyll content of plants. Growth-inhibiting concentrations of BA ( $10^{-5}$ - $10^{-4}$  M), on the other hand, diminish the chlorophyll level per weight of protein nitrogen in S. polyrrhiza (LE PABIC 1976a).

#### 2.5.8.4. Cinnamic acids

The aromatic amino acids phenylalanine and tyrosine are the source for biosynthesis of cinnamic acids and all their derivatives (phenylpropenoids). Phenylalanine-ammonia-lyase (PAL) is the key enzyme for this metabolic branch as it deaminates phenylalanine to cinnamic acid and - in several cases - deaminates tyrosine to p-coumaric acid (TAL activity). GORDON (1977) isolated the PAL from S. polyrrhiza, L. gibba, and L. aequinoctialis and showed the enzyme to possess both PAL and TAL activities. In S. polyrrhiza PAL activity is enhanced by  $Mn^{2+}$ . Very high lev-

els of PAL activity are induced by continuous UV radiation which is increasing anthocyanin accumulation. During continuous light (but not darkness) the PAL and TAL activities exhibit a circadian rhythmicity in S. polyrrhiza and L. aequinoctialis (GORDON and KOUKKARI 1978).

Up to now, hydroxycinnamic acids and their esters have been detected in 5 members of the Lemnoideae. In S. polyrrhiza, 5-p-coumaroylquinic acid, 5-caffeoylquinic acid, 1-feruloylglucose and 1-sinapoylglucose occur (KRAUSE 1978, KRAUSE and STRACK 1979a). Also in S. punctata (NG and THIMANN 1962) and L. gibba (UMEMOTO 1971) 5-caffeoylquinic acid, i.e., chlorogenic acid, has been indicated. L. trisulca and L. minor contain caffeic acid, and L. trisulca, in addition, ferulic acid (REZNIK and NEUHAEUSEL 1959). Interestingly, such substances are secreted from the plants to the medium, as has been demonstrated by JURD et al. (1957) and WALLACE (cited in McCLURE 1970).

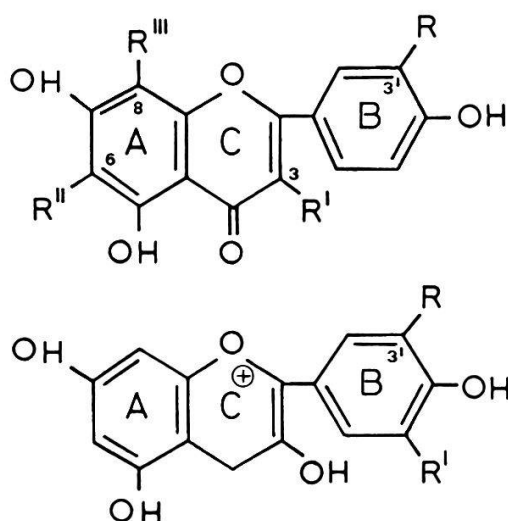
Certain phenylpropanes are intermediates on the way to prenylquinones, e.g., plastoquinone. 4-hydroxyphenylpyruvic acid, which is derived from tyrosine by an oxidase reaction, can be converted into homogentisic acid by a thylakoid preparation isolated from L. gibba (LOEFFELHARDT and KINDL 1979). The enzyme responsible for this reaction, 4-hydroxyphenylpyruvate dioxygenase, shows maximum activity, when  $\text{Fe}^{2+}$ , high concentrations of ascorbate and catalase are added to the incubation mixture.

The most important mechanism for formation of benzoic acids in plants is the side-chain degradation of cinnamic acids. The occurrence of benzoic acid in L. aequinoctialis and L. gibba was established by FUJIOKA et al. (1983a, 1985). The endogenous level of this compound is very much influenced by the composition and concentration of the culture medium, but the benzoic acid content in vegetative and in photoperiodically-induced flowering plants does not differ significantly. Salicylic acid, i.e., 2-hydroxybenzoic acid, could not be definitely identified in L. aequinoctialis. If it exists at all, the amount must be less than 1 ng/g fresh weight (FUJIOKA et al. 1983a).

Benzoic acids are known to be the source for production of phenols by oxidative decarboxylation. The ability to convert such phenols to the corresponding mono- $\beta$ -D-glucopyranosides for detoxification is widespread in angiosperms, but in L. minor and two other water plants (Elodea canadensis, Utricularia vulgaris) the ability to glucosylate quinol and resorcinol is lacking (PRIDHAM 1964). One can speculate that phenols may be released to the medium by water plants against the attack of microorganisms.

### 2.5.8.5. Flavonoids

Flavonoids are a large group of substances, which are all synthesized by combining a cinnamic acid with three acetate units. Ring A of the basic flavonoid structure (fig. 2.53) is formed from the three acetate units (via malonyl-CoA). The phenylpropan moiety gives rise to ring B and C-2, C-3, and C-4 of the heterocyclic ring C. The first  $C_{15}$  intermediate is the flavanone naringenin, which is in equilibrium with its chalcone.



