A preliminary investigation on interactions (competition, allelopathy) between some species of Lemna, Spirodela, and Wolffia

by JERZY WOŁEK

1. Introduction

The present author has shown in his recent article (WOŁEK 1974) that there was an increasing tendency to describe various minor pleustonic associations. These units were solely determined by phytosociological methods; however, no more detailed ecological investigations have been made.

Out of the ecological factors which play an important rôle in the formation and establishment of plant communities differing from one another in respect to their floristical composition, competition and allelopathy are supposed to be of an utmost importance. The significance of competition and allelopathy was generally accepted and stressed by numerous authors(ZARZYCKI 1965, MULLER 1966, TUKEY 1969, ODUM 1971, WHITTAKER and FEENY 1971). For this reason,it seemed advisable to investigate such interactions occurring between pleustonic species. It should be added that studies of the interactions between pleustonic plants *(Lemna, Spirodela, Wolffia, Salvinia)* have been carried out sporadically and to no great extent.

Pleustonic species are on the whole readily found and easily cultivated. They do not require large experimental areas and form communities consisting of not too many species. These characteristics and in particular the simplicity of the duckweed communities, make them suitable for studying the mechanics of the formation of plant associations.

Experimental investigations on the competition between pleustonic species mainly concerned the influence of plant density upon the growth. IKUSIMA, SHINOZAKI and KIRA (1955) studied the intraspecific competition in *Spirodela oligorrhiza*. These authors investigated the influence of density upon the growth of duckweed. They payed a special attention to the C-D effect (competition-density-effect) and expressed interrelation between yield per population, initial frond number and time in mathematical

terms.

CLATHWORTHY (1960 , cit.acc. to HARPER 1961) as well as CLATWORTHY and HARPER (1962) studied inter-and intraspecific competition between various species of *Lemna*, *Spirodela* and *Salvina*, cultivated in laboratory conditions in uncrowded and crowding cultures, with gradual renewal of the culture solution. The English authors pointed out that the success of a species in mixture could not be predicted from the parameters of growth in pure culture. They also stressed the fact that morphological features of the studied plants notably influenced the competition in mixed cultures.

CLATHWORTHY and HARPER studied competition for space and all species used in their investigations were floating on the water surface. On the other hand, BORNKAMM (1970) used *Lemna minor* which floated on the water surface and *L. trisulca* which was submerged. In doing so he stressed competition for light and not for space. BORNKAMM stated that in mixed cultures *L. trisulca* as the weaker species showed not only the lower production of dry matter and protein, but also the lower protein content and the higher ratio: carbohydrate/protein.

LANDOLT (1957) also investigated interactions between some species of the *Lemnaceae* family. According to this author, competition between duckweeds in nature means competition for space, light and mineral salts. The struggle for space and light is decided by the fastest growth and the struggle for salts is decided by the ability to get the ions from diluted solutions.

The allelopathy among higher water plants was not studied hitherto. Chemical interactions were only studied in relation to planctonic ecosystems (WHITTAKER and FEENY 1971). However, the influence of various chemical substances on the growth of *Lemnaceae* was studied by some authors (LANDOLT 1957, HILLMAN 1961 and others).

In the present study, the two types of interactions viz. competition and allelopathy were treated separately. According to the definition presented by MULLER (1969), competition is the process in which the reaction of a plant upon the habitat reduces the level of some necessary factor to the detriment of some other plant sharing the same habitat (either simultaneously or sequentially). Factors such as radiant energy, oxygen, carbon dioxide, mineral nutrients, and water are all capable of depletion by plants. Some, in turn,are capable of increase. Competition occurs, however, only if the reaction involves a reduction demonstrably deleterious to an-

other individual.

On the other hand, allelopathy is the process in which a plant releases into the environment a chemical compound which inhibits or stimulates the growth of another plant in the same or a neighbouring habitat (MULLER 1969, TUKEY 1969, WHITTAKER and FENNY 1971).

The objective of the experiment was to study the interaction between pleustonic species cultivated in laboratory conditions. The author used sterile mixed cultures of two species simultaneously inoculated. They were growing on an unrenewed nutrient solution under the conditions of a free culture and therefore competition for space and light was supposed to be excluded.

The chief aim of the experiment was to establish the character of interaction (competition, allelopathy, or both types of interactions), as well as to study in what way the observed forms of interaction will act upon the growth of the studied species.

Acknowledgements

The experimental part of study was carried out in the Geobotanical Institute of the Swiss Federal Institute of Technology in Zurich under the guidance of Prof. Dr. E. Landolt.

I am greatly obliged to the Geobotanical Institute, Rübel Foundation and the Swiss Federal Institute of Technology in Zurich for the grant which enabled me to stay in Switzerland for three months.

I am particularly grateful to Prof. Dr. E. Landolt, the Head of the Geobotanical Institute, for stimulating discussions, suggestions and critical remarks concerning my study as well as for never failing interest throughout the experiments. I would also like to thank all the colleagues of Geobotanical Institute for their advice and help, and for the kindness they showed me.

The results of the study were worked out in the Institute of Botany of the Polish Academy of Sciences in Kraków, Poland. I would like to thank Prof. Dr. K. Zarzycki and Doc. Dr. A. Lomnicki for remarks and suggestions concerning the interpretation of results.

2. Material and Methods

Experiments were carried out on mixed cultures of two species and on monocultures which were grown on mediums containing extract acquired from the individual plants studied. The outline of the experiment was presented in Table 1.