Agylcones	R	R'	R''	R'''
apigenin	H	H	H	H
luteolin	OH	H	H	H
vitexin	H	H	H	glucose
orientin	OH	H	H	glucose
isovitexin	H	H	glucose	H
isoorientin	OH	H	glucose	H
kaempferol	H	OH	H	H
quercetin	OH	OH	H	H
Agylcones	R	R'		
cyanidin	OH	H		
petunidin	OCH <sub>3</sub>	OH		

Fig. 2.53. Flavonoid nucleus, numbering system, and substitutions (R) of the flavone, glycoflavone, and flavonols (above) and of the anthocyanidins (below) detected in Lemnaceae (after McCLURE and ALSTON 1966).

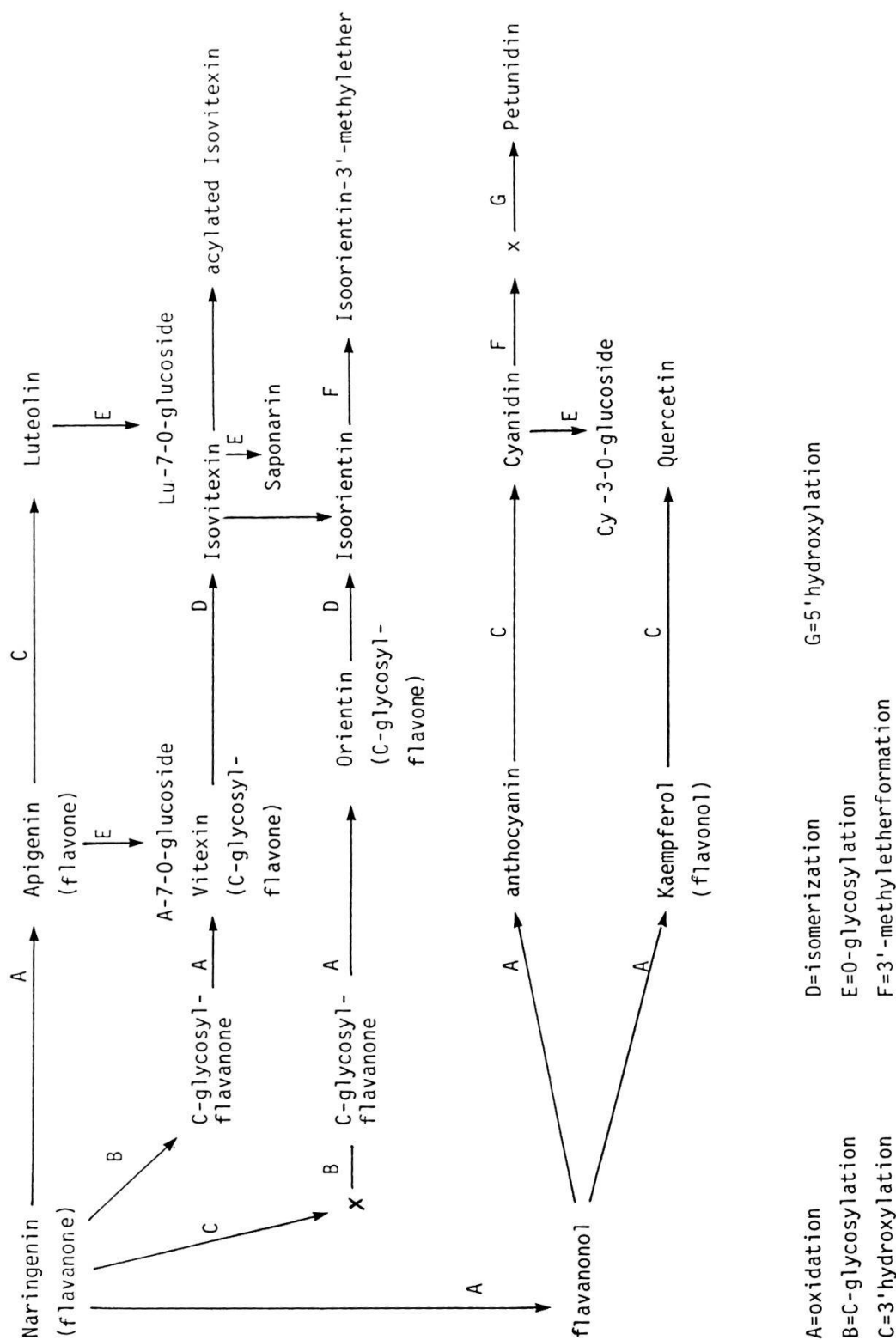


Fig. 2.54. Metabolic pathways leading to several subgroups of flavonoids in Lemnaceae, as elaborated essentially by WALLACE (see text).

Figure 2.54 summarizes the principal pathways leading to several subgroups of flavonoids in Lemnaceae. The scheme reflects the conclusions which can be drawn from the work of WALLACE (1967, 1969, 1973, 1975; WALLACE and ALSTON 1966a, 1966b; WALLACE et al. 1969; WALLACE and MABRY 1970; WALLACE and GRISEBACH 1973). Using  $^{14}\text{C}$ -labeled flavonoid aglycones WALLACE showed that Lemnoideae (at least S. polyrrhiza, S. punctata, L. minor, and L. obscura) are able to O-glycosylate supplied flavones. When  $^{14}\text{C}$ -apigenin is added to the nutrient medium, plants incorporate this flavone into apigenin-7-O-glucoside. Similarly, luteolin can be transformed to luteolin-7-O-glucoside. From these and other results it can be concluded that O-glycosylation of ring A and O-methylation of ring B evidently mark final stages of flavonoid biosynthesis. C-glycosylation of ring B, on the other hand, is an early biogenetic process occurring at the flavanone level. Naringenin but not apigenin or luteolin are incorporated into vitexin and orientin. Therefore, the enzyme oxidizing flavanone to flavone is irreversible. Furthermore, no labeled flavone is incorporated into an anthocyanin. The B-ring oxydation pattern of flavone-O-glucosides was determined at the flavone level of biosynthesis and was also essentially irreversible. The B-ring oxydation pattern of C-glycosyl flavones was noted to be prior to C-glycosylation, probably at the flavanone stage. Isomerization of the C-glycosyl flavones proceeds only from the 8-isomer to the 6-isomer (vitexin to isovitexin and orientin to isoorientin), but not in the reverse direction.

HOESEL et al. (1972) have shown that L. minor, which does not accumulate any flavonol, nevertheless can convert kaempferol and quercetin to the corresponding 2,3-dihydroxy flavanone. The authors argue such reaction could be the first step of a flavonol degradation, and conclude that a rapid turnover of flavonols may proceed even in L. minor. However, flavonols do not occur in the whole genus Lemna. Thus, the responsible enzyme in Lemna may use primarily another substrate instead of flavonols.

Especially the biosynthesis of anthocyanins depends on some environmental factors: light and the mineral content of the medium. The light requirement for anthocyanin formation is different in the three investigated species of Spirodela. In S. polyrrhiza the action of light is mediated by phytochrome and photosynthetic pigments. Five minutes of red light (far-red reversible) increase the anthocyanin content of dark-grown etiolated plants. When such plants are irradiated with white light

for 43 hours, the anthocyanin formation is inhibited by streptomycin (inhibition of chloroplast development), or by norflurazon (photooxidation of chlorophylls in the absence of carotenoids), or by CMU (inhibition of photosynthetic electron transport). Red and blue light, but not far red enhance anthocyanin accumulation during prolonged irradiation (MANCINELLI 1977, 1984; MANCINELLI and RABINO 1984). S. intermedia needs (in addition) high-energy blue light for anthocyanin formation. Under continuous red light anthocyanin production is completely lacking (McCLURE 1967b, 1968, 1975). S. punctata shows another type of light dependence, when plants have been pre-cultivated under light intensities of approximately 4000 lux. Under this condition the plants develop a low anthocyanin content. A terminating irradiation with single wave-lengths between 250 and 750 nm (before 40 hours of darkness) reveals that only far red, red, and ultraviolet can increase anthocyanin formation (maxima of the action spectrum at 705 nm and at 300 nm). Both blue and green light are relatively ineffective. Red and far-red exert additive effects only; no red/far-red antagonism can be obtained (NG et al. 1964).

The action of photosynthesis on anthocyanin formation in S. polyrrhiza can be replaced by adding sucrose to the cultures (MANCINELLI 1977, 1984, MANCINELLI and RABINO 1984). Promotive effects of sucrose on anthocyanin formation have been found also in S. punctata (THIMANN and EDMONDSON 1949, THIMANN et al. 1951, NG et al. 1964). THIMANN and RADNER then analysed the light requirement of anthocyanin formation in more detail. They found that RNA biosynthesis is needed for asserting the light effect on anthocyanin formation. Purine and pyrimidine analogs (THIMANN and RADNER 1955b, 1962) as well as ribonuclease (RADNER and THIMANN 1963) inhibit the production of anthocyanins in S. punctata. A further step in the reaction chain between light and anthocyanins seems to be the synthesis of riboflavine. Azaguanine inhibits not only anthocyanin but also riboflavine formation in the plants. Exogenous riboflavine can reverse the inhibiting effect of azaguanine (and some other inhibitors). Furthermore, the combined supply of riboflavine and sucrose can replace the light requirement completely (THIMANN and RADNER 1958). The inhibitory effect of methionine on anthocyanin formation (THIMANN and RADNER 1955a) also can be explained as an intervention in riboflavine synthesis. The conclusions which can be drawn from the results of THIMANN and co-workers are summarized in figure 2.55. The necessity for co-operation of photosynthates with riboflavine is stressed by the observation that