Table 1. Outline of experiments

Type of culture	Species		Code	Initial number of groups
Pure control cultures	*Wolffia arrhiza*		W/c	ca. 40
	Lemna minor		Lm/c	4
	Lemna gibba		Lg/c	4
	Spirodela polyrrhiza		Sp/c	4
Mixed cultures	*W. arrhiza* with	*L. minor*	W/Lm	ca. 20/2
		L. gibba	W/Lg	ca. 20/2
		S. polyrrhiza	W/Sp	ca. 20/1
	L. gibba with	*L. minor*	Lg/Lm	2/2
		S. polyrrhiza	Lg/Sp	2/1
	L.minor with	*S. polyrrhiza*	Lm/Sp	2/1
Cultures on medium with extracts	*W. arrhiza* on nutrient with added extract from	*W. arrhiza*	W/(W)	ca. 40
		L. minor	W/(Lm)	ca. 40
		L. gibba	W/(Lg)	ca. 40
		S. polyrrhiza	W/(Sp)	ca. 40
	S. polyrrhiza on medium with added extract from	*S. polyrrhiza*	Sp/(Sp)	2
		W. arrhiza	Sp/(W)	2
		L. minor	Sp/(Lm)	2
		L. gibba	Sp/(Lg)	2
	L. minor on medium with added extract from	*L. minor*	Lm/(Lm)	4
		L. gibba	Lm/(Lg)	4
		S. polyrrhiza	Lm/(Sp)	4
		W. arrhiza	Lm/(W)	4
	L. gibba on medium with added extract from	*L. gibba*	Lg/(Lg)	4
		L. minor	Lg/(Lm)	4
		S. polyrrhiza	Lg/(Sp)	4
		W. arrhiza	Lg/(W)	4

2.1. Experimental plants. The plants used to the experiments were kinly put at my disposal by Prof. Dr. E. LANDOLT. Four species were chosen for the experiments: *Lemna minor* L., strain 7402 (Nowogród. district Białystok, Poland; coll. F. KLÖTZLI), *L. gibba* L., strain 7107 (Berlin Germany; coll. R. KANDELER as strain G1), *Spirodela polyrrhiza* (L.) Schleiden, strain 7401 (Nowogród, district Białystok, Poland; coll. F. KLÖTZLI), and *Wolffia arrhiza* (L.) Wimm., strain 7014 (Hannoversches Wendland, Germany; Coll. R. TÜXEN). The experiment was intended to be carried out on the taxons of Polish origin; however, this was not always possible and the missing species had to be replaced by samples from neighbouring countries.

The chosen taxa were those floating on the water surface - thus occupying, despite differences in their size, a similar ecological niche. They were supposed to compete for the same nutrients in uncrowded culture (elimination of competition for space and light).

In order to guarantee an equal start to the individual species, an attempt was made to level out the differences in size of the fronds. The largest fronds were those of *Spirodela polyrrhiza* and this species was therefore chosen as a standard unit for comparison. Two groups of *S. polyrrhiza* corresponded in size to four groups of *Lemna minor* or *L. gibba* and to about 40 groups of *Wolffia arrhiza*. These proportions were taken into account during the preparation of flasks for pure control cultures and monocultures with extracts. Halved proportions were used for the mixed cultures consisting each of two species.

2.2. Nutrient solution. HUTNER's standard nutrient solution was used HILLMAN 1969). It was diluted to 1/5 of its normal concentration;sucrose was never added. The pH of the culture solution was adjusted to 5·5 by the addition of 5n KOH. Subsequently, 200 ml solution was poured into 500 ml. flasks. The flasks were plugged with cotton wool and sterilized.

During the experiment the medium was not mixed, for such procedures disturbed the arrangement of the fronds. Only during the rearranging of the flasks could the nutrient solutions undergo a small degree of mixing.

2.3. Extract. The plant material necessary for preparing the extract was acquired by laying out sixty monocultures of the studied species - 15 cultures of each species. After about two weeks, when the plants co-

vered the surface of the nutrient, each sample was separately prepared. The plants were crushed in a porcelain mortar and submerged in distilled water (about 20 ml.) for about 1 h and the extract was filtered through blotting paper. The solution was subsequently filtered through a chalk filter, to eliminate the chlorophil; clear filtrate was then centrifuged for 30 min. (3000 r.p.m.). The extracts were made up with distilled water to 160 ml. Then 10 ml.of extract plus 200 ml. of nutrient solution was added to each of the 64 flasks which subsequently were stoppered with cotton wool and sterilized. The pH of the nutrient solution hardly un - derwent a change after the addition of 10 ml. of extract.

2.4. Parameters of experiment. The experiment was carried out in the conditioned environment chamber of the Geobotanical Institute of the Swiss Federal Institute of Technology in Zurich. A 16 h photoperiod was applied. The light intensity 100% at plant level varied between 27'000 and 36'000 lux at various points in the chamber. The intensity of the light changed throughout the day: 4^{00} - 25%; 4^{30} - 50%; 5^{00} - 75%; 5^{30} - 100%... 18^{30} - 75%; 19^{00} - 50%; 19^{30} - 25%; 20^{00} - 0%. The light source was provided by 215 W Philips fluorescent lamps. Red light was emitted by four Philips lamps (120 W each). The air temperature during the night and day was $20^{\circ}C \pm 0{,}5$. The temperature in the nutrient solution with the light at 100% intensity, varied between $24^{\circ}C$ and $27^{\circ}C$. The moisture in the air during day and night was 50%. Every third day the flasks were rearranged in order to even out the light and temperature conditions.

2.5. The progress of the experiment. The experiment lasted 14 days, from 28.12.1972 to 11.1.1973. Beginning from the fifth day of the experiment, all the cultures were photographed every third day. The experiment was ended after 14 days when the plants covered almost the whole area of the nutrient solution. However, there was still no sign of overcrowding which might lead to competing for space and light.

2.6. Mathematical analysis of the results. The groups *(Wolffia arrhiza)* or all the visible fronds *(Spirodela polyrrhiza, Lemna minor, L. gibba)* were counted from the photographs. The groups of *Wolffia arrhiza* were mainly two-membered, more rarely they were three-membered or consisted

of a single frond. These data were plotted on a logarythmic scale and then used to draw out growth curves and also to calculate the exponential growth rates of individual species in control cultures, mixed cultures and cultures containing extract.

To calculate the growth curves, the method of least squares was used (Perkal 1963).

The relative growth-rates of the cultures were calculated according to the formula:

$$\text{Relative growth-rate} = \frac{\log N_t - \log N_o}{t - t_o} \times 1000$$

where N_t - represents the number of fronds (groups) in the time t; N_o - represents the number of fronds (groups) in the time t_o; t and t_o - represent time expressed in days (LANDOLT 1957, HILLMAN 1961). Each of the studied species manifested some variability in growth-rate, according to the type of culture used in the experiment. The average values calculated for nine days were compared by using the Duncan test (OKTABA 1965). The results are presented in Figures and Tables.

3. Results and discussion

3.1. Experiments with Wolffia arrhiza (Figs 1 and 2).

The growth curves of *Wolffia arrhiza* in individual cultures are presented in Fig. 1. The growth of *W. arrhiza* in control cultures and in cultures containing extract, was characterised by exponential growth during the fourteen days of the experiment. In mixed cultures, *W. arrhiza* displayed an exponential growth phase up to the eleventh day of experiment. On the fourteenth day a fall in the number of plants was observed, the smallest being in the cultures containing *Lemna minor*, the largest in the cultures with *Spirodela polyrrhiza*.

The growth-rate of *Wolffia arrhiza* in mixed cultures shows at first a tendency to rise, but falls rather soon. The highest fall in growth-rate in relation to control cultures, is marked in the cultures mixed with *Spirodela polyrrhiza*, a lower one in the cultures with *Lemna gibba*, and the lowest (and latest) fall in the cultures with *L. minor* (Fig. 2), in this last case, a test did not show any significant difference. However, a significant difference was observed between the growth-rate of

W. arrhiza on a nutrient solution with added *Lemna minor* extract, and the growth-rate of this species in a mixed culture with *L. minor*.

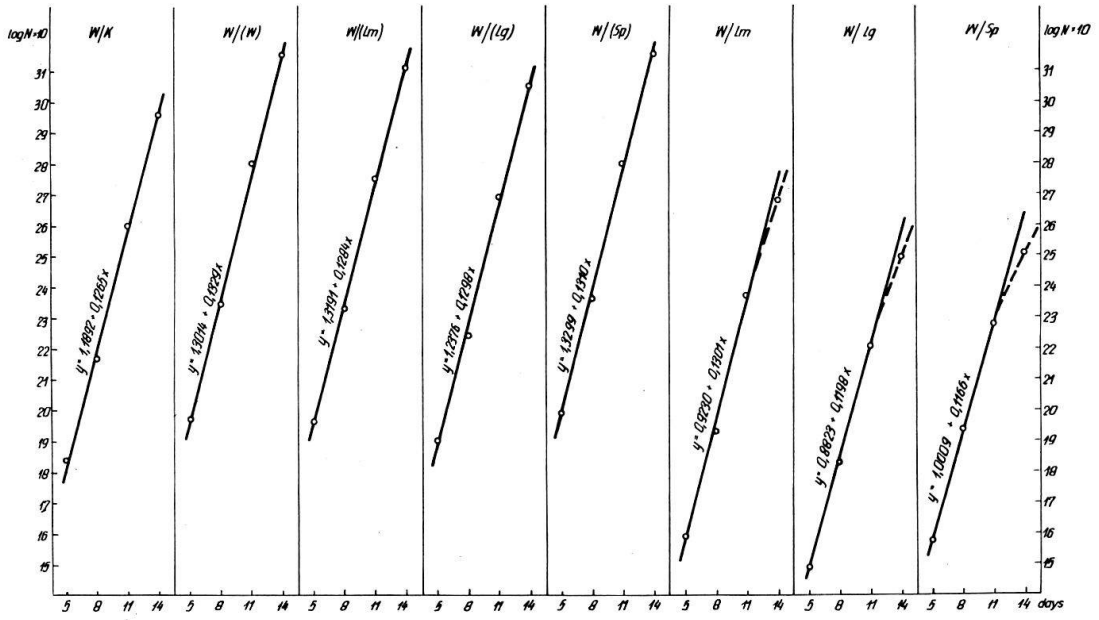

Fig. 1. Growth curves of *Wolffia arrhiza* in control cultures, mixed cultures and cultures with extract: circles represent the mean values of the 4(3) samples; N=the number of fronds.

Only in cultures with extract the growth-rate of *Wolffia arrhiza* displayed slightly higher values in the initial stages of the experiment as compared with the control cultures. However, these differences were not high enough to be statistically important. A significant rise in growth-rate, in comparison with control cultures, was only shown by *W. arrhiza* on a nutrient solution with added *W. arrhiza* extract.

The growth-rate of *W. arrhiza* in pure control cultures is presented in Table 2.

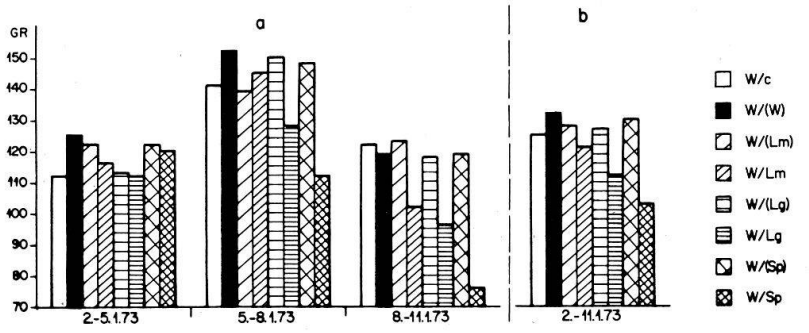

Fig. 2. The growth-rate (GR) of *Wolffia arrhiza* in control cultures, mimixed cultures and cultures with extract. Differences in the growth rates for 9 days-period were checked statistically; s=significant difference, n.s.= non significant difference; P=0,05.
W/c - W/(W) s. W/c - W/(Sp) n.s. W/c - W/Sp. s. W/(Sp) - W/Sp s.
W/c - W/(Lm) n.s. W/c - W/Lm n.s. W/(Lm) - W/Lm s.
w/c - W/(Lg) n.s. W/c - W/Lg s. W/(Lg) - W/Lg s.

Table 2. The growth-rate of studied species in pure control cultures.

	Lemna gibba	*Lemna minor*	*Wolffia arrhiza*	*Spirodela polyrrhiza*
Exponential growth-rate	155*	149	126	123
Mean values of growth-rate over a period of nine days	102	127	125	112

* Exponential growth-rate taken from the highest value.

3.2. Experiments with Lemna minor (Figs. 3 and 4).

The growth-curves of *Lemna minor* in individual cultures are represented in Fig. 3. The exponential growth phase of *L. minor* in control cultures and cultures with extract was up to about the eleventh day of experiment. On the fourteenth day, a drop in the number of plants was observed.