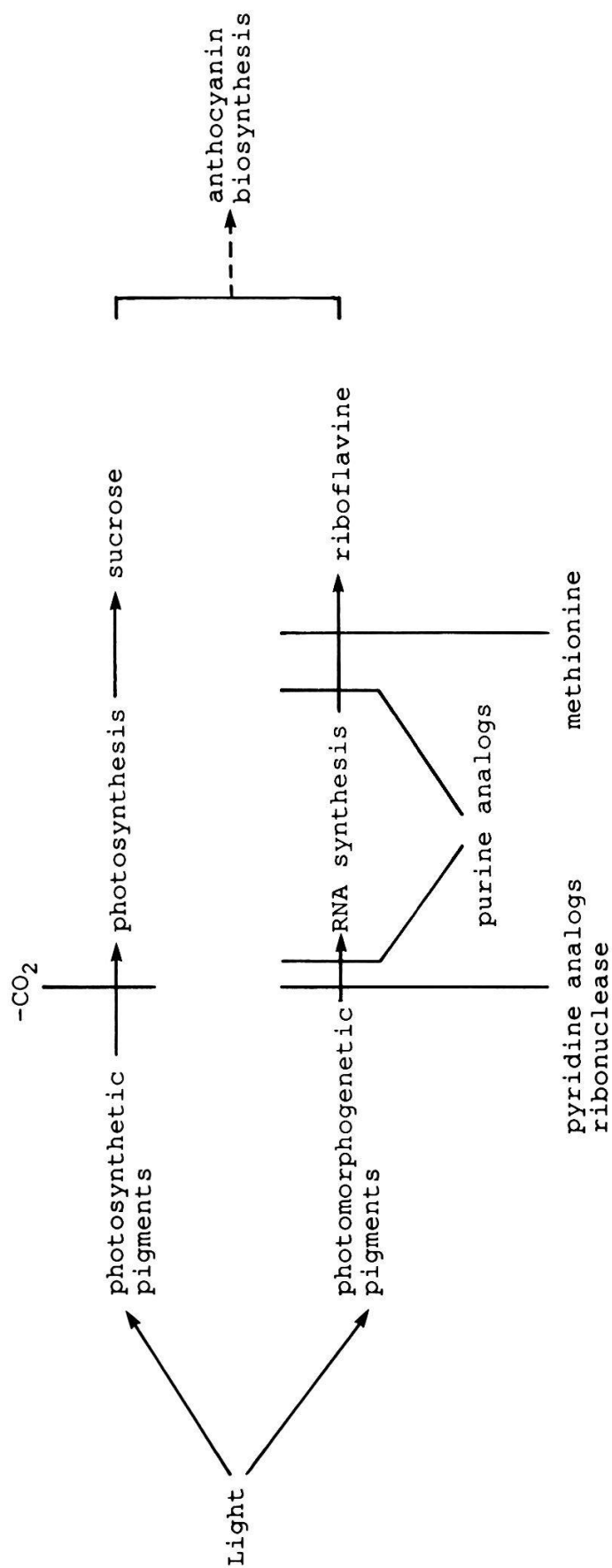


Fig. 2.55. Intermediate steps in the light action on anthocyanin biosynthesis in *Spirodela punctata*, as elaborated essentially by THIMANN and RADNER (see text).



absence of CO<sub>2</sub> during a preillumination period greatly reduces the effectiveness of riboflavine in the following dark period (THIMANN and RADNER 1958).

Light effects on other flavonoids have been investigated with S. intermedia by McCLURE and co-workers (see McCLURE 1968, 1970, 1975). Vitexin and kaempferol are synthesized to some degree even in complete darkness, but orientin and quercetin are stated to need low-energy blue or white light for accumulation.

Shortage of several mineral elements also influences anthocyanin formation. Low supply or deficiency of nitrogen (BALL et al. 1967, MENSCHIK 1970), phosphorus (THIMANN et al. 1951, BALL et al. 1967, JUNGNIKKEL 1978), iron (YOSHIMURA 1950), boron, zinc, and molybdenum (THIMANN and EDMONDSON 1949) promotes anthocyanin synthesis. In most, if not all cases a growth inhibition induced by the mineral deficiency may be the primary cause for the enhanced pigmentation. The effect of nitrogen deficiency in S. polyrrhiza can, but must not be connected with the induction of turions. Not only anthocyanin production is affected by nitrogen deficiency in S. polyrrhiza, but also the formation of other flavonoids (REZNIK and MENSCHIK 1969, MENSCHIK 1970). The relationship between growth inhibition (i.e., inhibition of protein synthesis) and enhanced anthocyanin formation exists also in experiments, in which copper (THIMANN and EDMONDSON 1949, EDMONDSON and THIMANN 1950) or a high concentration (1 mM) of EDTA (ELLIOTT 1977) were added to the medium. From three copper-chelating agents tested only one (phenylthiocarbamide) reduces anthocyanin formation without affecting plant growth (EDMONDSON and THIMANN 1950).

FURUYA and THIMANN (1964) investigated the effects of gibberellin A<sub>3</sub> on anthocyanin formation in S. polyrrhiza and S. punctata. In both species, anthocyanin synthesis is promoted by low GA<sub>3</sub> concentrations (10<sup>-7</sup>-10<sup>-6</sup>M) and inhibited by high GA<sub>3</sub> concentrations (10<sup>-4</sup>M) during the first 7-10 days of culture. After a further 10 days, all GA<sub>3</sub> concentrations are inhibitory. Then the sensitivity of plants to GA<sub>3</sub> is higher at lower light intensities. Under 3000 lux white light a concentration of 3x10<sup>-6</sup>M GA<sub>3</sub> is sufficient for 50% inhibition. At this level, GA<sub>3</sub> has no effect on growth.

A prerequisite for physiological investigations on flavonoids other than anthocyanins would be an effective and dependable method to separate and quantify these substances. STRACK and KRAUSE (1978) showed reversed-

phase high-performance liquid chromatography (HPLC) to be such a method for flavones and C-glycosylflavones from S. polyrrhiza. HPLC can also be used for separation of hydroxycinnamic acid conjugates (KRAUSE and STRACK 1979b).