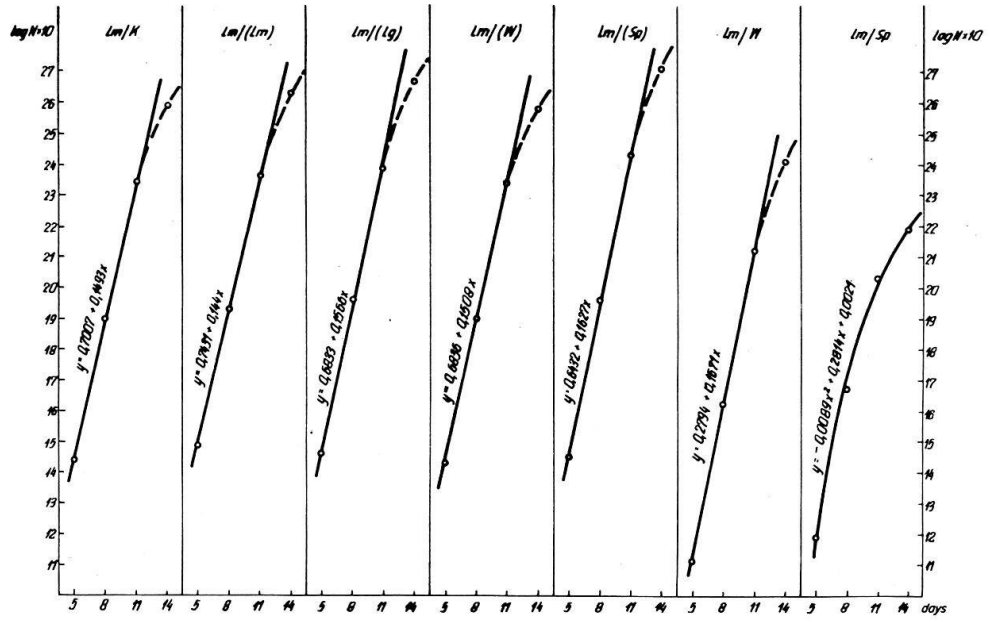

Fig. 3. Growth curves of *Lemna minor* in control cultures, mixed cultures and cultures with extract; circles represent the mean values of the 4(3) samples; N = the number of fronds.

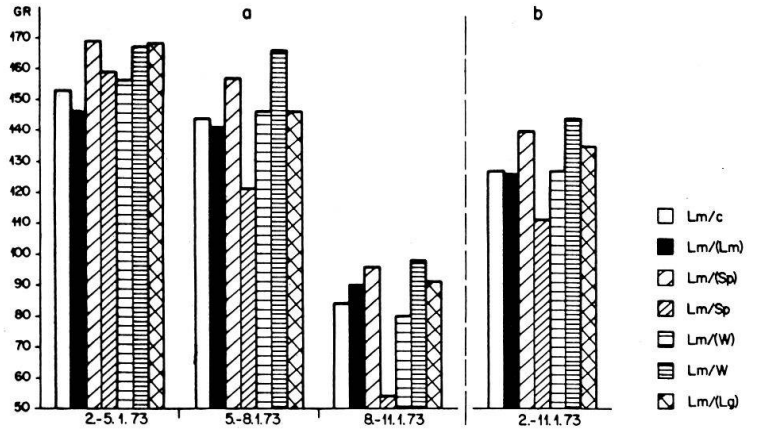

Fig. 4. Growth-rate (GR) of *Lemna minor* in control cultures, mixed cultures and cultures with extract. Differences in the growth rates for 9 days-period were checked statistically; s = significant difference, n.s. = non significant difference, P = 0,05.

Lm/c - Lm(Lm) n.s. Lm/c - Lm(W) n.s. Lm/c - Lm/Sp.s. Lm/(Sp)- Lm/Sp s.
Lm/c - Lm/(Sp)n.s. Lm/c - Lm(Lg) n.s. Lm/c - Lm/W s. Lm/(W) - Lm/W s.

An exponential growth of *Lemna minor* was also observed in the mixed cultures with *Wolffia arrhiza*. On the other hand, there was no satisfactory way to fit a straight line to the numerical data for *L. minor* in the mixed cultures with *Spirodela polyrrhiza;* therefore a parabola was fitted. It seems probably that the exponential growth phase for *L. minor* in the cultures with *Spirodela polyrrhiza* drops fairly rapidly, most probably around the eigth day of experiment. This would indicate the presence of a strong inhibitor limiting the growth of *L. minor*, and it might be *S. polyrrhiza* (Fig. 4) that causes a significant fall in the growth-rate of *L. minor* in comparison with control cultures. In cultures mixed with *Wolffia arrhiza*, the growth-rate of *L. minor* was significantly higher than in control cultures, and this tendency remained constant throughout the experiment.

Cultures cultivated on nutrient solutions with *Spirodela polyrrhiza* and *Lemna gibba* extract, showed significantly higher values for growth-rate in comparison with control cultures. On the other hand, the growth-rate of *L. minor* on nutrient solution with *L. minor* and *Wolffia arrhiza* extract, when compared with control cultures, did not display significant differences. The growth-rate of *L. minor* in pure control cultures is presented in Table 2.

Mixed cultures of *L. minor* and *L. gibba* were not considered, for none of the species could be clearly distinguished on the photographs. A direct counting of the fronds throughout the experiment proved to be impossible.

3.3. Experiments with Lemna gibba (Figs 5 and 6)

The growth curves of *Lemna gibba* in individual cultures are presented in fig. 5. In all the cases parabolas were fitted to the empirical data. The obtained results permit to assume that *L. gibba* shows exponential growth phase in the study period and it is likely to last till the eigth day.

The growth-rate of *L. gibba* cultivated on a nutrient solution with added extracts of *L. gibba* and *Spirodela polyrrhiza*, was higher than in control cultures and the differences were statistically significant. Only towards the end of the experiment the stimulating effect of the extract was brought to a halt (probably on account of the rise in the concentra-

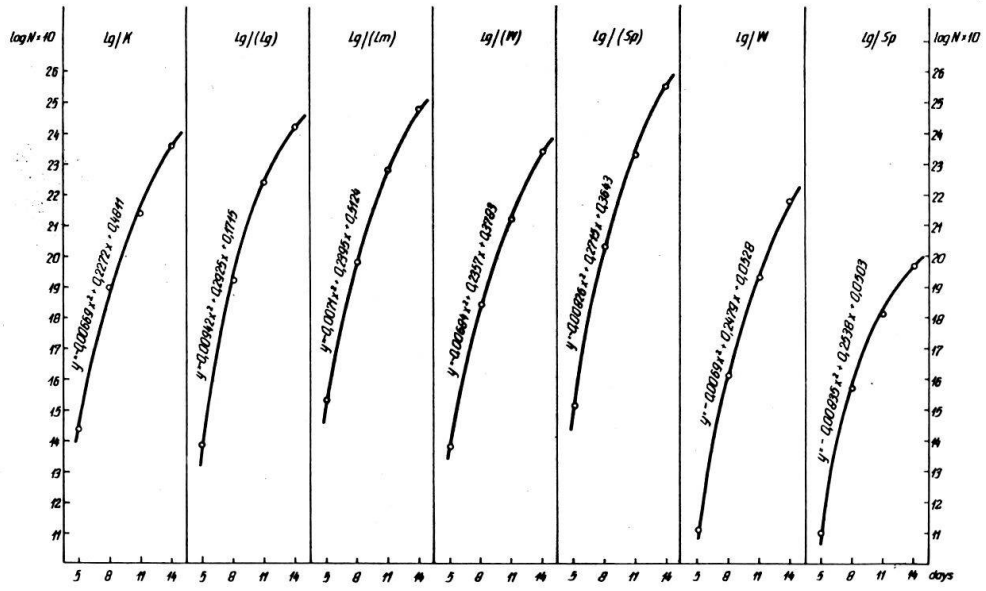

Fig. 5. Growth- curves of *Lemna gibba* in control cultures, mixed cultures and cultures with extract; circles represent the mean values of the 4(3) samples; N = the number of fronds.

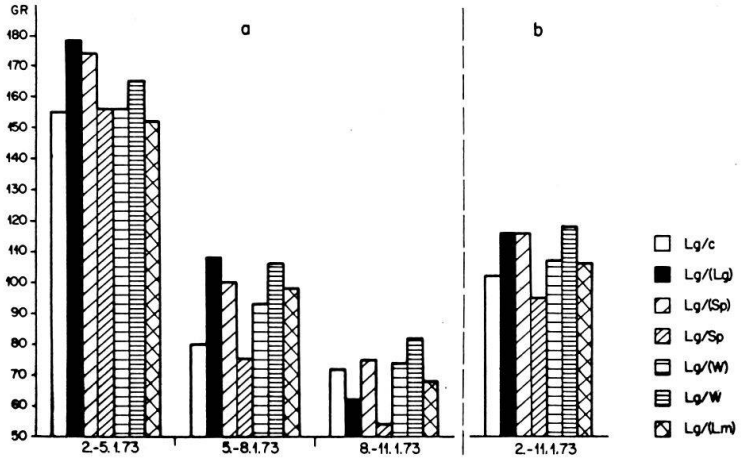

Fig. 6. The growth-rate (GR) of *Lemna gibba* in control cultures, mixed cultures and cultures with extract. Differences in the growth-rates for 9 days-period were checked statistically; s= significant difference, n.s. = non significant difference; P = 0,05.

Lg/c - Lg/(Lg) s. Lg/c - Lg/(W) n.s. Lg/c - Lg/Sp n.s. Lg/(Sp)-Lg/Sp s.
Lg/c - Lg/(Sp) s. Lg/c - Lg/(Lm) n.s. Lg/c - Lg/W s. Lg/(W) - Lg/W s.

tion of the metabolites) (Fig. 6). On the other hand, the growth of *L. gibba* on nutrients with added extracts of *Wolffia arrhiza* and *Lemna minor*, did not show significant differences when compared with control cultures, apart from certain oscillations.

The growth-rate of *L. gibba* in mixed cultures with *Spirodela polyrrhiza* did not show sighificant differences when compared with control oultures. This was because the fall in growth-rate in relation to control cultures was more marked in the last stages of the experiment.

In the cultures with *Wolffia arrhiza*, *L. gibba* showed a higher growth-rate than did the control cultures. The tendency was maintained throughout the experiment, and the difference between growth-rates was significant. The growth-rate of *L. gibba* in pure control cultures is presented in Table 2.

The mixed cultures of *Lemna gibba* with *L. minor* were not considered. It was found impossible to distinguish fronds of *L. gibba* from those of *L. minor* and behaviour of components in mixture could not be followed.

3.4. Experiments with Spirodela polyrrhiza (Figs 7 and 8)

The growth curves of *Spirodela polyrrhiza* in individual cultures are presented in Fig. 7. Only with some approximation could a straight line be fitted to the empirical data obtained from control cultures, from cultures cultivated on a nutrient solution containing *Lemna minor* extract, and from mixed cultures with *L. minor* and *Wolffia arrhiza*. This would indicate that in these cultures the exponential growth phase of *S. polyrrhiza* manifests itself up to the eleventh day of experiment. In the remaining cultures, the growth of *S. polyrrhiza* is represented by a parabola, and therefore the exponential growth phase should not last longer than up to the eigth day of experiment.

The growth-rate of *S. polyrrhiza* in cultures growing on nutrients with added extracts, did not significantly differ when compared with that of control cultures. Even when it was higher than in control cultures in initial stages of the experiment, it fell within a short period of time (Fig. 8).

In mixed cultures the growth-rate of *S. polyrrhiza* was significantly higher than in control cultures. Solely at the final stages of the experiment, the growth-rate of *S. polyrrhiza* in mixed cultures with *Lemna*