#### 2.5.9. Circadian rhythms in metabolism and photoperiodism

Plants are well adapted to the daily changes of light and darkness. Obviously, endogenous oscillators ('clocks') do exist, which adjust the metabolic activities in relation to the expected environmental changes. In this way, a 'temporal compartmentation' (HILLMAN 1979a) between alternate metabolic pathways is established. In Lemnaceae circadian rhythms of respiration, potassium and nitrate uptake, nitrate reductase, and RNA metabolism have been thoroughly investigated. The relation between metabolic rhythms and the sensitivity changes to photoperiodic light signals has also been taken into consideration.

In L. aequinoctialis 6746 diurnal oscillations of CO<sub>2</sub> output were demonstrated under heterotrophic conditions (HILLMAN 1970a). In darkness following either continuous dim red light or entrainment to a 12(12) light (dark) schedule, the rate of CO<sub>2</sub> output oscillates with a circadian periodicity, before damping after one, two, or three days (see below). In continuous red light, the rate is linear. In darkness the CO<sub>2</sub> output rhythm can be prolonged by entrainment with a daily short red light. This phytochrome-mediated light effect acts as the synchronizer for the endogenous rhythm (HILLMAN 1971c, 1975b). O<sub>2</sub> uptake in strain 7646 is essentially parallel to CO<sub>2</sub> output under all conditions tested (HILLMAN 1977c).

The course of CO<sub>2</sub> output rhythm depends on the kind and extent of nitrogen supply as well as on temperature. With low or lacking nitrogen or with aspartate as the sole nitrogen source one daily peak of CO<sub>2</sub> output appears. With higher concentrations of nitrate or ammonia or with glutamine as the sole nitrogen source two daily peaks of CO<sub>2</sub> output are recorded (HILLMAN 1971a, 1972a, 1975b, 1979a). Damping of the CO<sub>2</sub> output oscillation in continuous darkness proceeds in the presence of nitrate after one single circadian cycle, with medium without nitrogen after two or three cycles, and with ammonium-containing medium after three or more cycles (HILLMAN 1975b). Lowering of the temperature from 28°C to 21°C reduces the relative peak height of CO<sub>2</sub> output rhythm roughly by 70% when plants were cultivated in nitrate, ammonium, or aspartate medium, but only by 20-30% in nitrogen-deficient medium (HILLMAN 1979b).

Similar CO<sub>2</sub> output rhythms as in L. aequinoctialis 6746 have been demonstrated also for other Lemnaceae [several strains of L. aequinoctialis, strains G1 and G3 of L. gibba, and strain 6573 of L. turionifera (under

the name of L. minor)]. Not only the distinctness of rhythm varies from strain to strain, but also the dependence on the kind of nitrogen provision (HILLMAN 1972a, 1979a).

Using mixotrophic conditions, MIYATA and YAMAMOTO (1969) pointed out a diurnal rhythm of  $O_2$ -uptake in L. gibba G3. After exposure to 2 short days plants were exposed to continuous white light. Under this condition a circadian oscillation of  $O_2$ -uptake proceeds, damping after about three days. A similar damped oscillation was found for  $^{14}CO_2$  output after supply of glucose-1- $^{14}C$ , but not with glucose-2- $^{14}C$  or glucose-6- $^{14}C$ . Thus, the rhythm comes about by variations of activity of the pentose-phosphate pathway. The activities of NADP-linked and NAD-linked glyceraldehyde-3-phosphate dehydrogenase show diurnal changes in opposite directions, and an oscillation of acid phosphatase activity runs undamped for at least 3 days. Some other enzymes are active without rhythmical variations: NADP glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, pyruvate kinase, and NADP isocitric dehydrogenase.

Further investigations with L. gibba G3 were made under autotrophic conditions (MIYATA 1970, 1971a, 1971b). The light-on signal for the  $O_2$  uptake rhythm is mediated by phytochrome. The rhythm is set in motion after a lag-phase of 6 hours, in which the phytochrome has to be in the  $P_{fr}$  state. After the end of the lag-phase the expression of rhythm needs the presence of relatively high light intensities. Photosynthetic inhibitors such as CMU and DCMU abolish the  $O_2$  uptake rhythm after a lag of 1 day. Only a part of the respiration is subject to the circadian oscillation. This rhythmic component is sensitive to azide, but not influenced by exogenous substrate or 2,4-dinitrophenol.

The physiological basis for the mutually inverse rhythmic changes in activity of NADP-dependent and NAD-dependent glyceraldehyde 3-phosphate dehydrogenases (GPDs) has been worked out by GOTO (1978, 1979a,b, 1983, 1984). The NADP-specific enzyme is located almost completely in the chloroplast and the NAD-specific enzyme largely in the cytoplasm. The activity changes are caused by alteration of the apparent  $K_M$  values, not the  $V_{max}$  values, of the GPD reactions for their substrate and coenzymes. In the case of NADP-GPD there are two molecular species, the NADP-GPD I with 520 Kdalton and low affinity for NADP, and the NADP-GPD II with 170 Kdalton and high affinity for NADP. The decrease in  $K_M$  value of NADP-GPD comes about by the conversion of NADP-GPD I to NADP-GPD II, which can be manipulated in vitro by addition of NADP(H). This result induced GOTO to

examine the levels of NADP(H) and NAD(H) in L. gibba G3. In fact, in vivo levels of total NADP and total NAD display mirror-image circadian rhythms, with NADP(H) peaking at the same time as NADP-GPD (12 hrs after the beginning of continuous light), and NAD(H) peaking at the same time as NAD-GPD (24 hrs after the beginning of continuous light). The enzymes converting NADP and NAD into one another also oscillate diurnally. NAD kinase activity is maximum 6 hrs after the beginning of continuous light, and NADP phosphatase activity (as of other phosphatases, see above) 12 hours later. Moreover, in vitro treatments with EGTA (a  $\text{Ca}^{2+}$ -chelator), chlorpromazine and W7 (both inhibitors of calmodulin) are able to both inhibit NAD kinase from its highest level of activity to its minimal one and activate NADP phosphatase from its lowest level of activity to its maximal one. From these results GOTO concluded that the in vivo level of  $\text{Ca}^{2+}$ -calmodulin should oscillate in phase with the rhythm of NAD kinase activity, in this way inducing mirror-image circadian rhythms of activities of NAD kinase and of NADP phosphatase. If this hypothesis were right, then  $\text{Ca}^{2+}/\text{Ca}^{2+}$ -calmodulin, NAD kinase/NADP phosphatase, and NADP/NAD could possibly be envisaged as components of a circadian clock, which is self-sustained and light-entrainable, and which controls the activities of NADP-GPD, NAD-GPD, and possibly several other enzymes. Figure 2.56 summarizes all the data of GOTO and also his working hypothesis.

The diurnal NAD/NADP-rhythm and the concurrent rhythm of respiration may be the basis for several other metabolic oscillations. First of all, the investigations of KONDO on rhythmic changes of uptake and release of inorganic ions, especially potassium, should be mentioned. When L. gibba G3 after the start of continuous light is transferred to distilled water at different times, then the amount of electrolytes leaking out from the plants changes diurnally (KONDO 1978). Maximum leakage appears in the early subjective day and minimum leakage in the early subjective night. As in respiration, the rhythm is reset by a light-on signal and persists under relatively high light intensities (3000 lux) for at least 3-5 days. Potassium and nitrate are the major ions, which contribute to the change in electroconductivity of the ambient water (KONDO and TSUDZUKI 1978, KONDO 1982b). Magnesium and phosphate ions show circadian rhythmicity of uptake and release to a far lesser extent and calcium ions are consumed by plants without any rhythmical feature. The potassium and nitrate uptakes are not linked together by a common ion permeating system,

because the nitrate uptake rhythm is not affected by the lowering of potassium concentration, and the potassium uptake rhythm persists to some extent in the absence of nitrate (KONDO 1982c).

The attributes of potassium uptake rhythm were communicated in many further publications. The rhythm appears regardless of the potassium concentration in the culture medium (from 10  $\mu$ M to 2 mM) (TSUDZUKI and KONDO 1979). The amplitude of oscillation is dependent on light intensity (KONDO and TSUDZUKI 1978, TSUDZUKI and KONDO 1979). In total darkness,