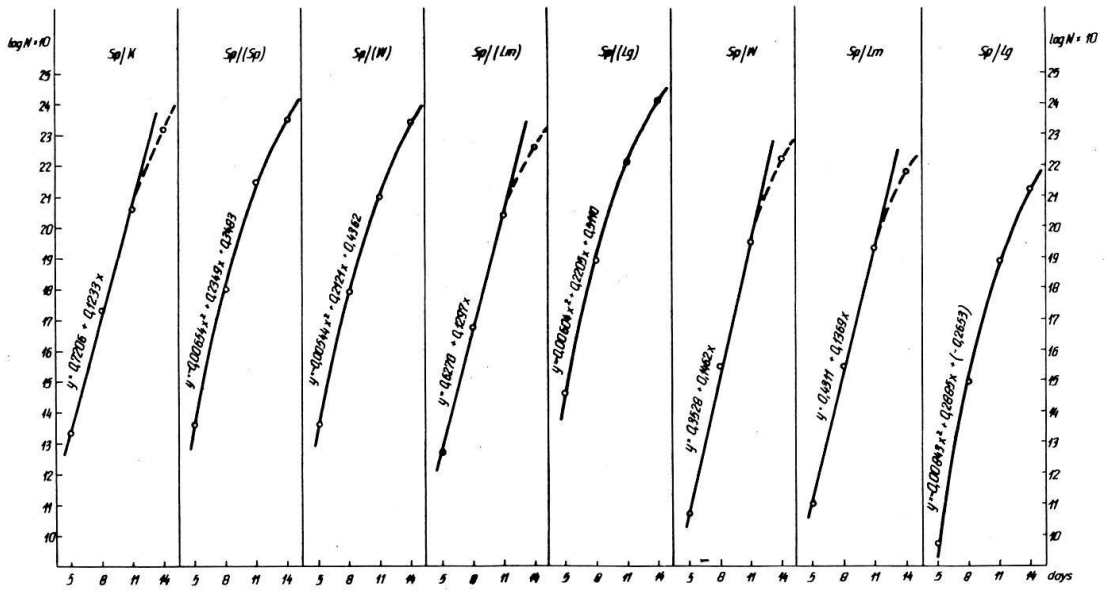

Fig. 7. Growth curves of *Spirodela polyrrhiza* in control cultures, mixed cultures, and cultures with extract; circles represent the mean values of 4(3) samples; N= the number of the fronds.

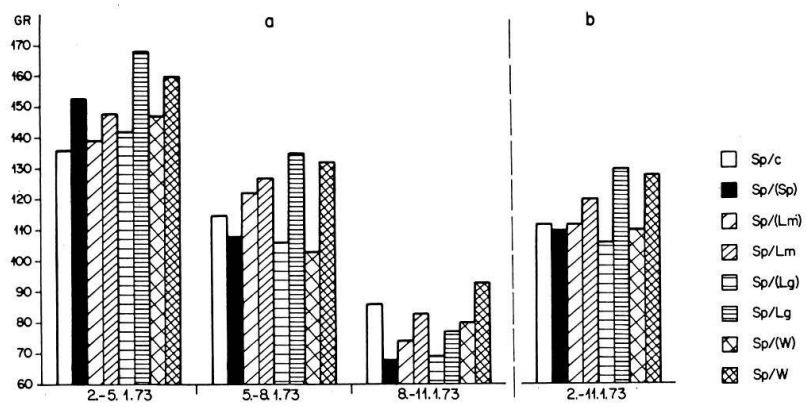

Fig. 8. The growth-rate (GR) of *Spirodela polyrrhiza* in control cultures, mixed cultures and cultures with extract. Differences in the growth-rates for 9 days period were checked statistically; s= significant difference, n.s.=non significant difference; P = 0,05.

Sp/c - Sp/(Sp) n.s. Sp/c - Sp/(W) n.s. Sp/c - Sp/W s. Sp/(W) - Sp/W s.
Sp/c - Sp/(Lm) n.s. Sp/c - Sp/ Lm s. Sp/(Lm)-Sp/Lm s.
Sp/c - Sp/(Lg) n.s. Sp/c - Sp/Lg s. Sp/(Lg)-Sp/Lg s.

gibba and *L. minor* was slightly lower than in the control. Nevertheless, the test showed the stimulating effect of mixed cultures on the growth of *S. polyrrhiza*. The growth-rate of *S. polyrrhiza* in pure control cultures is presented in Tabl. 2.

3.5. Interpretation of results (Fig. 9, Tab.3)

The results obtained on mixed cultures may be explained on the basis of the differences in morphology of the studied species.

All four species float freely on the water surface, but differ from each other in respect to shape and size of the submerged part (Fig. 9). This factor seems to decide, under experimental conditions, which species will be more effective in drawing mineral salts from the solution, and therefore will appear as stronger competitor in a mixed culture of two species. Fig. 9 explains why the lowest growth-rate is shown by *Wolffia arrhiza* in a mixed culture with *Spirodela polyrrhiza*, and why it yields to *Lemna minor* only towards the end of the experiment.

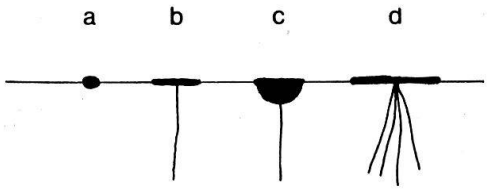

Fig. 9. *Wolffia arrhiza* (a), *Lemna minor* (b), *Lemna gibba* (c) and *Spirodela polyrrhiza* (d). c. 1 1/2 life size.

As to the factors affecting the growth of *Lemna minor* in mixed cultures with *Spirodela polyrrhiza*, the experiment showed that the competition for nutrient salts is stronger interaction than allelopathy; it is capable of halting the stimulating allelopathic influence of *S. polyrrhiza* on *L. minor* (see p. 7).

The results of the cultivation of *Lemna minor* on a nutrient solution with *Wolffia arrhiza* extract and in a mixed culture with *W. arrhiza* deserve a special mention. In the first case the growth-rate of *L. minor* does not show a significant difference as compared to that of control cultures. However, in a mixed culture *W. arrhiza* has a stimulating effect on the growth of *L. minor* (Tab. 3). It is unlikely that this stimulating effect is influenced by micro- or macroelements released by *W. arrhiza*

Table 3. Behaviour of *Wolffia arrhiza* (W), *Lemna minor* (Lm), *L. gibba* (Lg) and *Spirodela polyrrhiza* (Sp) cultivated in cultures with extracts and in mixed cultures (based on figures 2,4,6,8).