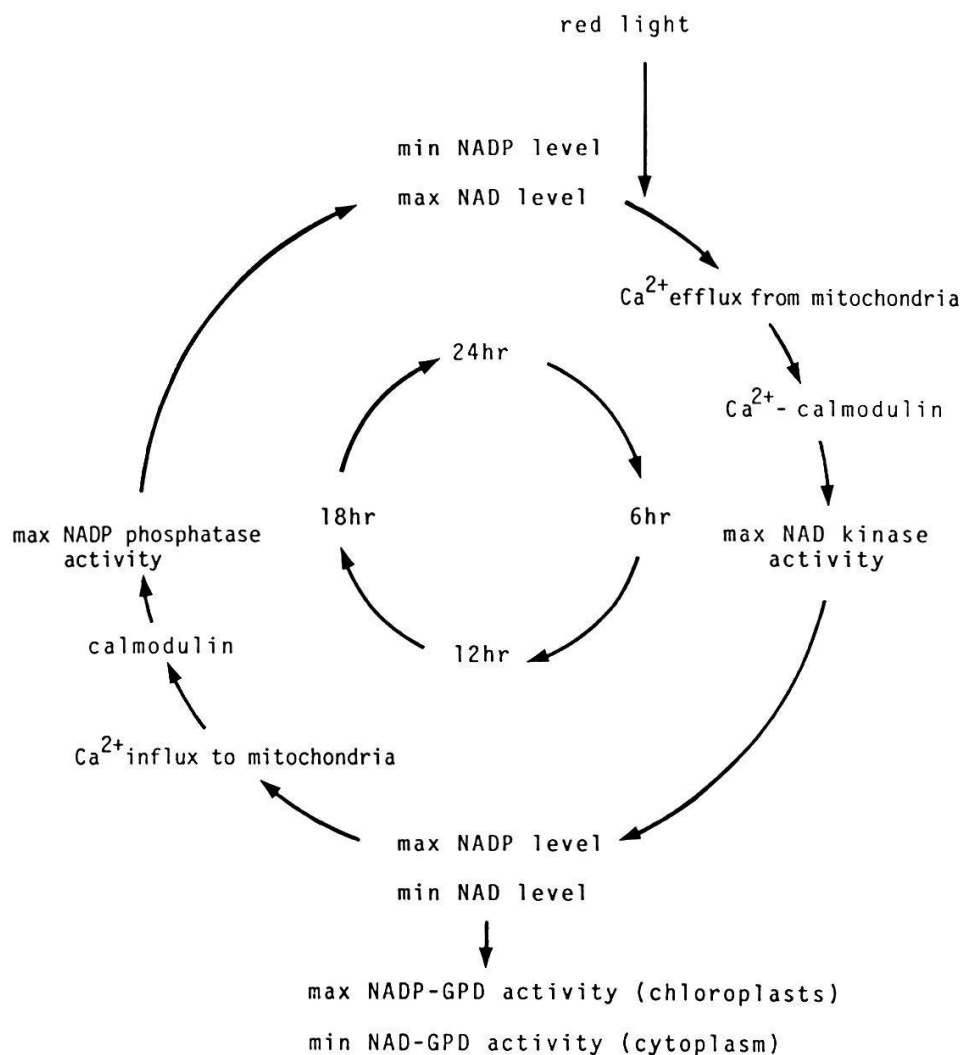


Fig. 2.56. Hypothetical scheme for a self-sustaining and light-entrainable rhythm working in Lemna gibba G3 (after data of GOTO, see text).

the rhythm fades out in c. 48 h, and under low-intensity light (below 700 lux) the rhythm is damped rapidly. With 13000 lux, on the other hand, the rate of potassium uptake increases almost linearly, probably due to the accelerated plant growth under this strong light. Therefore, a good rhythmic behaviour can be observed between 1500 and 8000 lux light intensity. Exogenous sucrose can largely replace the light requirement. A daily light pulse (4200 lux for 15 min) is sufficient under such conditions to sustain the rhythm (KONDO 1982a). Respiratory inhibitors or uncouplers (NaCN, 2,4-dinitrophenol, CCCP) reduce the average rate of potassium uptake without remarkably decreasing the amplitude of the rhythm.  $\text{NaN}_3$ , however, preferably diminishes the amplitude at a concentration as low as  $10^{-6}\text{M}$  (KONDO and TSUDZUKI 1980b). Factors changing the period of the free-running potassium uptake rhythm are lithium, rubidium, and ethanol (KONDO and TSUDZUKI 1980c, KONDO 1984b). Whereas  $\text{Li}^+$  ( $\geq 0.3\text{ mM}$ ) and  $\text{Rb}^+$  [ $\text{Rb}^+ / (\text{Rb}^+ + \text{K}^+) = 0.9$ ] lengthen the oscillation period under the routinely used experimental conditions, ethanol (0.3-3%) has a shortening effect. The lengthening of the period of potassium uptake rhythm due to  $0.5\text{ mM Li}^+$  can be removed by addition of  $20\text{ }\mu\text{M}$  sodium (KONDO 1984a).

Phase resetting of the potassium uptake rhythm can be managed by light, darkness, extreme temperatures, and azide (KONDO 1982d, 1983a,b, KONDO and TSUDZUKI 1980a). Under conditions of continuous light a pulse of high temperature ( $39^\circ\text{C}$ ) shifts the phase in the same way as a pulse of high-intensity light (15000 lux): both pulses cause a phase delay when given early in the subjective night, but cause a phase advance when given late in the subjective night, with a distinct discontinuity (break-point) in the phase response curve about the middle of the subjective night. A pulse of darkness, or of low temperature ( $5$  or  $10^\circ\text{C}$ ), however, causes a phase shift that is the inverse of that caused by high-intensity light or high temperature. Temperature steps from  $20$  to  $30^\circ\text{C}$  or from  $30$  to  $20^\circ\text{C}$  do not modify the phase of the rhythm. A 6-hour application of  $1\text{ mM}$  azide to plants has the same effect as the 6-hour pulse of darkness or low temperature.

In one publication, KONDO and TSUDZUKI (1978) investigated the circadian rhythm of potassium uptake not only in *L. gibba* G3, but also in *L. aequinoctialis* 6746. In correspondence to what is known about the respiration rhythm (see above), *L. aequinoctialis* 6746 displays a  $\text{K}^+$ -uptake rhythm only in the dark and not in the light. The rhythm does not persist beyond the second cycle.



Diurnal fluctuations of nitrate reductase activity with a peak in the forenoon were found in L. aequinoctialis (DEVI and MAHESHWARI 1979) and W. microscopica (BAKSHI et al. 1978, 1979). In both species, the rhythmicity persists under extended dark conditions as well as under continuous light, at least for one, two, or three cycles. In W. microscopica, the phase of the rhythm shifts under inverted light-dark cycles. As shown by inhibitor studies, the increase in enzyme activity seems to depend on the new synthesis of both RNA and protein. Despite the fact that nitrate reductase is induced by its substrate (see chapter 2.5.6.4), there is no correlation between enzyme activity and the level of tissue nitrate. The nitrate level in the tissue declines constantly during a 24-h period of analysis, whereas the nitrate reductase level shows normal fluctuations. In relation to the NAD/NADP-cycle, however, it is of interest that the nitrate reductase extracted from W. microscopica is protected by NADH (BAKSHI 1983).

Other enzymes which display a circadian rhythmicity are phenylalanine ammonia-lyase (=PAL, determined from S. polyrrhiza and L. aequinoctialis 6746) and tyrosine ammonia-lyase (determined from L. aequinoctialis). The oscillations of enzyme activity can be demonstrated under continuous light, but not - at least the PAL activity in L. aequinoctialis - in continuous darkness (GORDON and KOUKKARI 1978). Recently, investigations on PAL rhythmicity in S. punctata have been made by KNYPL et al. (1986). Diurnal as well as 2-3-day rhythms have been found, when plants were held under continuous light and were provided with glucose or sucrose. For interpretation of some of the results it should be taken into account that plants may release cinnamic acids and ABA into the medium (see chapter 2.5.2.3.4). Trans-cinnamic acid is an inhibitor of PAL, and ABA has been shown to shift the PAL oscillation.

A circadian rhythm of RNA synthesis (indicated by  $^3\text{H}$ -uridine incorporation into RNA) has been shown to occur in L. gibba G3 under continuous light conditions (NAKASHIMA 1976). Uridine incorporating activity is reaching its highest level at 18 h and its lowest at 6 h after the beginning of continuous light. The length of the period of the rhythm is not affected by temperature over a range of  $16^{\circ}$  to  $30^{\circ}\text{C}$ . The distinctness of amplitude of the rhythm depends on the quantity of light (duration and intensity) during the first half of the cycle, and on the  $P_{fr}$  state of phytochrome during the second half of the cycle (KATO and NAKASHIMA 1979). The diurnal oscillation comprises the synthesis of ribosom-

al RNAs in the nucleus as well as in the chloroplasts (NAKASHIMA 1975, 1978). As stated already in chapter 2.5.7.5.4, in nuclei only RNA polymerase I, but not RNA polymerase II activity changes diurnally (NAKASHIMA 1979a).

As in other plants, circadian clocks work in Lemnaceae not only to arrange the timing of certain metabolic activities, but also to permit day-length measurement in connection with photoperiodic control of flowering. In chapter 2.4.3.1.5 it was already mentioned that the effectivity of short light pulses to induce or to inhibit flowering, changes diurnally during prolonged dark periods. In a series of papers HILLMAN (1963, 1964, 1972b, 1976a, 1976b, 1976c) pointed out that in heterotrophically grown L. aequinoctialis 6746 the light sensitivity of flowering and the oscillation of respiration may be dependent on the same clock. In both cases, the rhythm is entrained in the same way by "filled skeleton photoperiods" (that is a series of 2, 4, or 6 short light pulses equally distributed during the regular photoperiod). A 6 h increase in length of the photoperiod delays the time of maximal light sensitivity for flowering and the time of maximal CO<sub>2</sub> output by 3.6 h and about 3 h, respectively.

The mechanism of the clock which controls the effectivity of light signals for regulation of developmental processes is unknown, but several details of its attributes are known. During the last decade OOTA has examined thoroughly, whether PITTENDRIGH's (1966) model of the photoperiodic clock may be applicable to the oscillator(s) responsible for light regulation of flowering in L. gibba G3 (OOTA 1970, 1971, 1975a,b, 1981, OOTA and HOSHINO 1979, OOTA and NAKASHIMA 1978, OOTA and TSUDZUKI 1979) and L. aequinoctialis 6746 (OOTA 1983a, 1983b, 1984, 1985). In both species, a great number of special light-dark schedules have been tried out. For example, in experiments with 24 h-light-dark cycles and night breaks given additionally not only the position of the night break (short light pulse) but also the duration of the main light period have been varied systematically. From a wealth of data the following conclusions have been drawn. In plants entrained by light-dark cycles a light-on oscillator works. A light pulse starts the circadian cycle, resetting the clock to its "L1-phase". In the following phase the clock runs independently whether plants are exposed to light or not. This means that complete or skeleton photoperiods are equally effective. After several hours the "L2-phase" is reached, which is identical with the terminating

part of the critical light phase. The L2-phase is light-sensitive and decides on the developmental effect (flower induction). In the long-day plant L. gibba G3 the critical light phase amounts to about 12 hours. Lightening of the L1- and L2-phase induces flowering. In the short-day plant L. aequinoctialis 6746 the critical light phase amounts to 14 hours. Lightening of the L1- and L2-phase prevents flowering. Light pulses given between a main light period and the light-sensitive L2-phase are registered as "false dusk", in this way establishing an "asymmetric skeleton photoperiod". Light pulses, however, which are supplied after the L2-phase, act as "false dawn". They constitute an asymmetrical skeleton photoperiod together with the following main photoperiod and shift the clock to the L1-state. After one (L. aequinoctialis) or two (L. gibba) transient cycles the entrainment is accomplished.

Under conditions of continuous light, the light-on oscillator described in L. aequinoctialis becomes inactive. Then another clock is set in motion, when plants are transferred to darkness (OOTA 1985). The light-off signal starts the L1-phase of a light-off oscillator. The terminating part of the critical dark phase (L2-phase) is reached 7 hours later. For flower induction in L. aequinoctialis especially this light-sensitive L2-phase has to be darkened. Flower inhibition during the L2-phase is effected by red as well as far red light.