+++ strong stimulation --- strong inhibition
++ moderate stimulation -- moderate inhibition
+ weak stimulation - weak inhibition
O no reaction

\+ stimulation - inhibition O no reaction

Species	Type of interaction		Period of experiment 2.-5.1.73	5.-8.1.73	8.-11.1.73	2.-11.1.73
Wolffia arrhiza	Extract from	W	++	++	-	+
		Lm	++	O	O	0
		Lg	O	+	-	0
		Sp	++	+	-	0
	Competitor	Lm	+	+	--	0
		Lg	O	--	---	−
		Sp	+	---	---	−
Lemna minor	Extract from	W	+	O	-	0
		Lm	-	O	+	0
		Lg	++	O	+	+
		Sp	++	++	++	+
	Competitor	W	++	++	++	+
		Sp	+	---	---	−
Lemna gibba	Extract from	W	O	++	O	0
		Lm	-	++	-	0
		Lg	+++	+++	--	+
		Sp	++	++	+	+
	Competitor	W	++	+++	++	+
		Sp	O	-	--	0
Spirodela polyrrhiza	Extract from	W	++	-	--	0
		Lm	+	+	--	0
		Lg	+	-	--	0
		Sp	++	-	--	0
	Competitor	W	+++	++	+	+
		Lm	++	++	-	+
		Lg	+++	++	-	+

into the nutrient solution. It seems probable that this influence is of an allelopathic nature, and takes place when not too high a concentration of organic substances is released into the nutrient solution. A higher concentration of these substances does not have such an effect upon the growth-rate of *L. minor*. In this case, allelopathic reactions have a greater influence on the growth of the studied species than has the competition for nutrient salts. This could be explained by the fact that, in accordance with the proposed hypothesis, *L. minor* is the stronger competitor because its root can penetrate the nutrient solution zone beyond the reach of *Wolffia arrhiza*. Consequently, one could suggest that if competing for mineral salts is no great problem for *L. minor*, this interaction does not hide allelopathic influences. This seems also to be the case of *Lemna gibba*.

In the culture with *Spirodela polyrrhiza*, the stimulating allelopathic influence upon the growth of *L. gibba* was not noticeable. However, it is interesting to note that it was observed in the *L. gibba* culture growing on a nutrient with *S. polyrrhiza* extract (Tab. 3). It seems probable that also in this case *S. polyrrhiza* appears as a stronger competitor, because of its developed underwater part; therefore the competition for nutrient salts is a more important factor than the allelopathic activity, and for this reason allelopathy cannot reveal itself. On the other hand, *L. gibba* is a strong enough competitor, so that a clear lowering in its growth-rate in comparison with control cultures is only noticeable towards the end of the experiment.

The results are somewhat different in the mixed culture with *Wolffia arrhiza*. *Lemna gibba* is a stronger competitor than *W. arrhiza*, and therefore competing for mineral salts does not halt its growth. This brings about the possibility of a stimulating allelopathic influence, but only if smaller amounts of metabolites are released into the nutrient solution by *W. arrhiza*. This phenomenon was not noted when the metabolites were present in a higher concentration (extract from *W. arrhiza)* (Tab.3).

The stimulating effect of mixed cultures on the growth of *S. polyrrhiza* once more seems to support the earlier hypothesis that, out of the studied species, *S. polyrrhiza* is the strongest competitor in the conditions provided in the experiment, for it has the most developed underwater part.

On the other hand, it can be assumed that particular morphological characters of *Lemna minor* and *L. gibba* permit them to compete to some extent with *S. polyrrhiza* (see p. 154). Another explanation may be found in the particular sensitivity of *S. polyrrhiza* to rises in the concentration of organic substances with this species and this competitors release into the nutrient solution.

It seems that there exists an indirect proof supporting the opinion that the stimulation of the growth of stronger species observed in the experimental conditions could be explained by allelopathic reactions. Firstly, the plants were grown in uncrowded cultures - they therefore had sufficient amount of nutrients. Secondly, *Lemna minor*, *L. gibba* and *Wolffia arrhiza* cultivated on nutrient solutions with added extracts manifested a significant stimulation of the growth-rate, in spite of the fact that their monocultures were inoculated with the same number of groups as the corresponding control flasks. The latter observation suggests that the stimulation resulting from less intense intraspecific competition of the stronger species (ZARZYCKI 1965) does not occur. However, this problem requires further investigations.

3.6. Comparison with results of other workers

The importance of the morphological features determining the results of competition for space (=light), was stressed by CLATWORTHY and HARPER (1962). For instance, gibbosity of *Lemna gibba* meant that this species always turned out victorious when it was cultivated in mixed cultures with *Spirodela polyrrhiza*. The well developed aerenchyma meant that *L. gibba* occupied the surface layer of the frond matting as soon as the culture became crowded. This caused the overshadowing of the fronds of *S. polyrrhiza*, which eventually died from lack of light.

In the conditions studied by the present author, the same well developed aerenchyma made *Lemna gibba* to a stronger competitor than *Wolffia arrhiza* and *Lemna minor*, but a weaker competitor than *Spirodela polyrrhiza*; one might assume that the same morphological feature may express various competitive values depending on the particular conditions.

The importance of the concrete ecological situation for the result of competition, has already been emphasized by GAUSE, NASTUKOVA and ALPATOV (1934), in their studies on the influence of the metabolite contaminated

environment upon the growth of the population *Paramecium caudatum* and *P. aurelia*, and upon the competition between them. In 1948 PARK (cit. acc. to ŁUCZAK 1956) confirmed the results of these experiments on the basis of his own findings.

The modifying influence of the environment was also studied over several years by CROMBIE (1947) who used insects of *Rhizoptera*, *Tribolium* and *Oryzaephilus* for this purpose. He discovered that competition for food occured in an unrenewed environment with a limited supply of food and contaminated with the products of metabolism. The background to this interaction was the destructive influence of the metabolites on competing populations. However, the action of metabolites became the most important factor when there was enough food.

An identical pattern was found in mixed cultures of two species. Chemical interaction stimulated growth of the stronger species(very weak competition for mineral salts or its total absence). In the case of the weaker species the allelopathic influence on its growth was not in evidence because competition for mineral salts was a more important factor.

TUKEY (1969) states that many laboratory experiments concerning the allelopathic action of plants on one another were not successfully demonstrated in the natural conditions. It seems possible that appearance of both considered interactions is strongly influenced by ecological factors.

The behaviour of species studied in the course of the present work supports the opinion that the growth-rate in pure cultures cannot be the factor that decides in favour of the competitive abilities of given taxon. In this respect, the present results stay in agreement with the previous data of CLATWORTHY and HARPER (1962) as well as with those of GILL (1972) in *Paramecium*.

4. Conclusions

The experiments showed two types of interactions to exist: competition for mineral salts and allelopathic action of plants on one another.

1. Taking into account the various criteria, the studied species can be arranged in the following orders:

a) Exponential growth-rates in control cultures

Lemna gibba > *Lemna minor* > *Wolffia arrhiza* > *Spirodela polyrrhiza*

b) Mean values of growth-rate in control cultures over a period of nine days

Lemna minor > *Wolffia arrhiza* > *Spirodela polyrrhiza* > *Lemna gibba*

c) Success in mixed cultures, based on five types of experiments:

W. arrhiza + S. polyrrhiza, W. arrhiza + L. gibba, W. arrhiza + L. minor, S. polyrrhiza + L. gibba, S. polyrrhiza + L. minor, *Spirodela polyrrhiza* > *Lemna gibba* > *Lemna minor* > *Wolffia arrhiza*

It is impossible to foresee which species will be the stronger competitor from the speed of growth-rate in pure control cultures. The present results point to a decisive rôle of morphological characteristics; species with longer roots or stronger developed underwater parts that reach deeper into the nutrient solution appear to be better competitors in experimental conditions.

2. The interspecific competition for mineral salts inhibits the growth-rate of weaker competitor in mixed undrowded cultures consisting of two species. On the other hand, the stronger species does not seem to be disturbed in any significant way.

3. The allelopathic influence of metabolites released by plants into the nutrient solution, is stimulating the growth of the studied species if the metabolites concentration is low. Higher concentration of metabolites ceased to be stimulating and caused either an indifferent reaction or an inhibiting one.

4. A stimulating allelopathic influence upon the studied species may not appear in the presence of a stronger competitor for the competition, for nutrient salts represent a more important factor.

Summary

The present work deals with experimental studies on interactions between *Lemna minor, L. gibba, Spirodela polyrrhiza* and *Wolffia arrhiza*.

The plants were cultivated in laboratory conditions, in sterile cultures simultaneously inoculated and occupying more or less equal surface at the start. In order to eliminate competition for space and light the plants were grown in uncrowded cultures. Nutrient solutions were unrenewed. A 16 h photoperiod was applied. The average light intensity at plant level varied between 27'000 and 36'000 lux at various points in a control-

led environment room. The light source was provided by 215 W Philips fluorescent lamps. Red light was emitted by four Philips lamps (120 W each). The air temperature during the night and day was 20°C ± 0,5. The temperature in the nutrient solution with the light at 100% intensity, varied between 24°C and 27°C. The plants were grown on 1/5 strength Hutner's nutrient solution.

Two types of interactions were found to exist: competition for nutrient salts and allelopathic action of plants on one another

The competitive strength of studied species falls into the following order:

S. polyrrhiza > *L. gibba* > *L. minor* > *W. arrhiza*. One can never foresee which species will be the stronger competitor from the speed of growth-rate in pure control cultures. The present results point to a decisive rôle of morphological characteristics: species with longer roots or stronger developed underwater parts reach deeper into the nutrient solution and appear to be better competitors in experimental conditions.

The competition for mineral salts inhibits the growth-rate of weaker competitor in mixed uncrowded cultures consisting of two species. On the other hand, the stronger competitor does not seem to be disturbed in any significant way.

The allelopathic influence of metabolites released by plants into the nutrient solution, is stimulating the growth of the studied species, if the metabolites concentration is low. Higher concentration of metabolites ceased to be stimulating and caused either an indifferent reaction or an inhibiting one.

A stimulating allelopathic influence upon the studied species may not appear in the presence of a stronger competitor,for the competition for nutrient salts represents a more important factor.

Zusammenfassung

Die vorliegende Arbeit umfasst experimentelle Untersuchungen über Wechselwirkungen zwischen *Lemna minor, L. gibba, Spirodela polyrrhiza* und *Wolffia arrhiza*.

Die Pflanzen wurden in Klimakammern auf sterilen Lösungen nach Hutner/ 1/5 verdünnt, kultiviert. Die neuen Kulturen wurden gleichzeitig so beimpft, dass die Arten bei Versuchsbeginn ungefähr gleiche Flächen einnamen . Um die Konkurrenz um Raum und Licht auszuschalten, wurden die Versuche jeweils abgebrochen, bevor die Pflanzen die ganzen Flächen überdeckten. Die Nährlösungen wurden während des Versuches nicht erneuert. Als Lichtquelle wurden 215 W Philips-Fluoreszentröhren verwendet, denen rotes Licht durch 120 W Philips - Lampen beigefügt wurde. Die Tageslängen betrugen 16 Stunden, die Lichtintensität schwankte innerhalb der Klimakammern auf Pflanzenhöhe zwischen 27'000 and 36'000 Lux. Die Lufttemperatur war während des Tages und der Nacht 20°C ± 0,5. Die Temperatur der Nährlösung während des Tages variierte zwischen 24° und 27°C.

Zwei Wechselwirkungen zwischen den Pflanzen konnten beobachtet werden: Konkurrenz um Nährsalze und allelopathische Wirkung der Pflanzen aufeinander.

Die Wettbewerbskraft der untersuchten Arten sinkt in der folgenden Reihe:

S. polyrrhiza > *L. gibba* > *L. minor* > *W. arrhiza*. Es ist unmöglich, nach der Wachstumsrate in Reinkultur im voraus zu beurteilen, welche Art konkurrenzkräftiger ist. Die vorliegenden Ergebnisse weisen auf die Bedeutung von morphologischen Eigenschaften hin: Arten mit langen Wurzeln oder gut entwickeltem Sprossteil unter Wasser tauchen tiefer in die Nährlösung ein und scheinen unter den Versuchsbedingungen konkurrenzkräftiger zu sein.

Die Konkurrenz um Nährsalze verzögert das Wachstum des schwächeren Partners in Mischkulturen, die die Oberfläche noch nicht überdecken. Dagegen wird der stärkere Partner durch die Konkurrenz offenbar nicht wesentlich beeinflusst.

Stoffwechselprodukte, die durch die Pflanzen in die Nährlösungen ausgeschieden werden, stimulieren in niederer Konzentration das Wachstum der untersuchten Arten. Hohe Konzentrationen hatten entweder keinen Einfluss oder hemmten das Wachstum.

Ein stimulierender allelopathischer Einfluss auf die untersuchten Arten kann bei Anwesenheit eines stärkeren Konkurrenten nicht immer in Erscheinung treten, weil die Konkurrenz um Nährsalze stärker wirkt.

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